Czech Annual Cancer Research Meeting

November 24–26 2025 | Olomouc, CZ

20th Czech Annual Cancer Research Meeting

20th Molecular Pathology Days

4th Conference of the National Institute for Cancer Research

ABSTRACT BOOK

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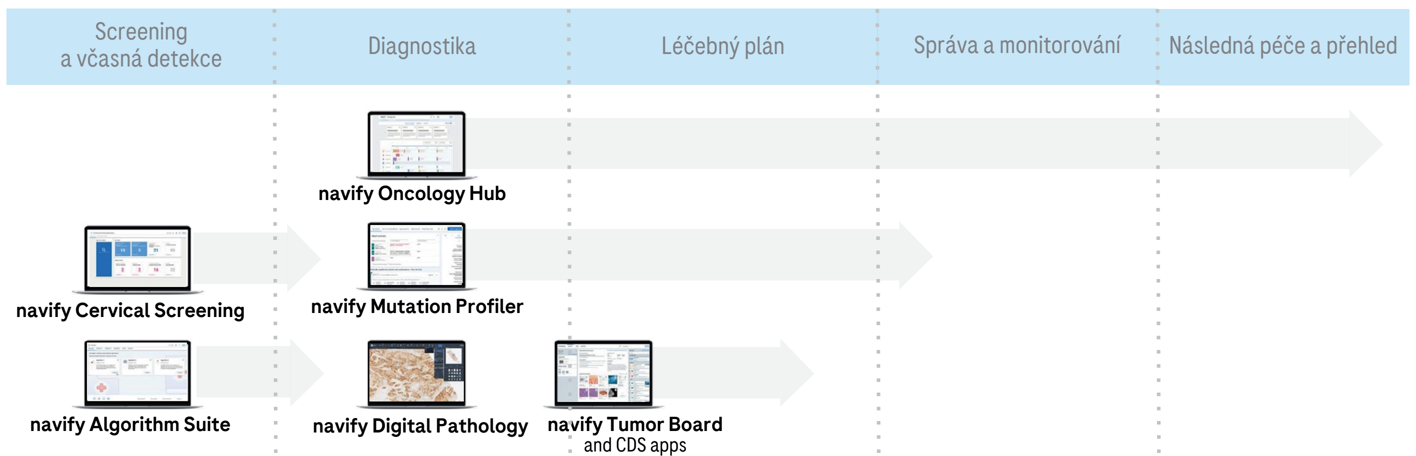

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CZECH RECOVERY PLAN

MINISTRY OF EDUCATION, YOUTH AND SPORTS

MONDAY NOVEMBER 24 2025

13:00 - 13:15 **GRAND OPENING**

Precision Oncology in Colorectal Cancer: Genetics, Immunity, and Gender

Chairs: Aleksi Sedo, Josef Srovnal

13:15 - 13:45	Germline Genetics of Colorectal Cancer **Asta Forsti**
13:45 - 14:00	Difference in TMB Distribution and Specific Mutation Types in CRC Patients with Different Times of Metastasis **Filip Ambrozkiewicz**
14:00 - 14:15	Distribution and Prognostic Significance of Macrophages in Colorectal Cancer Between Adjacent Non-Tumor Mucosa, Primary Tumor and Liver Metastasis **Andriy Trailin**
14:15 - 14:30	Beyond the Diagnosis: Gender Aspects in Rectal Cancer Management **Ludmila Boublikova**
14:30 - 14:45	Functional Single-Cell Analysis to Advance CAR-T and TCR-T Therapies Through Beacon Discovery **Tiago Santos**
14:45 - 15:00	Advancing Cancer Research With Accessible Single-Cell Technologies **Lukas Valihrach**

15:00 - 15:30 **COFFEE BREAK**

Molecular Targets And Anticancer Drugs I

Chairs: Karel Smetana, Alexander Doemling

15:30 - 16:00	Protein Regulation in Multiple Myeloma **Jan Kronke**
16:00- 16:30	Spatial Profiling of Cancer-Associated Fibroblast Heterogeneity in Head and Neck Cancer **Daniela-Elena Costea**
16:30 - 16:45	Towards Precision Medicine: PROTAC-Based Selective Degradation of P53-Y220c **Imma Capriello**
16:45 - 17:00	Development of Fully Characterized Human 3D Cancer Cell Lines for *In Vitro* And *In Vivo* Modeling of Pancreatic and Colon Cancer **Jan Strnadel**
17:00 - 17:15	The Hox Code is Respected by Adult Human Cancer-Associated Fibroblasts **Karel Smetana ml**
17:15 - 17:30	Regulation of Microtubule Organization and Migration in Glioblastoma Cells by GIT Proteins **Vadym Sulimenko**

TUESDAY NOVEMBER 25, 2025

Molecular Mechanisms and Emerging Therapeutic Targets in Cancer I

Chairs: Ludmila Boublikova, Luca Vannuci

9:00 - 9:30	Metabolic Reprogramming in Liver Cancer **Dirk Mossmann**
9:30 - 9:45	Molecular Profiling of the Cervical Microbiome for Predicting Lesion Progression **Veronika Holubekova**
9:45 - 10:00	The Microbiome in Tumor Tissue and Adjacent Mucosa from Colorectal Cancer Patients **Pavel Vodicka**
10:00 - 10:15	Biologically Active Polysaccharides as Modulators of Tumour Microenvironment and Microbiome: A Possible Nutraceutical Support for Cancer Patients **Luca Vannucci**
10:15 - 10:30	Towards Biomarker Discovery: Multiomics Analysis of Endometriosis **Lukas Najdekr**
10:30 - 11:00	**COFFEE BREAK**

Molecular Mechanisms and Emerging Therapeutic Targets in Cancer II

Chairs: Sarka Pospisilova, Marek Mraz

11:00 - 11:30	Tertiary Lymphoid Structures and B Cells Determine Clinically Relevant T Cell Phenotypes in Ovarian Cancer **Jitka Fucikova**
11:30 - 11:45	Repression Of MicroRNA miR-29 via Myc Leads To Increased CD40 Signaling in Transformed Follicular Lymphoma (FL) And Unfavourable Prognosis in FL **Marek Mraz**
11:45 - 12:00	A Novel EPO Promoter Variant Causes Hereditary Erythrocytosis Through Non-Renal Erythropoietin Expression **Lucie Lanikova**
12:00 - 12:15	Genome-Wide CRISPR Screening Reveals Cullin-1 As A Novel Therapeutic Target Improving The Efficacy Of CD19-Directed Immunotherapy In B-Cell Malignancies **Michal Smida**
12:15 - 12:30	Low-Attomolar Detection of miRs Related to Myelodysplastic Neoplasms Using Novel Digital Surface Plasmon Resonance Biosensor **Tomas Springer**
12:30 - 13:15	**LUNCH**

13:15 - 14:00	**POSTER PRESENTATION OF GROUP I – More Info at the End of the Program**

TUESDAY NOVEMBER 25, 2025

Molecular Targets And Anticancer Drugs II

Chairs: Marian Hajduch, Tomas Etrych

14:00 - 14:30	A Little History Of Kinase Drug Discovery **Doriano Fabbro**
14:30 - 15:00	New CDK4/6/9 PROTACs for Pediatric Cancers **Jan Molenaar**
15:00 - 15:15	Antibody Polymer Drug Conjugates with Increased Drug to Antibody Ratio: An Innovative Nanomedicine for the Advance Neoplastic Treatment **Tomáš Etrych**
15:15 - 15:30	Novel Semi-Synthetic Triterpenoids with Selective Cytotoxic Activity **Milan Urban**
15:30 - 15:45	Controlled Release of Gemcitabine from HPMA Conjugates: A Strategy to Improve Antitumor Efficacy **Katerina Behalova**
15:45 - 16:15	**COFFEE BREAK**

Cancer Immunology and Immunotherpy I

Chairs: Juan De Sanctis, Marek Kovar

16:15 - 16:45	The Role of SHP Inhibitors in T and NK Signal Transduction and Cytotoxic Activity **Juan Bautista De Sanctis**
16:45 - 17:00	IL-2 is a Crucial Cytokine for Expansion of Antigen-Primed $CD8^+$ but not $CD4^+$ T cells: A Paradigm Shift in T Cell Biology and Implication for Cancer Immunotherapy **Marek Kovar**
17:00 - 17:15	CD25-biased IL-2 Immunocomplex as a Potent Anti-Cancer Immunotherapy and Its Mechanism of Action **Milada Sirova**
17:15 - 17:30	Myeloid-Derived Suppressor Cells (MDSCs) as Major Inducers of Immunosuppression in Melanoma **Viktor Umansky**
19:00 - 23:00	**SOCIAL EVENT**

WEDNESDAY NOVEMBER 26, 2025

Molecular Targets And Anticancer Drugs III

Chairs: Milos Petrik, Jan Bouchal

9:00 - 9:30	Surface Targets and Treatment Efficacy of Antibody – Drug Conjugates in Testicular Germ Cell Tumors **Lucia Kucerova**
9:30 - 9:45	Platelets, Chromogranin A, and C-Reactive Protein Predict Therapy Failure of Metastatic Hormone-Sensitive Prostate Cancer **Jan Bouchal**
9:45 - 10:00	The Pros and Cons of 161Tb-labelled Monoclonal Antibodies for Cancer Imaging and Therapy **Zbynek Novy**
10:00 - 10:15	Spatial Analysis of Tumor Microenvironments via the Novel SignalStar™ Multiplex Immunohistochemistry Assay **Flora Guarnotta**
10:15 - 10:30	Mitoxantrone-Bearing Polymeric Conjugates Can Successfully Induce Immunogenic Cell Death in Androgen Independent Prostate Carcinoma Cells *In Vitro* and *In Vivo* **Daniil Starenko**
10:30 - 11:00	**COFFEE BREAK**

Molecular Targets And Anticancer Drugs IV

Chairs: Petr Dzubak, Daniel Rosel

11:00 - 11:30	Establishment of an Organoid-Based Platform Combining High-Throughput Phenotypic Drug Screening With a WNT Signaling Reporter to Identify Novel Stemness Targeting Drugs Against Colorectal Cancer **Martina Zowada**
11:30 - 11:45	Multicomponent Peptide Stapling and Miniaturization Driven Tool for the Development of Inhibitors of Protein–Protein Interactions **Thimmalapura Marulappa Vishwanatha**
11:45 - 12:00	Spirooxindole Scaffolds: Unlocking the Potential for Novel Therapeutics for Cancer Treatment **Assem Barakat**
12:00 - 12:15	A Novel Migrastatic Compound: Molecular Mechanisms and Anti-Metastatic Effects **Daniel Rosel**
12:15 - 12:30	Colchicine-BODIPY Probes: Evidence for the Involvement of Intracellular Membranes in the Targeting of Colchicine to Tubulin **Jiri Rehulka**
12:30 - 13:15	**LUNCH**
13:15 - 14:00	**POSTER PRESENTATION OF GROUP II** – More Info at the End of the Program

WEDNESDAY NOVEMBER 26, 2025

Molecular Mechanisms and Emerging Therapeutic Targets in Cancer II

Chairs: Stjepan Uldrijan, Martin Mistrik

14:00 - 14:15	Uncovering the Role of Translational Control in Growth Signaling and Therapy Resistance in Malignant Melanoma **Stjepan Uldrijan**
14:15 - 14:30	Autophagy Inhibition: A Key to Overcoming Lung Cancer Resistance **Anezka Teissingova**
14:30 - 14:45	Global Interactome Mapping Reveals Pro-tumorigenic Interactions of NF-κB in Breast Cancer **Petr Lapcik**
14:45 - 15:00	Spatiotemporal Organization of Biomolecules in Cancer Cells by Phase Separation **Martin Sztacho**
15:00 - 15:15	**CLOSING CEREMONY – BEST LECTURE AND POSTER AWARDS**

PARALLEL SESSIONS / Evropa Hall Small

MONDAY NOVEMBER 24, 2025

10:00 - 12:30	**EDU_NICR**
13:45 - 14:45	**Project meeting of the NICR Research Program 2 –** Research and Development of Anticancer Pharmaceuticals and Therapeutic Methods
15:30 - 18:00	**Czech Genomic Platform Meeting (hybrid)**

TUESDAY NOVEMBER 25, 2025

Project meeting of the NICR Research Program 1 – Molecular Basis of Cancer and Molecular Targets

Project meeting of the NICR Research Program 3 – Biomarkers of Cancers and Cancer Diagnostics

Project meeting of the NICR Research Program 4 – Early Detection and Prevention of Tumours

Project meeting of the NICR Research Program 5 – Translational Oncology: Verification Clinical Studies of the Proof-of-concept Type

Exact Times of the Meetings Will Be Specified Soon

WEDNESDAY NOVEMBER 26, 2025

13:15 - 14:45	**EATRIS-CZ and EATRIS-SK Joined Meeting**

PARALLEL SESSIONS / Ventana Lounge

MONDAY NOVEMBER 24, 2025

14:00 - 15:00	**NICR_ADMIN Meeting**
18:00 - 21:00	**NICR Board Meeting + Dinner**

PARALLEL SESSIONS / VIP Lounge in the NH Restaurant

WEDNESDAY NOVEMBER 26, 2025

18:00 - 21:00	**ISAB Meeting + Dinner**

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MONDAY NOVEMBER 24, 2025

Chairs: Aleksi Šedo, Josef Srovnal

Germline Genetics of Colorectal Cancer

Asta Försti[1,2], Kari Hemminki[3,2]

[1]Hopp Children's Cancer Center (KiTZ), Heidelberg, Germany. [2]German Cancer Research Center (DKFZ), Heidelberg, Germany. [3]Charles University, Pilsen, Czech Republic

Twin studies have estimated that heritability accounts for 35% of the variation in susceptibility to colorectal cancer (CRC) and Swedish Family-Cancer Database studies have shown that about 15% of CRC patients have first-degree relatives affected by the same malignancy. Linkage analysis in large CRC families have identified the main CRC predisposition genes, including the mismatch repair genes (MSH2, MLH1, MSH6 and PMS2) that cause Lynch syndrome and APC, a regulator of the WNT signaling, that causes familial adenomatous polyposis (FAP). More recently, next generation sequencing, together with linkage analysis, has identified further predisposition genes, including polymerase proofreading genes, POLE and POLD1, and other genes related to DNA repair, TGFβ and WNT signaling pathways. Altogether germline mutation in the high-to-moderate penetrance genes explain only a few percent of familial clustering of CRC. Genome-wide association studies have further identified more than 200 common, low-penetrance variants, that in combination as a polygenic risk score, may be associated with equally high risk of CRC as the high-penetrance mutations. Finally, genes predisposing to other hereditary cancers and syndromes may also predispose to CRC, as has been noticed both in register-based and genetic studies.

Difference in TMB distribution and specific mutation types in CRC patients with different times of metastasis

Filip Ambrozkiewicz[1], Marie Rajtmajerova[1], Viktor Hlavac[2,1], Andriy Trailin[1], Lenka Cervenkova[1], Jan Bruha[1], Pavel Soucek[2,1], Vaclav Liska[1], Kari Hemminki[1]

[1]Faculty of Medicine in Pilsen, Charles University, Plzen, Czech Republic. [2]Toxicogenomics Unit, National Institute of Public Health, Praha, Czech Republic

Background and Aims: Colorectal Cancer (CRC) is the second most deadly cancerous disease worldwide. Early diagnosis is vital for successful treatment, as survival gradually drops from 90 % at stage I to below 20% for stage IV. CRC metastasizes frequently to the liver, and it can be diagnosed simultaneously with the primary tumor (synchronous metastasis) or after primary tumor (pCRC) resection (metachronous metastasis). The relationship between cancer and tumor mutational burden (TMB) arises from the concept that higher mutational loads often lead to increased generation of neoantigens. Tumors characterized by high TMB are therefore more susceptible to immune surveillance. Moreover, in different cancer types, TMB has been proven to be a useful tool in predicting response to the immune-checkpoint inhibitor therapy. The evolving role of TMB in clinical practice is under active investigation, with ongoing studies aiming to refine its predictive value. In the present study, we report for the first time TMB and specific mutation types differences in tumor progression from primary CRC (pCRC) to liver metastases (LM) in our unique set of paired samples, with consideration of different metastasis time.

Methods: Whole exome sequencing was performed on 87 samples of pCRC and its paired LM. From this group, we excluded from further analysis patients who received neoadjuvant treatment and had TMB >10. Among 73 selected patients, we could distinguish 32 synchronous and 41 metachronous samples. TMB was calculated as the total number of somatic non-synonymous mutations (missense and nonsense mutations) per MegaBase sequenced, whose functional effect was classified as either "high" or "moderate" according to the Ensembl Variant Effect Predictor tool. TMB assessment was done in the maftools package. Statistical analysis was performed in the R environment (v.4.3.2). TMB differences between pCRC and LM were assessed by a paired Wilcoxon test. Differences between synchronous and metachronous groups were assessed by the Mann-Whitney test. Spearman correlation was used to evaluate relations between TMB in pCRC, LM, and clinical data. Data for specific types of genetic changes: frameshift deletions and insertions, in-frame deletions and insertions, missense, nonsense, and nonstop mutations, and mutations in splice sites and translation start sites were extracted with the maftools package. Due to the number of nonstop and translational site mutations being lower than 10, they were excluded from further analysis. Similarly, Spearman correlation was applied to assess the correlation between specific mutation types in pCRC and LM in CRC of different chronicity. Univariate Cox regression was performed with the Finalfit package. Kaplan-Meier analysis was performed with the survival package, and plots were generated with the survminer package.

Results: We didn't observe differences in TMB distribution in pCRC and LM of the synchronous

or metachronous group. Mutation types were distributed almost equally in pCRC and LM, with no regard to chronicity. With missense and nonsense mutations present in approximately 100% samples and other types being present in 50 to 90 % of samples. However, specific mutation types frequencies differed between pCRC, leading to synchronous or metachronous LM. Frameshift insertions had a lower frequency in the synchronous group than in the metachronous group (59% and 80% respectively). These differences were statistically significant (p = 0.01). Reverse observation was made for in-frame insertions, which presented a higher frequency in synchronous than in the metachronous group (72% and 59%) although it was not statistically significant. Positive correlations between pCRC and LM for frameshift insertion (R=0.39, p=0.03), exclusively for the synchronous group. On the other hand, in the metachronous group we observed a positive correlation between in-frame deletions (R=0.41, p=0.009), frameshift deletions (R=0.36, p=0.02), and splice site mutations (R=0.6 p= 0.00004) in pCRC and LM. Nonsense mutations were positively correlated between pCRC and LM in both synchronous (R=0.43, p=0.01) and metachronous (R=0.32, p=0.04) groups. We observed that higher TMB was associated with tumors localized on the right side of the colon in the synchronous (p=0.04) and metachronous group (p=0.002). Additionally, for pCRC, age at the time of diagnosis significantly correlated with higher TMB in the synchronous group (R=0.36, p=0.04), which was not observed for the metachronous group (R=0.2, p=0.2). Similar observation was made for the size of primary tumor, which was correlated for synchronous (R=0.44, p=0.04) but not for metachronous group (R=0.12, p=0.6). For LM, we explored grade, size, and number of metastases; none of them was correlated or associated with clinical data. Higher TMB in pCRC was associated with longer overall survival from liver surgery in the synchronous (HR=0.22, p=0.008) group but not in the metachronous group. No association between TMB and overall survival was observed for LM.

Conclusion: The results suggest that distinct tumor progression pathways account for different chronicity and may have an ultimate impact on the patient's prognosis. Moreover, based on our results, TMB in pCRC could be a useful tool in predicting the time of metastasis.

Distribution and prognostic significance of macrophages in colorectal cancer between adjacent non-tumor mucosa, primary tumor and liver metastasis

Andriy Trailin[1], Esraa Ali[1], Wenjing Ye[1], Sergii Pavlov[1], Lenka Červenková[2], Ondřej Vyčítal[2,3], Filip Ambrozkiewicz[1], Ondřej Daum[2,4], Václav Liška[2,3], Kari Hemminki[1,5]

[1]Laboratory of Translational Cancer Genomics, Biomedical Center, Faculty of Medicine in Pilsen, Charles University, Pilsen, Czech Republic. [2]Laboratory of Cancer Treatment and Tissue Regeneration, Biomedical Center, Faculty of Medicine in Pilsen, Pilsen, Czech Republic. [3]Department of Surgery, University Hospital and Faculty of Medicine in Pilsen, Charles University, Pilsen, Czech Republic. [4]Bioptická Laboratoř s.r.o., Pilsen, Czech Republic. [5]Department of Cancer Epidemiology, German Cancer Research Center, Heidelberg, Germany

Introduction. Colorectal cancer (CRC) is one of the leading causes of cancer-related morbidity and mortality worldwide, with liver representing the most common and clinically significant site of distant metastases. Macrophages are key innate immune cells that infiltrate tumors and surrounding tissues and demonstrate remarkable phenotypic plasticity in response to local signals. Based on their activation status, macrophages are broadly classified into two phenotypic extremes: classically activated (M1 type), which are pro-inflammatory and anti-tumorigenic, and alternatively activated (M2 type), which are immunosuppressive and promote tumor growth, angiogenesis, and metastasis.

We aimed to assess the role macrophages in the metastatic process by comparing densities and evaluating prognostic associations of several subsets of macrophages in the tumor adjacent non-tumor colorectal mucosa (NM), primary colorectal cancer (pCRC) and liver metastases (LM) in patients with synchronous and metachronous metastases of CRC.

Patients and methods. We retrospectively identified CRC patients, who underwent resection of both pCRC and LM in Pilsen University Hospital between 1999 and 2022. 55 patients presented at the time of diagnosis with LM (stage IV, synchronous) and 44 patients (stage II/III) developed LM later (metachronous). After immunohistochemical staining of FFPE sections and whole slide scanning densities of macrophages CD68+ (M0, nonpolarized), CD80+ (M1), CD206+ and CD163+ (both M2) were quantified in NM, pCRC and LM using QuPath software. In pCRC and LM cell densities were estimated in tumor center (TC), inner invasive margin (IM), outer invasive margin (OM) and peritumor (PT) region. IM and OM were defined as 500 μm on each side of the tumor border towards TC or PT region. Immune cell densities were correlated with clinical and pathological variables and tested as prognostic variables for overall survival (OS) since liver surgery.

Results. Densities of macrophages followed the pattern: CD163+ > CD206+/CD68+ > CD80+ in NM and TC of pCRC and LM, with significantly smaller densities of all cell types in tumors vs NM. For all analyzed types of macrophages

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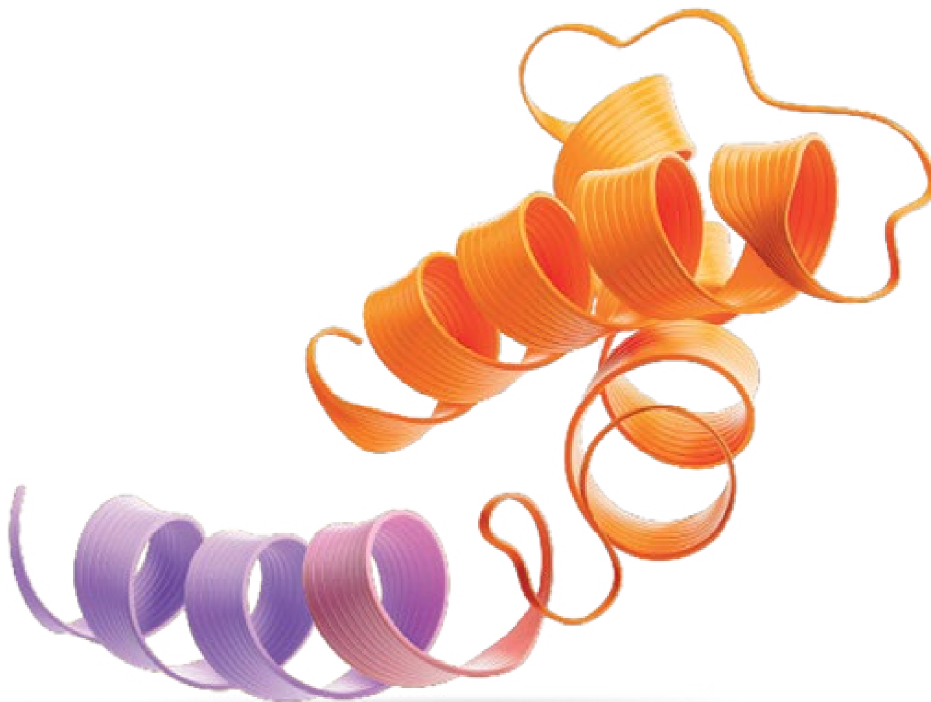

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in both pCRC and LM, the tumor core (TC and IM) exhibited smaller densities compared to OM and PT regions across both synchronous and metachronous groups. LM exhibited significantly higher densities of CD68+ cells across all regions compared to pCRC in both patient groups. A similar trend was observed for CD163+ cells, with significantly higher densities in OM and PT. In contrast, an opposite trend was observed for CD80+ and CD206+ macrophages, with higher densities in pCRC compared to LM across all regions in both groups. Densities of CD163+ macrophages were significantly greater in OM and PT of metachronous LM compared to respective regions in synchronous group.

In the synchronous group high densities of CD163+ macrophages in NM were associated with longer OS (hazard ratio (HR)=0.45, P=0.04). High densities of CD68+ cells in OM of LM were associated with shorter OS (HR=2.09, P=0.048). In the metachronous group high densities of CD80+ macrophages in NM (HR=0.24, P=0.001) and in OM of pCRC (HR=0.36, P=0.020), and high densities of CD68+ cells in PT region of pCRC (HR=0.40, P=0.038) were associated with longer OS.

Conclusion. M2-type macrophages predominated over M1 in all tissues. Development of CRC is accompanied by decreased densities of macrophages in TC of pCRC and LM compared to NM irrespective of stage of the disease. Macrophages of all types were more abundant in the tumor exterior both in pCRC and LM. In synchronous disease CD163+ M2 macrophages in NM associate with longer survival whereas CD68+ M0 macrophages in LM associate with shorter survival. In metachronous disease CD80+ M1 macrophages in NM and pCRC and M0 macrophages in pCRC associate with longer survival. We demonstrated a difference in prognostic significance of macrophages with respect to their polarization, localization and stage of the disease. These findings underscore the importance of evaluating the tumor immune microenvironment beyond the primary tumor itself for prognostic biomarker discovery in CRC.

Beyond the Diagnosis: Gender Aspects in Rectal Cancer Management

Ludmila Boublikova, Sona Krivonoskova, Anna Oplustilova, Veronika Makajevova, Radka Lohynska

1.LF UK and Thomayer University Hospital, Prague, Czech Republic

The prognosis of locally advanced rectal cancer (LARC) has changed dramatically over the past years with the implementation of novel, precise radiotherapy techniques and intensified induction chemotherapy. This so-called total neoadjuvant therapy (TNT) has improved the local relapse as well as distant metastatic rates, and allowed for non-operative management and sphincter-preserving approaches. The largest recent phase 3 studies that confirmed the role of TNT in rectal cancer (PRODIGE 23, RAPIDO, OPRA) enrolled around 35% of females in the study populations, but no gender subanalyses of the results have been carried out.

We analyzed a single-center cohort of patients with LARC treated by TNT to address the potential gender aspects and their projection into the treatment outcomes. Among 60 enrolled patients, 19 (32%) were females. While the age, disease characteristics, and treatment schemes were comparable in both sexes, and the achieved overall complete response to TNT (30%) corresponded with that reported in the abovementioned clinical trials (28%), the females showed higher rates of complete responses than males (37 vs. 27%, $p = 0.55$).

The potential variables of all treatment modalities have been explored. Effective doses and individual exposure to systemic therapy can be assessed indirectly only and may vary slightly between males and females due to the formulas used for dose calculation based on body surface or weight, and differences in cytostatic distribution and metabolism. Local treatment modalities, however, are more prone to and influenced by inter-individual and inter-sexual variability, with the sex-specific anatomy of the lesser pelvis entailing major consequences for radiotherapy and surgery. The volumes and doses calculated from radiotherapy plans for target volumes (PTVs) and organs at risk (OaR) have revealed distinct patterns in male and female patients, with better PTV 98% coverage in females ($p < 0.05$) but also higher doses to OaR - bowels ($p < 0.001$) and femur heads ($p < 0.05$) in females compared to males. There were no significant differences in postoperative complications and quality of life, in particular the reported LARS (low anterior resection syndrome) between males and females, with median score 33.5 (18 – 39) vs. 36 (11 – 41), respectively, but women responded better to physiotherapy and pelvic floor exercises.

Overall, although not previously studied and reported, gender-specific anatomical and physiological characteristics may play an important role in rectal cancer management and influence the patients' outcome and quality of life. Larger studies are warranted to define the gender-associated factors worth respecting and adjusting for in the individual treatment plans to improve the survival and quality of life of patients with rectal cancer.

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Functional Single-Cell Analysis to Advance CAR-T and TCR-T Therapies through Beacon Discovery

Tiago Santos

Bruker Cellular Analysis, Lisabon, Portugal

The development of effective CAR-T and TCR-T therapies depends on the ability to rapidly identify and select immune cells with potent, specific, and durable anti-tumor activity. Beacon Discovery is a next-generation platform enabling high-throughput, multiparametric analysis of live single cells—capturing dynamic cytotoxicity, cytokine secretion, and surface phenotype in real time. By providing rapid, functional insights into engineered cell products, Beacon streamlines candidate selection, validation, and optimization with unmatched resolution. This presentation will showcase real-world applications, including Prof. Eichmüller's work on patient-specific TCR discovery and Prof. Zamora's studies on CAR-T functionality, illustrating how Beacon accelerates the path from concept to clinic in advanced cell therapy development.

Advancing Cancer Research with Accessible Single-Cell Technologies

Lukas Valihrach

Institute of Biotechnology of the Czech Academy of Sciences, Vestec, Czech Republic

The heterogeneity of tumors and their microenvironment presents a major challenge in cancer research. While single-cell transcriptomics is the ideal tool to address this complexity, its widespread adoption has been historically hindered by high costs, complex logistics, and challenging data analysis.

In this talk, I will present a portfolio of next-generation technologies that are breaking down these barriers. New solutions drastically reduce per-sample costs and are complemented by flexible sample preservation methods that greatly facilitate collaboration. I will provide an overview of methods that, combined with decreasing sequencing costs and more intuitive analysis software, are removing these historical obstacles. To further enhance cellular maps, I will also highlight recent advances in spatial transcriptomics. Finally, I will share key insights from our work at GeneCore, a specialized academic facility where we implement these technologies to advance cancer research projects of varying scope.

The accessibility of these high-throughput methods is fundamentally transforming cancer research. They enable researchers to tackle previously intractable questions with unprecedented resolution, paving the way for groundbreaking discoveries in tumor evolution, immune infiltration, and therapeutic resistance.

MONDAY NOVEMBER 24, 2025

Chairs: Karel Smetana, Alexander Doemling

Protein regulation in multiple myeloma

Jan Krönke

Protein regulation is central to biology and therapy of multiple myeloma and cancer more broadly, yet most omics research focuses on DNA and RNA, missing critical post-transcriptional mechanisms—especially the ubiquitin-proteasome system (UPS)—that control protein stability and drug response. In a multi-omics study including global (phospho)proteomics, transcriptomics, and genomics of 138 patient samples covering myeloma and healthy controls, malignant plasma cells showed widespread deregulation of proteins, often driven by post-transcriptional mechanisms. Importantly, a prognostic protein signature was identified that predicts aggressive disease independent of standard risk factors, while functional genomics and single-cell analyses revealed deregulated pathways and protein targets unique to multiple myeloma. Although most proteins remain undruggable, targeted protein degradation represents a breakthrough, enabling the elimination of disease-associated proteins previously considered inaccessible by the UPS. Approved therapies such as thalidomide analogs (IMiDs), alongside novel agents like PROTACs and deubiquitinating enzyme inhibitors, now allow selective targeting of these proteins. The identification of cancer-selective proteins and the development of drugs against them are laying the foundation for personalized therapies and improved outcomes in multiple myeloma and other cancers

Spatial profiling of cancer-associated fibroblast heterogeneity in head and neck cancer

Stian Tornaasa, Lorena Larios Salazar[a], Karoline Fjeldstada, Dimitrios Kleftogiannisa[,b], Siren Fromreide[a], Hilde Ytre-Hauge Smelanda,[c], Hans Jørgen Aarstad[d], Olav Karsten Vintermyrc, Lars A. Akslena[,c], Harsh Nitin Dongre[a] and Daniela Elena Costea[a,c]

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Background:

Cancer-associated fibroblasts (CAFs) represent a key component of the tumor microenvironment in head and neck squamous cell carcinoma (HNSCC), yet their phenotypic and spatial diversity remain incompletely understood. Distinct CAF subsets may differentially shape tumor progression and immune contexture.

Objectives:

To dissect CAF heterogeneity and spatial organization in primary HNSCC using high-dimensional imaging mass cytometry (IMC).

Methods:

Multiplex IMC was performed on formalin-fixed paraffin-embedded HNSCC tissues using a 24-marker panel including epithelial, fibroblastic, endothelial, and immune markers (E-cadherin, EGFR, Ki-67, α-SMA, CD140β, FAP, CD90, FSP-1/S100A4, integrin α-11, collagen-1, tenascin-C, caveolin-1, YAP-1, CD146, CD31, CD4, CD8, CD20, CD68, CD163, FoxP3, granzyme B, and podoplanin). Single-cell segmentation and unsupervised clustering were followed by biological curation of CAF metaclusters.

Results:

IMC revealed a highly heterogenous and complex stromal compartment comprising six CAF phenotypes, several of which partially aligned with previously recognized functional states. CAF-1 (α-SMAhigh, CAV-1med, CD140βhigh, FAPhigh, ITGA11neg, Col-1high, CD90high) and CAF-2 (α-SMAlow, CAV-1low, CD140βhigh, FAPhigh) corresponded to classical myofibroblastic and secretory/immune-modulatory CAFs, respectively, both enriched at the tumour–stroma interface. CAF-3 (α-SMAlow, CD140βmed, FAPmed, Col-1high) represented an ECM-producing subtype broadly distributed throughout the stroma, whereas CAF-4 (α-SMAlow, ITGA11low, Col-1low, CD90med) showed features of interstitial or pre-CAF-like fibroblasts. CAF-5 (α-SMAneg, Col-1high, CD90neg) displayed a quiescent ECM-depositing phenotype, while CAF-6 (α-SMAhigh, CAV-1high, ITGA11high, Col-1low, CD90low) reflected an actomyofibroblastic subtype enriched at invasive fronts and spatially associated with cytotoxic $CD8^+$ T cells infiltrating tumour islands.

Conclusions:

Spatial single-cell profiling of HNSCC delineates six phenotypically and topographically distinct CAF subsets encompassing contractile, secretory, ECM-remodelling, and quiescent states. This integrated framework links stromal heterogeneity to immune cell infiltration and clinical outcome in head and neck cancer.

Towards Precision Medicine: PROTAC-Based Selective Degradation of p53-Y220C

Imma Capriello[1,2], Thimmalapura Marulappa Vishwanatha[1,2], Dora Yuen Lam[3], Jan Krönke[3], Alexander Dömling[1,2]

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p53 is a crucial tumor suppressor protein, often referred to as the „guardian of the genome“ for its role in maintaining genomic integrity. Under normal conditions, p53 can stop the cell cycle, activate DNA repair, or trigger apoptosis, ensuring that damaged cells do not turn into cancer. Unfortunately, mutations in the p53 gene occur in more than half of all human cancers, severely impairing these protective functions. Within this broad spectrum of alterations, the Y220C mutation stands out, as it replaces a tyrosine with a cysteine residue, creating a surface cavity. This newly formed, druggable pocket offers a unique opportunity for therapeutic intervention.

In this study, we focus on the development of carbazole-based PROTACs (Proteolysis Targeting Chimeras) designed to selectively degrade p53 Y220C. By harnessing the PROTAC strategy, these molecules simultaneously engage the destabilized mutant and recruit E3 ubiquitin ligases, driving its ubiquitination and subsequent proteasomal degradation. This approach aims to eliminate the dominant-negative and oncogenic effects of mutant p53, thereby restoring the functionality of the p53 pathway. Preliminary results highlight the potential of carbazole scaffolds to confer high affinity and selectivity for the Y220C pocket, paving the way for rationally designed degraders as innovative therapeutic options against p53-driven cancers.

Building on this opportunity, we have synthesized a focused library of 48 carbazole-based PROTACs in parallel, employing a direct-to-biology (D2B) approach. This strategy enables rapid progression from molecular design to biological testing by evaluating compounds directly in their crude form, prior to full purification. Comparative analyses between crude samples and their purified counterparts are underway to assess whether early-stage, high-throughput biological readouts align with results obtained from fully characterized molecules. Such parallel synthesis and side-by-side evaluation aim to accelerate hit identification, reduce cycle times, and provide valuable insights into the efficiency and reliability of the D2B workflow in the context of targeted protein degradation.

This work was supported by the project National Institute for Cancer Research (Programme EXCELES, ID Project No. LX22NPO5102) - Funded by the European Union – Next Generation EU.

Development of fully characterized human 3D cancer cell lines for *in vitro* and in vivo modeling of pancreatic and colon cancer

Jan Strnadel

Biomedical Centre Martin, Jessenius Faculty of Medicine in Martin, Comenius University in Bratislava, Martin, Slovakia

In vitro cell models or cell lines represent the fundamental tools and the gold standard for any area of biomedical research. The quality and availability of cell lines is therefore a critical point for novel therapy testing. Although many cancer cell lines already exist and are provided by repository centers, there is a need for the development of new *in vitro* models as already existing cell lines gain mutations during the continuous culture and sometimes lost their ability to represent the original tumor. At Biomedical Centre Martin, Jessenius Faculty of Medicine in Martin we utilize the technology of 3D culture and cell reprogramming together with our optimized protocol for the development of novel cancer cell lines, derived from patients with colorectal cancer, pancreatic ductal adenocarcinoma or neuroendocrine tumors. Newly derived cancer cell lines are then characterized by flow cytometry, immunocytochemistry, Western blot analysis, PCR, STR fingerprinting, karyotype analysis, genome sequencing and in vivo xenotransplantation into athymic nude mice.Fully characterized cell lines are then published in scientific journals and registered in selected cell line repository centers (like ATCC). Through these repository centers our cell lines are available to the researchers from the field. Recently, cancer cell lines developed by our team were used for the development of the first humanized large animal model of pancreatic ductal adenocarcinoma (pig). Due to the significant anatomical and physiological similarities with human, this large animal model with growing transplanted human cancer cells represents more relevant preclinical model for novel therapy testing.

The HOX code is respected by adult human cancer-associated fibroblasts

Lucie Pfeiferová[1], Michal Španko[2], Kenneth J Pienta[3], Aleksi Šedo[4], Petr Bušek[4], Michal Kolář[1], Karel Smetana ml[2]

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Fibroblasts, the most abundant cell type in the human body, play crucial roles in biological processes such as inflammation and cancer progression. They originate from the mesoderm or neural-crest-derived ectomesenchyme. Ectomesenchyme-derived fibroblasts contribute to facial formation and do not express HOX genes during development. The expression and role of the HOX genes in adult fibroblasts is not known. We investigated whether the developmental pattern persists into adulthood and under pathological conditions such as cancer. We collected adult fibroblasts of ectomesenchymal and mesodermal origins from distinct body parts. The isolated fibroblasts were characterized by immunocytochemistry and their transcriptome was analysed by whole genome profiling. Significant differences were observed between normal fibroblasts from the face (ectomesenchyme) and upper limb (mesoderm), particularly in genes associated with limb development, including HOX genes, e.g. HOXA9 and HOXD9. Notably, the pattern of HOX gene expression remained consistent postnatally, even in fibroblasts from pathological tissues, including inflammatory states and cancer associated fibroblasts from primary and metastatic tumours. Therefore, the distinctive HOX gene expression pattern can serve as an indicator of the topological origin of fibroblasts. The influence of cell position and HOX gene expression in fibroblasts on disease progression warrants further investigation.

The study was supported by the project National Institute for Cancer Research (Programme EXCELES, ID project no. LX22NPO5102) – funded by the European Union – Next Generation EU.

Regulation of microtubule organization and migration in glioblastoma cells by GIT proteins

Vadym Sulimenko[1], Eduarda Dráberová[1], Vladimíra Sládková[1], Taťána Sulimenko[1], Věra Vosecká[1], Omar Skalli[2], Pavel Dráber[1]

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Glioblastomas are the most aggressive form of glioma in adults, with no effective treatments currently available. Microtubules play a well-established and critical role in cell division and mesenchymal migration, directly influencing the viability and invasiveness of malignant tumors. Previously, we demonstrated that ARF GTPase-activating proteins GIT1 and GIT2 form complexes with γ-TuRC proteins, key regulators of microtubule nucleation, in glioblastoma cell lines. We report that, in interphase glioblastoma cell lines, GIT proteins associate with both focal adhesions and centrosomes; however, GIT2 preferentially localizes to centrosomes. Accordingly, GIT1 and GIT2 exhibit distinct subcellular distributions in glioblastoma cells. Depletion of GIT2 enhances centrosomal microtubule nucleation and exerts a more pronounced and opposite effect on this process compared with GIT1. In contrast, depletion of either GIT1 or GIT2 similarly impairs cell migration, as demonstrated by wound-healing assays. We further show that the ArfGAP domain of GIT2 and protein kinase C (PKC) are important for regulating microtubule nucleation in glioblastoma cell lines, which are characterized by overexpression of PKCα. We identified serine 46 (S46) within the ArfGAP domain as a PKCα phosphorylation site and demonstrated that phosphorylation of GIT2 at S46 promotes microtubule nucleation. These findings suggest that PKC-mediated phosphorylation of centrosomal GIT2 enhances microtubule nucleation, thereby promoting the invasive properties of glioblastoma cells.

Supported by Ministry of Education, Youth and Sports of the Czech Republic (Program EXCELES, Project LX22NPO5102, grant LUC23123).

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TUESDAY NOVEMBER 25, 2025

Chairs: Ludmila Boublikova, Luca Vannuci

Metabolic reprogramming in liver cancer

Dirk Mossmann[a,b], Christoph Müller[a], Sujin Park[a,c], Marco Colombi[a], and Michael N. Hall[a]

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Background:

Progress in the last decade has revealed that cancer is a metabolic disorder in which several, if not most, major metabolic pathways are rewired to enhance cell proliferation. However, the upstream mechanisms and downstream targets of metabolic reprogramming in cancer are largely unknown. Here, I present examples of how alterations in (I) acetyl-CoA, (II) arginine, and (III) polyamine metabolism are controlled in and impact liver cancer.

Objectives:

Detect and investigate metabolic alterations in liver cancer.

Methods:

Multi-omic analyses of mouse and human liver cancer models and patient-derived tissues combined with functional cellular assays and in vivo validation experiments.

Results:

(I) Acetyl-CoA levels and non-histone protein acetylation are decreased in liver cancer due to the transcriptional downregulation of all acetyl-CoA synthesis pathways. We identified the transcription factors TEAD2 and E2A to globally repress acetyl-CoA synthesis genes. Functionally, TEAD2 and E2A promote proliferation and dedifferentiation by reducing acetyl-CoA levels.

(II) We found increased arginine levels in liver cancer despite suppression of the urea cycle. Increased uptake and decreased conversion to polyamines maintain high arginine. Mechanistically, arginine binds the transcription regulator RBM39 to promote oncogenic metabolism. This includes promoting ASNS expression and asparagine synthesis which further enhances arginine uptake, creating a positive feedback loop. Therapeutically, RBM39 dependency in liver cancer could be exploited by molecular glue degraders.

(III) Polyamine levels are increased in liver cancer despite suppression of arginine-to-polyamine conversion. We found elevated polyamine uptake in liver cancer and are now investigating mechanisms of transport. While, historically, polyamine transport was suggested to involve endocytosis and endosomal exporters, we found a highly regulated plasma membrane transporter to facilitate polyamine import.

Conclusions:

Oncogenic signalling and metabolic alterations are interrelated in cancer cells to control cell growth and metabolism. We found low levels of acetyl-CoA and high levels of arginine and polyamines to promote liver cancer tumorigenicity and identified mechanisms of metabolic reprogramming, including a novel role for arginine as a second-messenger-like metabolite.

Molecular Profiling of the Cervical Microbiome for Predicting Lesion Progression

Veronika Holubeková[1], Zuzana Kolková[1], Andrea Hornáková[1], Dušan Loderer[1], Tomáš Rokos[2], Ivana Kašubová[1], Terézia Pribulová[2], Erik Kozubík[2], Kamil Biringer[2], Erika Halašová[1], Erik Kúdela[2]

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Introduction: The composition of the cervical microbiome is increasingly recognized as an important factor influencing the progression of precancerous cervical lesions to invasive carcinoma. Molecular biology and genomic approaches provide powerful tools for characterisation this microbial community and identifying predictive biomarkers of disease outcome.

Aim: In our work, we apply complementary methods including quantitative PCR, Sanger sequencing, and next-generation sequencing to investigate the cervical microbiome in women with varying degrees of cervical intraepithelial neoplasia.

Material and methods: DNA was extracted from cervical swabs, and examined for the presence of HPV viruses by Sanger sequencing. Quantitative PCR (qPCR) was employed in the sensitive detection and quantification of specific bacterial taxa. Recently, next-generation sequencing was selected to characterise all microbial strains in cervical swabs.

Results: Sanger sequencing allowed us to get an overview of the prevalence of HPV genotypes in our region. The most prevalent was HPV16 (31%, 74/237), HPV52 (5%, 13/237), HPV66 (4%, 9/237) and HPV45 (3%, 6/237). qPCR identified the presence of bacterial/fungal DNA and next-generation sequencing provides a comprehensive overview of microbial diversity and relative abundance, uncovering shifts in community structure that may be associated with progression risk.

Conclusion: By integrating these approaches, we aim to delineate microbial signatures that correlate with lesion severity and potential for malignant transformation. Such insights can contribute to the development of predictive models

for cervical cancer risk stratification and may support future preventive and therapeutic strategies in clinical practice.

Acknowledgement: This work was supported by the project no. APVV-23-0428 funded from Ministry of Education, Science, Research, and Sport of the Slovak Republic.

The microbiome in tumor tissue and adjacent mucosa from colorectal cancer patients

Pavel Vodička[1,2], Safaa Andarawi[3], Petr Hanák[4], Michal Kroupa[5], Václav Liška[1], Ludmila Vodičková[1,2], Alessio Naccarati[6], Klára Kostovčíková[7]

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Dysbiosis of bacterial and fungal communities in the bowel has been associated with inflammatory diseases and cancer. Since most of the studies deal with the luminal samples of the gut content, we focused on the microbes closely associated with the mucosa. We collected samples of colorectal cancer tissue and adjacent non-affected mucosa from 125 patients and processed them for sequencing of 16S rRNA and ITS1 genes for bacterial and fungal microbiota profiling, respectively. In the biopsies from CRC patients, we have also investigated relative telomere length (RTL), general and oxidative DNA damage.

We evaluated the relationships among DNA damage, RTL and microbiota/fungi, as analysed in intestinal mucosa and CRC tumors. Tumor-associated microbiota was enriched with potential pathogens, such as genera Fusobacterium, Treponema, Campylobacter and Selenomonas, whereas adjacent tissue exhibited increased relative abundance of order Bacteroidales and genera Blautia, Faecalibacterium, Odoribacter and Dorea. Tumor tissue was markedly resided by fungal genera Pseudopithomyces and Peniophora, suggesting environmental origin. Stratification to gastrointestinal tract compartments showed that tumor tissues from the left side of the colon and rectosigmoideum had the highest relative abundance of genus Fusobacterium and Streptococcus, respectively. Genus Selenomonas was significantly and specifically enriched in the tumor tissue from the right side of colon. We found marked positive correlation of genus Parvimonas with Peptostreptococcus ($r=0.85$, $p=7.6x10-15$), Campylobacter ($r=0.82$, $p=4.6x10-13$), Dialister ($r=0.55$, $p=4x10-5$) and Fusobacterium ($r=0.54$, $p=5.4x10-5$) in adjacent tissue. Whereas we found the only significant difference in RTL between tumors (shorter RTL) and adjacent mucosa, there was no association with either localization or microbial settlement. Interestingly, the levels of both general and oxidative DNA damage were significantly higher in tumor tissues than in adjacent mucosa

Colorectal cancer-associated dysbiotic microbiome differs between colon compartments, and certain genera, such as Fusobacterium, Campylobacter, Parvimonas and Selenomonas have potential to improve colorectal cancer detection.

Biologically active polysaccharides as modulators of tumour microenvironment and microbiome: A possible nutraceutical support for cancer patients

Luca Vannucci[1], Leonid Sushytskyi[1], Lenka Rajsiglová[1], Pavol Lukáč[1,2], Paolo Tenti[1,2], Andryi Synytsya[3], Dmitry Stakheev1,4, Daniel Smrž[1,4], Petr Šíma[1]

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Biologically active polysaccharides—especially β-glucans from diverse sources— can shape innate and adaptive immunity, with implications for nutrition and oncology. Microalgal cell wall polysaccharides, alongside cereal-derived β-D-glucans, were characterized, linking molecular architecture (glycosidic linkages, branching, and molecular weight) to solubility, viscosity, and immunomodulatory potency. Across experimental systems, β-glucans activate pattern-recognition pathways (notably dectin-1, TLRs, and complement receptor 3), reprogram macrophages and dendritic cells, enhance NK and cytotoxic T-cell functions, and modulate cytokine profiles toward antitumor and host-protective responses. Dietary perspectives highlight the differential impact of low- to high–molecular–weight oat β-glucans on physiological endpoints, including glycemic control, lipid metabolism, and mucosal immunity, while acting as prebiotics that influence microbial communities and the production of short-chain fatty acids. Integrating insights from microbiome and virome research, the work highlights how polysaccharide-driven shifts in gut ecology can modulate systemic inflammation, potentially mitigating the smouldering inflammation that underlies carcinogenesis. Translational avenues include nutraceuticals, vaccine adjuvants, and stromal/immune co-targeting strategies in cancer, contingent on rigorous standardization of source material, purity, and structure–function analytics (e.g., FTIR/NMR, linkage analysis, and rheology). The authors identify key gaps—dose–response calibration, bioavailability, and interindividual microbiome variability—as priorities for future mechanistic and clinical studies.

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Towards Biomarker Discovery: Multiomics analysis of Endometriosis

Sára Filipová[1], Jan Macháň[1], Richard Masař1, Tomáš Oždian[1], Pavlína Javorová[1,2], Lukáš Najdekr[1]

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Endometriosis is a chronic, estrogen-dependent inflammatory disease characterised by the presence of endometrial-like tissue outside the uterus. Affecting approximately 10% of reproductive-age women, its diagnosis is often delayed due to nonspecific symptoms and a lack of reliable biomarkers. In this study, we applied untargeted metabolomics and lipidomics as well as proteomics to explore plasma-based molecular signatures associated with endometriosis. Plasma samples from patients (Grades I–IV), suspected cases, and matched controls underwent extraction protocols optimised for both metabolite and lipid profiling, followed by high-resolution LC-MS/MS in positive and negative ion modes.

Data preprocessing included peak picking, quality control-based signal correction (QC-RSC), and statistical filtering using the open-source PySPRESSO pipeline. Principal Component Analysis (PCA) and supervised Partial Least Squares Discriminant Analysis (PLS-DA) revealed clear group separations, particularly between control and diagnosed cases. Multi-omic Factor Analysis (MOFA) highlighted latent factors that correlated with disease class and diagnosis. Candidate features were selected based on volcano plots and statistical metrics.

Structural elucidation of lipids, including phosphatidylcholines, was performed using ultraviolet photodissociation (UVPD) fragmentation on an Orbitrap Ascend platform, enabling precise localisation of double bond positions. Notably, lipids such as PC(16:0_20:5) showed differential abundance and fragmentation patterns between groups. These findings underscore the diagnostic potential of untargeted omics approaches in endometriosis and demonstrate the utility of UVPD for structural lipidomics. Future work will focus on expanding compound annotation, integrating proteomics, and refining predictive models for clinical translation.

The project National Institute for Cancer Research (Programme EXCELES, ID Project No. LX22NPO5102) - Funded by the European Union – Next Generation EU. & JG_2024_026 (UPOL) & IGA LF 2025_006

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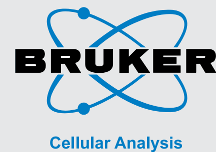

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TUESDAY NOVEMBER 25, 2025

Chairs: Sarka Pospisilova, Marek Mraz

Tertiary lymphoid structures and B cells determine clinically relevant T cell phenotypes in ovarian cancer

Jitka Fucikova

Sotio Biotech, Prague, Czech Republic. 2nd Medical School, Charles University, Prague, Czech Republic

Intratumoral tertiary lymphoid structures (TLSs) have been associated with improved outcome in various cohorts of patients with cancer, reflecting their contribution to the development of tumor-targeting immunity. Here, we demonstrate that high-grade serous ovarian carcinoma (HGSOC) contains distinct immune aggregates with varying degrees of organization and maturation. Specifically, mature TLSs (mTLS) as forming only in 16% of HGSOCs with relatively elevated tumor mutational burden (TMB) are associated with an increased intratumoral density of CD8+ effector T (TEFF) cells and TIM3+PD1+, hence poorly immune checkpoint inhibitor (ICI)-sensitive, CD8+ T cells. Conversely, CD8+ T cells from immunologically hot tumors like non-small cell lung carcinoma (NSCLC) are enriched in ICI-responsive TCF1+ PD1+ T cells. Spatial B-cell profiling identifies patterns of in situ maturation and differentiation associated with mTLSs. Moreover, B-cell depletion promotes signs of a dysfunctional CD8+ T cell compartment among tumor-infiltrating lymphocytes from freshly isolated HGSOC and NSCLC biopsies. Taken together, our data demonstrate that – at odds with NSCLC – HGSOC is associated with a low density of follicular helper T cells and thus develops a limited number of mTLS that might be insufficient to preserve a ICI-sensitive TCF1+PD1+ CD8+ T cell phenotype. These findings point to key quantitative and qualitative differences between mTLSs in ICI-responsive vs ICI-irresponsive neoplasms that may guide the development of alternative immunotherapies for patients with HGSOC.

Repression of microRNA miR-29 via MYC Leads to Increased CD40 Signaling in Transformed Follicular Lymphoma (FL) and Unfavourable Prognosis in FL

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Introduction: Follicular lymphoma (FL) is a generally indolent disease; however, FL patients are at risk of disease transformation to high-grade lymphoma (tFL). FL transformation occurs with a frequency of 1-3% per year and is associated with a very dismal prognosis. While genetic alterations have been implicated in tFL, the role of microenvironmental interactions and epigenetic regulation by non-coding RNAs remains poorly understood.

Results: We performed the first matched profiling of non-coding RNAs (miRNAs) and mRNAs in paired FL and tFL samples (n=10 pairs). This identified differential

expression of 1075 mRNAs and 19 miRNAs, including repression of the miR-29 family in tFL (miR-29a/b/c), which we further focused on. We uncovered that MYC activity is uniformly induced in tFL (GSEA, FDR=0.016) and represses miR-29 by binding to its promotor, and MYC silencing (siRNA) led to miR-29s induction in lymphoma cell lines (P<0.05). RNAseq in FL-tFL pairs revealed changes in multiple molecular pathways potentially controlled by miR-29s, including CD40 signaling being strongly activated in tFL (GSEA, IPA). The CD40 pathway is a major pro-proliferative factor in normal and FL lymph nodes, and increasing its propensity would provide FL/tFL cells with a proliferative advantage. scRNAseq data reanalysis (Roider et al, 2019) revealed that CD40L is amongst the 10 most active ligands in tFL. Increased CD40 activation in tFL contrasted with the reduced CD4+ and CD8+ T-cell numbers in tFL niches (IHC in 10 FL-tFL pairs, CIBERSORTx from FL-tFL RNAseq). To directly identify miR-29 targets, we performed RNA profiling in 2 cell lines engineered for miR-29c overexpression, revealing 20 putative miR-29 targets downregulated in both cell lines. This included TRAF4, which has been previously linked to CD40 signaling. miR-29c overexpression leads to ~50% reduction of TRAF4 levels via miR-29c binding to its 3'UTR. Importantly, cell lines constitutively overexpressing miR-29c or transfected with synthetic miR-29c (1000 nM) were less responsive (↓ pIKKa/b) to recombinant CD40L or HS-5 cells engineered for CD40L expression. Altogether, MYC-mediated miR-29 repression results in increased TRAF4 and CD40 signaling. Importantly, TRAF4 levels were increased in tFL compared to paired FL (n=11 pairs). Ki67 correlated positively with TRAF4 and negatively with miR-29s levels (FL/tFL n=46), and TRAF4 and MYC were concurrently induced in tFL (R=0,8, P=0.004). Lower levels of all miR-29s(a/b/c) were associated with shorter OS and PFS in FL in univariate (n=185) and multivariate analysis (age, FLIPI, Hgb, LDH, Ann Arbor, B sympt.). Lower miR-29c levels were associated with shorter OS also in a validation FL cohort (n=92) from an R-CHOP arm of a clinical trial (NCT00006721), but not in DLBCL (n=174).

Conclusions: The first whole-genome miRNA profiling in tFL showed that MYC represses miR-29s levels, leading to increased TRAF4 and stronger CD40 signaling propensity. This likely represents an adaptive response to reduced CD40L availability from T-cells in the tFL niches. Moreover, low levels of miR-29c can be used as an FFPE-based biomarker of unfavorable FL prognosis.

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A Novel EPO Promoter Variant Causes Hereditary Erythrocytosis Through Non-Renal Erythropoietin Expression

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Hereditary erythrocytosis remains genetically unexplained in a significant subset of patients. We investigated a five-generation pedigree with autosomal dominant erythrocytosis and identified a novel non-coding variant in the EPO gene promoter (c.-136 G>A) segregating with disease. This germline variant introduces a de novo hypoxia response element (HRE) motif on the reverse DNA strand, suggesting a gain-of-function mechanism that enhances erythropoietin (EPO) expression outside the kidney.

Functional studies in CRISPR/Cas9-edited Hep3B cells harboring the EPO c.-136 G>A mutation demonstrated increased EPO transcription and protein secretion under both normoxic and hypoxic conditions, in contrast to wild-type controls. Luciferase reporter assays confirmed the mutation enhances transcriptional activity, particularly in the presence of HIF-2α, although chromatin immunoprecipitation failed to detect direct HIF binding, implying alternative regulatory mechanisms.

To evaluate whether this mutation affects the source of EPO production, we analyzed EPO glycoforms in patients plasma and urine. Isoelectric focusing (IEF) revealed a shift toward more basic isoforms, characteristic of non-renal EPO, which lacks sulfation found in renal-derived EPO. This indicates a switch to non-renal, likely hepatic, EPO production. These findings were further supported by SAR-PAGE, which showed no molecular weight increase, ruling out exogenous EPO use.

Importantly, the variant's gain-of-function effect persisted in homozygous and heterozygous cell clones, driving EPO production even under normoxia. Bioinformatic analysis revealed that c.-136 G>A creates potential binding motifs for additional transcription factors (e.g., SOX18, KLF9/13/17), whose contribution to the observed phenotype cannot be excluded.

Our data suggest that EPO c.-136 G>A leads to constitutive, ectopic EPO expression in non-renal tissues, overriding kidney-specific regulation and causing erythrocytosis. This mutation expands the known genetic landscape of hereditary erythrocytosis beyond coding regions and underscores the importance of analyzing regulatory sequences in patients with unexplained erythrocytosis and normal-to-elevated EPO levels. The distinct glycosylation profile of EPO in affected individuals may also have implications for anti-doping testing and the understanding of EPO biosynthesis.

Genome-wide CRISPR screening reveals Cullin-1 as a novel therapeutic target improving the efficacy of CD19-directed immunotherapy in B-cell malignancies

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Background:

Cancer immunotherapy using ex vivo engineered chimeric antigen receptor (CAR)-T cells has achieved remarkable initial responses in acute lymphoblastic leukemia, certain B-cell lymphomas or multiple myeloma. Yet, its efficacy in other B-cell malignancies is below expectations and the long-term response rates are rather disappointing. One of the key resistance mechanisms to CD19-targeted CAR T cells is the antigen escape through the loss of CD19 surface expression. However, the mechanisms governing CD19 protein stability, its intracellular trafficking and degradation remain poorly understood.

Aims:

We aimed to identify molecular regulators of CD19 surface protein stability and to test therapeutic strategies to restore CD19 surface expression and immunotherapy-based target cell killing in B cell malignancies.

Methods:

We performed a genome-wide CRISPR/Cas9 knockout screen in CD19$^+$ Ramos cells co-cultured with primary CD19 CAR-T cells at a low effector-to-target ratio that preserves most B cells, while resulting in their CD19 loss. We then sorted out B cells retaining CD19 expression under this immune pressure and detected their knocked-out genes using NGS. Among the top hits, we identified Cullin-1 (CUL1), a scaffold component of the CRL1 (Cullin-RING Ligase 1) E3 ubiquitin ligase complex, which is responsible for targeting numerous proteins for ubiquitin-mediated degradation. CUL1 was validated with individual sgRNAs across independent biological replicates. Pharmacologic inhibition of the CRL1 complex was achieved using pevonedistat, a clinical-grade inhibitor of the NEDD8-activating enzyme (NAE), which inactivates Cullin-RING ligases by blocking their neddylation.

CD19 expression was assessed using both surface and intracellular flow cytometry, and by western blotting. Immune cytotoxicity was quantified using flow-based assays in co-cultures with CAR-T cells, CAR-NK cells, and CD16$^+$ NK effectors combined with the therapeutic CD19 monoclonal antibody Tafasitamab for antibody-dependent cellular cytotoxicity (ADCC). Experiments were extended to multiple B cell malignancy models (Ramos, HG3, MEC1, Raji) and primary chronic lymphocytic leukemia (CLL) patient samples.

Results:

CRISPR screening revealed CUL1 as one of the key regulators of CD19 surface expression upon CAR-T cell mediated pressure. CUL1 knockout resulted in significant upregulation of CD19 at both the surface and total protein levels, without significant changes in mRNA. Treatment with pevonedistat mimicked the CUL1 knockout, significantly enhancing CD19 expression across various B cell lines.

Functional co-culture assays of pevonedistat-treated B cells exhibited their increased susceptibility to immune killing not only in response to CAR-T cells, but also by CAR-NK and Tafasitamab-mediated ADCC, compared to CAR-T or CAR-NK alone. Notably, pevonedistat enhanced immune-mediated cytotoxicity also in primary CLL samples using both primary T cells engineered with CD19 CAR as well as CD19-CAR-NK cells, underscoring the therapeutic relevance of this approach. Across models, CD19 mRNA levels remained unchanged, strongly supporting post-translational regulation via the CRL1 axis.

Conclusions:

Our study identifies Cullin-1 as a novel post-translational regulator of CD19 protein stability, with implications for immune escape in B cell malignancies. Pharmacologic inhibition of Cullin-1 through pevonedistat stabilizes CD19 and significantly enhances immune cytotoxicity mediated by CAR-T cells, CAR-NK cells, and antibody-dependent effector mechanisms. These findings pave a way to overcome antigen escape and enhance CD19-targeted immunotherapies in resistant or relapsed B cell leukemias and lymphomas.

This research was supported by the grant from Czech Science Foundation (project no. 22-35273S), project MUNI/A/1685/2024 and project NICR (EU program EXCELES, No. LX22NPO5102).

Low-attomolar detection of miRs related to myelodysplastic neoplasms using novel digital surface plasmon resonance biosensor

Tomáš Špringer[1], *Jan Bukáček*[1], *Zdeněk Krejčík*[2], *Ludmila Jurgová*[1], *Lucie Peštová*[1], *Michaela Dostálová Merkerová*[2], *Jiří Homola*[1]

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Microribonucleic acids (miRs) are short noncoding RNAs that play important roles in various biological processes and have been linked with a multitude of human pathologies, including myelodysplastic neoplasms (MDS). MDS is a heterogeneous group of hematological malignancies that can progress to acute myeloid leukemia. The relevant miRs circulate in blood at extremely low levels (aM

- pM range), which makes their quantification rather challenging. Herein, we report on a novel method for the ultra-sensitive detection of miRs related to MDS in human blood plasma. This method combines a new surface plasmon resonance (SPR) imaging platform based on a diffraction grating and an advanced nanoparticle release (NPR) assay. The SPR imaging platform is used to visualize individual binding events on a sensor surface via gold nanoparticles (AuNPs). In the NPR assay, analyte (hsa-miR-451a) is captured on a sensor surface and forms a sandwich complex with gold nanoparticles. Then, DNA of a special design is injected to release the AuNPs bound to the captured miRs to obtain the specific signal from these AuNPs. This approach allows for suppressing the interferences from AuNPs nonspecifically adsorbed on the sensor surface and thus improves the specific to non-specific sensor response ratio. We showed that the method enables the detection of hsa-miR-451a with a limit of detection of 10 aM (~ 6000 hsa-miR-451a in 1 mL). In addition, we detected hsa-miR-451a in human blood plasma samples and observed decreased levels of hsa-miR-451a in the samples from MDS patients when compared to healthy controls. This work presents the most sensitive SPR biosensor-based detection of miRs that provides the level of sensitivity comparable to that of the standard polymerase chain reaction (PCR), while eliminating the need for miR isolation, reverse transcription, and PCR amplification steps, and shows the potential of this detection method and hsa-miR-451a for MDS diagnosis. This work was supported by the project National Institute for Cancer Research (Program EXCELES, ID Project No. LX22NPO5102) - Funded by the European Union – Next Generation EU, by the Czech Science Foundation (contract 20–23787X), and by the Ministry of Health of the Czech Republic (MH CZ-DRO UHKT 00023736).

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TUESDAY NOVEMBER 25, 2025

Chairs: Marian Hajduch, Tomas Etrych

A little history of kinase drug discovery

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Background:

Since the first approvals, kinase inhibitors have reshaped modern therapeutics and much of this progress stems from a large amount of structure-guided chemical changes that delivered outsized clinical impact.

Objectives:

The goal of this lecture is to illustrate the evolution of kinase drug discovery -from the stone age to the golden ages- through two case studies:

Inhibitors of BCR-ABL as well as PI3K/mTOR. In the case of BCRABLI how hinge binding, DFG-in/out preference, and gatekeeper mutations shaped successive BCR-ABL generations, and how allosteric strategies rewrote the playbook, while for the PI3K/mTOR program the development of PQR309 including its clinical application as topical formulations will be showcased.

Methods: Best-practice medicinal chemistry (SBDD, SAR, PK/PD optimization) and translational development typical of Big Pharma and biotech portfolios.

Results: A rich pipeline of kinase inhibitors has emerged, with many (>70) approved by FDA with many progressing to FDA approval and standard-of-care impact.

Conclusions: Kinase inhibition remains a prolific modality. New technologies—such as molecular glues and PROTACs—are extending the playbook and accelerating next-generation therapies.

New CDK4/6/9 PROTACs for pediatric cancers

Jan Molenaar

Antibody polymer drug conjugates with increased drug to antibody ratio: an innovative nanomedicine for the advance neoplastic treatment

Ondřej Lidický[1], Robert Pola[1], Nicol Renešová[2], Júlia Kudláčová[1], Marcela Filipová[1], Petra Kovaříková[2], Eva Pokorná[2], Lucia Kučerová[3,4], Natália Udvorková[3,4], Michal Mego[3], Pavel Klener[2], Tomáš Etrych[1]

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The current requirement in clinical oncology is the need of highly effective therapeutics with proper biodistribution and pharmacokinetic profiles. Recently, the concept of Antibody Drug Conjugates (ADCs) has been intensively studied as an innovative and highly effective treatment modality [1]. Generally, an ADC consists of three main elements: a monoclonal antibody (mAb), a short linker containing a stimuli-sensitive pattern (spacer) and a highly toxic drug molecule. The enzymatically cleavable linker, containing a combination of a dipeptide and a self-immolating motive, para-amino benzyl carbamate (PAB), such as Val-Cit-PAB, was developed specifically for the ADC concept. For the therapy of hematological malignancies, there are currently seven FDA-approved ADCs targeting CD33, CD30, CD79b, CD22 (two different ADCs), CD19, and B-cell maturation antigen (BCMA) [2]. Despite the clinical success of the ADC concept, there are still some limitations. Due to issues with solubility and immunogenicity, ADCs can carry only a limited number, from 2 to 6, of attached molecules per antibody. One possible approach to increase the Drug to Antibody Ratio (DAR) without negatively affecting the ADC is to employ a biocompatible, non-fouling hydrophilic polymer as a spacer between the drug and the antibody.

The Antibody-Polymer Drug Conjugate (APDC) concept [3] expands ADC capabilities by involving a biocompatible, water-soluble polymer chain as a carrier of the toxic drug payload. In general, an APDC should result in conjugates with a low degree of conjugation (DOC) but a very high drug-to-antibody ratio (DAR), which could be up to 10 times higher compared to an ADC, without the negative effects caused by the hydrophobicity of the linker or the drug.

In this study, we designed innovative APDCs based on the anti-CD38, anti-CD20, anti-CD19 and anti-FOLR1 monoclonal antibodies, with either doxorubicin attached via a hydrazone bond or MMAE attached via Val-Cit-PAB spacer linked to an HPMA-based biocompatible polymer. The prepared APDCs reached DARs from 13 to 50 thus highly exceeding the DARs in ADC, while keeping physico-chemical properties and targeting efficacy of the monoclonal antibody preserved. The therapeutic efficacy of the new APDC compared to the ADC was validated on a series of patient-derived xenograft (PDX) models and cell-derived models. In the study, we confirmed that designed APDCs

are highly effective nanomedicines for the experimental treatment of chemotherapy-refractory lymphoma [4] and choriocarcinoma. Indeed, the tumor-stimuli sensitive behavior made the developed APDCs highly safe, as no adverse effects were observed throughout the in vivo treatment experiments. The developed APDC could serve as a promising new candidate for advanced neoplastic therapy, based on highly innovative, actively targeted drug-delivery systems.

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Novel semi-synthetic triterpenoids with selective cytotoxic activity

Milan Urban[1,2], Ivo Frydrych[1], Jan Bachořík[2,1], Šimon Orság[2,1], Ondřej Jurčeka[2,1], Soňa Gurská[1], Petr Džubák[1], Marián Hajdúch[1]

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Pentacyclic triterpenoids are natural compounds with significant biological activities.[1] During our previous research, we discovered that the majority of the compounds with selective cytotoxicity are modified with a heterocycle at the A-ring. [2]

In this work, we focused on the synthesis of a number of new triterpenoid structures containing a heterocycle condensed to their skeleton at the A-ring or replacing the isopropenyl moiety in lupanoid structure. The synthesis started from natural compounds that were subjected to multiple-step reaction pathways to obtain several sets of new compounds. Altogether, more than 150 derivatives were prepared, isolated and fully characterized. All new compounds were tested for their *in vitro* cytotoxic activity in 6 cancer cells and two non-cancer fibroblasts. The most active compounds from each set was subjected to more studies towards the mechanism of action. All of these derivatives trigger apoptosis via intrinsic pathway, although the detailed mechanism may be variable.

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Controlled Release of Gemcitabine from HPMA Conjugates: A Strategy to Improve Antitumor Efficacy

Katerina Behalova[1], Natalie Klusova[1], Martin Ivancik[1], Milada Sirova[1], Robert Pola[2], Tomas Etrych[2], Pavol Lukac[3], Luca Vannucci[3], Marek Kovar[3]

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Gemcitabine (Gem) is a widely used chemotherapeutic agent for treating breast, lung, pancreatic, and ovarian cancers. Despite its potency, its clinical use is hindered by extremely rapid metabolic degradation via cytidine deaminase, resulting in a very short half-life. To address this, we developed four N-(2-hydroxypropyl)methacrylamide (HPMA) copolymer-based conjugates (P-Gem1–4), each differing in the nature of the linker between the polymer backbone and Gem. These structural variations allow for controlled drug release kinetics and protect the active drug from degradation in circulation.

The antitumor efficacy of the conjugates was assessed *in vitro* across several cancer cell lines and in vivo using murine models of breast (4T1), lung (LL2), and pancreatic cancer. For pancreatic tumors, both syngeneic (Panc02) and human xenograft (MIA PaCa-2) models were employed. In all systems, slower drug release (P-Gem3 and P-Gem4) correlated with improved pharmacokinetics, reduced systemic toxicity, and significantly enhanced antitumor efficacy. In triple-negative breast cancer (TNBC) model 4T1, slow-releasing conjugates led to complete tumor remission in up to 30% of treated animals and significantly reduced lung metastases. Promising therapeutic effects were also observed in pancreatic cancer models, highlighting the potential of these conjugates in treating aggressive and difficult-to-manage malignancies.

Importantly, we also observed immunomodulatory effects of slow-release conjugates. P-Gem3 and P-Gem4 reduced levels of myeloid-derived suppressor cells (MDSCs)

and regulatory T cells (Tregs) in spleens and tumors, suggesting a favorable shift in the tumor immune microenvironment. Gene expression profiling confirmed upregulation of pro-apoptotic and anti-proliferative markers and downregulation of immunosuppressive factors in treated tumors.

Overall, our data demonstrate that drug release kinetics critically determine both the therapeutic and immunological outcomes of P-Gem conjugates. Slow-releasing P-Gem nanotherapeutics emerge as promising candidates for improving the efficacy of gemcitabine therapy, particularly in hard-to-treat solid tumors such as pancreatic cancer and TNBC.

Acknowledgement:

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TUESDAY NOVEMBER 25, 2025

Chairs: Juan De Sanctis, Marek Kovar

Myeloid-derived suppressor cells (MDSCs) as major inducers of immunosuppression in melanoma

Viktor Umansky

German Cancer Research Center (DKFZ), Heidelberg, Germany

Myeloid-derived suppressor cells (MDSCs) represent a heterogeneous population of myeloid cells enriched and activated in tumor microenvironment under chronic inflammatory conditions. They substantially contribute to immunosuppression in many cancer types including melanoma and represent thereby a valuable therapeutic target. It has been recently demonstrated that such MDSC accumulation in the tumor-bearing host could be mediated not only by a long-term production of soluble inflammatory factors, leading to an impairment of normal differentiation of myeloid cells but also via the acquisition of immunosuppressive properties by mature myeloid cells. This conversion of normal myeloid cells into MDSC could be influenced by extracellular vesicles (EV) secreted by tumor cells that contain a broad range of proteins, mRNA, microRNA and lipids and are considered as mediators of intercellular communication. Furthermore, the EV cargo, including HSP90a, S100A8/A9 and HMGB1 could contribute to this process of MDSC generation.

The mechanisms of conversion of mature myeloid cells into MDSC and possible approaches for MDSC targeting in preclinical mouse tumor models and in melanoma patients will be discussed.

The role of SHP inhibitors in T and NK signal transduction and cytotoxic activity

Viktor Valentini[1], Helena Besta Smrčková[2], Jenny Garmendia[2], Hana Duchová[1], Marián Hajdúch[1], Juan Bautista De Sanctis[1]

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Introduction: The role of SHP phosphatases is to generate negative signals through inhibitory receptors. PD-1/PDL1/L2 interaction activates SHP phosphatases, which inhibit the tyrosine kinases involved in cell activation and cytoktoxic responses

Material and Methods: Lymphocytes were isolated from the buffy coat using Ficoll-Hypaque. Purified lymphocytes and nano-bead-purified CD8 and NK cells were used in different experiments, non-activated, PMA/ionomycin or anti-CD3 activated and treated with the SHP inhibitors TPI-1 (inhibitor of SHP-1), and PTP1B (inhibitor of protein tyrosine phosphatase 1B). As a control, the samples were treated with dasatinib, a broad tyrosine kinase inhibitor. The cytotoxic assays were conducted by exposing the stimulated cells to different cell lines, including HCT116, HCT116KO p53, and A549 in 2D and 3D conditions and leukemic cells in 2D conditions.

Results: The phosphatase inhibitors partially reversed the PD1/PDL1/L2 effect on the cells, and it is clear that a second stimulation in the presence of the inhibitors induced more cell exhaustion and cell death. Separated CD8 cells were more susceptible to exhaustion than NK cells upon continuous stimulation.

Conclusions: The effect of phosphatase inhibitors in lymphocytes, NK, and CD8 cells was partial. Despite the partial effect, they were able to reverse the inhibitory effect of the PD1 pathway. More research needs to be conducted to enhance the effectiveness of these inhibitors.

IL-2 is a crucial cytokine for expansion of antigen-primed CD8+ but not CD4+ T cells: A paradigm shift in T cell biology and implication for cancer immunotherapy

Irfan Baki Kilic, Petra Weberová, Vladyslav Mazhara, Milada Šírová, Marek Kovář

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IL-2, mainly produced by primed T cells, promotes the proliferation, survival, and differentiation of antigen-primed T cells into effector and memory T cells, as well as NK cell cytolytic activity. Effective T cell priming requires recognition of the peptide/MHC complex by TCR and the interaction of costimulatory molecules on APCs with counter-costimulatory receptors on T cells, such as CD80/86 interacting with CD28. In turn, such primed T cells induce the expression and secretion of IL-2, which serves as a T-cell growth factor in an autocrine/paracrine manner. The IL-2 receptor (IL-2R) comprises up to three subunits: IL-2Rα (CD25), IL-2Rβ (CD122), and IL-2Rγ (CD132). IL-2 binding to the intermediate-affinity dimeric receptor (CD122/CD132; Kd ~ 1 nM) or the high-affinity trimeric receptor (CD25/CD122/CD132; Kd ~ 10 pM) triggers JAK1/3-STAT5, PI3K pathway and MAP kinase pathway downstream signaling. Dimeric receptor is a dominant receptor form

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found on CD122high populations, namely memory CD8+T cells (CD3+CD8+CD44highCD122high) and NK cells (CD3-NK1.1+NKp46+). Trimeric receptor is found predominantly on activated T cells and T regulatory cells (Treg; CD3+CD4+CD25+Foxp3+). CD25 (Kd ~ 10 nM) is not involved in signal transduction but induces a conformational change in IL-2 upon binding, thus increasing its affinity for CD122. CD25 is absent on resting naïve and central memory T cells. Its expression is induced upon T cell activation, and IL-2 signaling keeps a high level of CD25 expression, thus making positive feedback.

It has been demonstrated in vivo biological activity of IL-2 could be dramatically increased by forming complexes of IL-2 with anti-IL-2 mAb (IL-2co henceforth). Moreover, IL-2co selectively stimulate different subsets of IL-2-responsive cells depending on the clone of anti-IL-2 mAb used. Complexes of mouse IL-2 and anti-IL-2 mAb S4B6 (IL-2/S4B6) predominantly, but not exclusively, target CD122high populations, thus being highly stimulatory for memory CD8+T and NK cells and moderately stimulatory also for Treg cells, γδ T, and NKT cells. Conversely, complexes of mouse IL-2 and anti-IL-2 mAb JES6-1A12 (IL-2/JES6) stimulate highly selectivelyCD25high populations and thus induce vigorous expansion of Treg cells but have no effect on CD122high populations. Moreover, we previously demonstrated that both kinds of IL-2co are able to vigorously drive the expansion of TCR-stimulated CD8+T cells in vivo 30 though IL-2/JES6 being more potent than IL-2/S4B6.

We have noticed within our previous work that both CD122-biased IL-2/S4B6 and CD25-biased IL-2/JES6 IL-2co providing strong sustained IL-2 signal in vivo surprisingly did not stimulate expansion of antigen-primed CD4+T cells. Robust expansion of antigen-primed CD8+T cells was observed under the same experimental conditions as expected. Adoptively transferred (AT) OT-I CD8+ and OT-II CD4+T cells showed comparable expansion (5-10 times) upon immunization with chicken ovalbumin (OVA) in comparison to control. However, IL-2co (1.5 μg IL-2/dose) administered for 4 days upon OVA injection dramatically boosted OT-I CD8+T cell expansion (100-200 times relative to control) without effect on OT-II CD4+T cells. To exclude that no stimulatory effect for antigen-primed CD4+T cells is caused by so far unrecognized feature of IL-2co, we treated mice also with free IL-2 (10 μg IL-2/dose) and found no increased expansion as well. We observed very significant expansion of both OT-I CD8+ and OT-II CD4+T cells when we treated OVA-injected mice with IL-1β. IL-1β-induced expansion of OT-II CD4+T cells was comparable to that seen with IL-2co in OT-I CD8+T cells. Interestingly, combination of IL-2co with IL-1β increased expansion of OT-I CD8+T cells in comparison to either cytokine alone while it considerably decreased expansion of OT-II CD4+T cells in comparison to IL-1β alone. Importantly, free IL-2 also impeded IL-1β-stimulated expansion of OT-II CD4+T cells. To confirm these findings under the identical experimental condition for both CD4+ and CD8+T cells together with inclusion of possibility that antigen-primed CD8+ and CD4+T cells may affect each other similarly to physiological polyclonal T cell response, we performed adoptive co-transfer (AcT) of OT-I CD8+ and OT-II CD4+T cells and treated the mice as previously, except not using free IL-2. This experiment confirmed that strong sustained IL-2 signal does not stimulate expansion of antigen-primed CD4+T cells and it eventually inhibits their IL-1β-stimulated expansion. Further, we made similar finding for antigen-primed CD4+T cells in AT experiments where IL-1β was replaced by polyI:C. We also demonstrated that administration of αCD25 mAb eliminates the inhibitory effect of CD25-dependent IL-2/JES6 but not CD25-independent IL-2/S4B6 in IL-1β-stimulated expansion of antigen-primed CD4+T cells.

Next, we performed a series of *in vitro* experiments with purified naïve polyclonal CD8+ and CD4+T cells stimulated with plate-bound αCD3 mAb or αCD3 + αCD28 mAbs. Indeed, IL-1β showed powerful stimulatory activity for activated CD4+T cells. On the other hand, when activated CD8+ and CD4+T cells were exposed to constant concentration of IL-1β in combination with titrated concentrations of IL-2, CD8+T cells well responded to IL-2 but CD4+T cells did not.

These results strongly suggest distinct requirements of antigen-primed CD4+ and CD8+T cells for IL-2 and IL-1β in vivo, i.e. IL-2 is important for CD8+T cells but dispensable for CD4+T cells while IL-1β potently stimulates expansion of both subsets though it is probably more important cytokine for CD4+T cells. Current immunology considers the IL-2 to be equally important for both activated CD8+ and CD4+T cells. Thus, our work aims to change this current paradigm in the field that IL-2 is a crucial cytokine for proliferation and survival of both CD4+ and CD8+ antigen-primed T cells. We found that IL-2 is vital for activated CD8+T cells, but it is a dispensable cytokine for activated CD4+T cells and it can even dampen their expansion under some circumstances in direct or indirect fashion. To enhance CD4+T cell activity in anticancer immunity, we propose utilizing IL-1β alongside IL-2/anti-IL-2 mAb complexes and immune checkpoint inhibitors or employing IL-2-based immunocytokines containing attenuated IL-1β thereby presenting it selectively to IL-2R expressing cells in cis in cancer immunotherapy.

CD25-biased IL-2 immunocomplex as a potent anti-cancer immunotherapy and its mechanism of action

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Agonists targeting CD25 aimed at high-affinity IL-2 receptors mediate strong stimulation of regulatory T cells (Treg). Therefore, they were primarily considered for the treatment of autoimmune diseases. However, recent findings suggest that IL-2 agonists acting through CD25 may concurrently effectively stimulate tumor-specific $CD8^+$ T cells and provide better anti-tumor efficacy and safety as compared to approaches targeting IL-2 receptors without involving CD25. In this study, we show that the CD25-targeted IL-2 agonist, immunocomplex consisting of IL-2 and anti-IL-2 antibody JES6.1 (IL-2/JES6.1), stimulates proliferation of tumor-specific $CD8^+$ T cells in both *in vitro* and in vivo systems. In the absence of Treg cells, we showed an expansion of CD8+ cytotoxic T cells leading to effective anti-tumor immune response and tumor regression. As the experimental model sytem, we used the MC38-OVA mouse model of adenocarcinoma expressing ovalbumin antigen. Indeed, in immunocompetent mice bearing the MC38-OVA tumors, IL-2/JES6.1 induced a significant expansion of Treg cells. Despite that, combination treatment with checkpoint inhibitors anti-PD-1 and anti-CTLA-4, followed by the IL-2/JES6.1 immunocomplex supported also expansion of effector $CD8^+$ T cells and promoted eradication of large MC38-OVA tumors. Detailed analysis of T cell populations after the treatment revealed activation of effector $CD8^+$ T cells and increase in memory-like CD8+ cells. Upon treatment, Treg cells do not suppress KILR cells and do not limit expansion of „better effectors“. In addition, the combination treatment led to an expansion of effector $CD8^+$ cells also in non-immunogenic model of B16-F10 melanoma. Together, the findings show therapeutic potential of CD25-biased IL-2 immunocomplexes as an effective anti-cancer therapy.

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Chairs: Milos Petrik, Jan Bouchal

Surface Targets and Treatment Efficacy of Antibody – Drug Conjugates in Testicular Germ Cell Tumors

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Background: Testicular germ cell tumor (TGCT) patients who relapse after platinum-based regimen and present with refractory disease currently do not have any effective treatment option. Moreover, they do not benefit from already available novel targeted modalities yet. This represents an unmet medical need and therefore we focused on identification of druggable surface antigens overexpressed in TGCT. In addition, we evaluated the cytotoxic efficacy of selected antibody-drug conjugates (ADC) to identify suitable candidates for clinical application.

Methods: Expression of Erb-b2 receptor tyrosine kinase 2 (HER2), folate receptor 1 (FRα), trophoblast cell-surface antigen 2 (Trop2), nectin cell adhesion molecule 4 (Nectin 4) and receptor tyrosine kinase-like orphan receptor 1 (ROR1) was determined in histology subtypes of the TGCT in publicly available datasets. Protein levels of the target antigens were determined by immunohistochemical staining in our patient cohort (n=71). Cytotoxicity in cisplatin-resistant TGCT was assayed by multiplexing viability assays. Efficacy of the ADC treatment was further evaluated in preclinical study on the experimental metastasis model.

Results: In our analysis, we covered all histology subtypes of the TGCT including seminoma (SEM), embryonal carcinoma (EC), yolk sac tumor (YST), choriocarcinoma (ChC) and teratocarcinoma (TER). Majority of the patient samples and model cell lines express surface target antigens such as HER2, FRα, TROP2, Nectin4 and ROR1. Our proof-of-concept studies *in vitro* demonstrated high efficacy of the ADCs Sacituzumab govitecan and mirvetuximab soravtansine in preclinical spheroid models. Specifically, we identified high expression of FRα and TROP2 in JEG3 and JAR (ChC) and TCam2 seminoma (SEM) cells. Accordingly, these were the most sensitive to the treatment by FRα-targeting or Trop2-targeting ADCs *in vitro*. ROR1 expression was high in the TGCT cell lines, associated with significantly lower disease-free survival in TGCT patients and the IC50 for the ROR1-targeting ADC zilovertamab vedotin was 2.5 μg/mL in SuSa (TER) cells. Nectin 4 protein is significantly higher expressed in TGCT in comparison to normal testis tissue and exhibits also high expression in EC and ChC cell lines thus representing promising target for the ADC enfortumab vedotin. HER2 protein is highly expressed in some TGCT and the efficacy of the ADC trastuzumab deruxtecan in TGCT model cells will be presented.

Conclusions: Our analysis offers a rationale for repurposing clinical-stage ADC and development of novel ADC target multiple-relapsed/ refractory TGCT. Our results suggested high potency of the ADC in the treatment of cisplatin- resistant solid tumors overexpression target antigens. Next clinical trials investigating benefit of these modalities in relapsing refractory TGCT patients with progressive disease are warrantied.

Keywords: Antibody-drug-conjugate; Testicular germ cell tumors; chemoresistance; targeted chemotherapy, surface antigen.

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Platelets, Chromogranin A, and C-Reactive Protein Predict Therapy Failure of Metastatic Hormone-Sensitive Prostate Cancer

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Androgen deprivation therapy has long been the first-line treatment for hormone-sensitive prostate cancer (HSPC). After progression to castration-resistant prostate cancer (CRPC), androgen receptor pathway inhibitors (ARPIs) are commonly used. Recently, combined therapy with androgen deprivation and an ARPI has been recommended for metastatic HSPC patients. Novel markers are urgently needed for monitoring this disease and for making therapeutic decisions.

Plasma samples were collected from 140 patients with either metastatic HSPC (n = 72) or CRPC (n = 68) before the start of ARPI therapy. Digital PCR was used to assess AR gene amplification, while the expression levels of miR-375 were measured by quantitative PCR. Sixteen other clinical markers were also evaluated, including prostate specific antigen (PSA), chromogranin A (CGA), alkaline phosphatase (ALP), lactate dehydrogenase (LDH), C-reactive protein (CRP), lymphocyte-to-monocyte ratio, and platelet count. A semi-targeted lipidomic analysis was performed in a subset of mHSPC patients, encompassing 900 lipids.

A multivariate analysis, adjusted for

age and metastatic dissemination, identified miR-375 expression and lymphocyteto-monocyte ratio to be the independent negative predictors of ARPI therapy failure in CRPC patients. Regarding the HSPC patients, we found the independent negative predictive value of platelet count, CRP, and CGA for the failure of combined androgen deprivation therapy and ARPI. Preliminary analysis also found 125 lipids with significant differences across three groups of mHSPC patients: (i) ARPI therapy <1 year, high volume disease (n = 12); (ii) ARPI >1 year, high volume (n = 22); (iii) ARPI >1 year, low volume (n = 18).

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The pros and cons of 161Tb-labelled monoclonal antibodies for cancer imaging and therapy

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Introduction: The monoclonal antibodies – „magic bullets“ – are explored as radiotracer for at least last 40 years. The idea of combing very specific targeting molecule with suitable radionuclide was and still is very attractive especially in the field of cancer imaging and therapy. But several drawbacks have appeared on the way to great new radiotracers.

Methods: We have performed preclinical biological tests mainly focused to in vivo methods. The ex vivo biodistribution studies in healthy and tumor-bearing mice were the first stage of the testing followed by SPECT/CT imaging with tumor-bearing animals. The last stage of the tracer evaluation was therapy efficacy study with tumor mice. The tested compounds included several different antibodies aimed at different targets – pertuzumab (antiHER2), ramucirumab (antiVEGFR2), bevacizumab (antiVEGF) and amivantamab (antiEGFR/MET), all of them were labelled with terbium-161 and/or lutetium-177.

Results: All tested monoclonal antibodies shared certain biodistribution patterns – high liver and spleen uptake, surprisingly high kidney and lung uptake. The tumor uptake differed among the tested antibodies with pertuzumab being the most promising one. SPECT/CT imaging results were in good accordance with ex vivo biodistribution data, nevertheless they showed the promising potential (appropriate image contrast) just for 161Tb-pertuzumab. The therapy efficacy study was still under investigation at the time of abstract submission.

Conclusions: Despite initial high expectations, most of tested 161Tb-antibodies failed to show promising properties for tumor imaging or therapy. The only exception was the case of 161Tb-pertuzumab, which revealed very high tumor uptake and low accumulation in non-targeted organs like liver, spleen, kidneys. But further studies are needed to confirm its clinical potential.

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Spatial analysis of tumor microenvironments via the novel SignalStar™ multiplex immunohistochemistry assay

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Understanding the complex interactions within the Tumor Microenvironment (TME) is critical for advancing cancer diagnostics and designing effective immunotherapies. Traditional immunohistochemistry (IHC) is limited to analyzing only one or two targets per tissue section, obscuring the vital spatial context and co-expression patterns of multiple biomarkers. Existing multiplex IHC (mIHC) methods require panel optimization and validation which can take weeks to months. In this presentation we will introduce Cell Signaling Technology‘s (CST) SignalStar™ Multiplex IHC, a technology that uses antibodies, oligonucleotides, and fluorophores to interrogate cellular presence, location, function, and target co-expression patterns. SignalStar enables the robust, simultaneous detection and spatial quantification of up to eight protein targets in formalin-fixed, paraffin-embedded (FFPE) tissue samples. We will detail the unique, two-round signal amplification workflow that ensures high sensitivity for low-abundant biomarker and allows for flexible, user-defined panel design with minimal optimization time. Case studies will demonstrate SignalStar‘s application in profiling the immune infiltrate (e.g., macrophage polarization, T-cell exhaustion markers like PD-L1 and PD-1) and cellular phenotypes within solid tumors. Ultimately, SignalStar accelerates translational cancer research by providing reproducible, high-throughput spatial data, which is essential for identifying novel prognostic biomarkers and guiding precision medicine strategies.

Mitoxantrone-bearing polymeric conjugates can successfully induce immunogenic cell death in androgen independent prostate carcinoma cells *in vitro* and in vivo

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Immunogenic cell death (ICD) is one of the most extensively studied processes in recent cancer research. When ICD is induced, dying tumor cells start to express danger signals such as HSP70/90 or calreticulin on their surface or secrete soluble factors (extracellular ATP, HMGB1), which cause enhanced phagocytosis by APCs, mainly DCs, and induce their maturation. Subsequently, antigens from tumor cells are presented to and activate T cells. Mitoxantrone (MTX) is an anthracendione chemotherapeutic approved for palliative treatment of prostate cancer, mainly its androgen independent form (AIPC) resistant to hormonal therapy. MTX is also one of the most potent ICD inducers out of conventional cytostatic drugs. Ability to induce ICD can prove beneficial if combined with immunotherapy such as checkpoint inhibitors or IL-2 immunocomplexes. In our current research, we decided to prepare a drug-delivery system (DDS) based on N-(2-hydroxypropyl) methacrylamide (HPMA) bearing MTX derivative covalently bound via pH-sensitive hydrazone bond (P-MTXD). Use of HPMA-based DDS allows to improve pharmacokinetics of MTX and increase its release from polymeric carrier in acidic tumor microenvironment further amplified by enhanced permeability and retention effect providing higher accumulation of macromolecules in tumor tissue due to leaky vasculature and poor lymphatic drainage. We assessed cytostatic and cytotoxic effects of P-MTXD *in vitro* and its toxicity and antitumor activity in vivo in models of mouse and human AIPC. We also aimed at evaluating the ability of P-MTXD to induce ICD in prostate cancer cells *in vitro* and in vivo by measuring expression of ICD surface and soluble markers and ability to protect mice from developing tumors after vaccination with P-MTXD-treated cells. Additionally, we tested P-MTXD anticancer potential in other types of tumors treated with MTX (e.g. triple negative breast cancer and acute myeloid leukemia). Application of DDS bearing ICD inducer can be a promising strategy for treatment of AIPC and potential combination with immunotherapy.

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Chairs: Petr Dzubak, Daniel Rosel

Establishment of an organoid-based platform combining high-throughput phenotypic drug screening with a WNT signaling reporter to identify novel stemness targeting drugs against colorectal cancer

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Colorectal cancer (CRC) is a tumor entity of high incidence and mortality, which is caused by metastasis formation, resistance, and relapse, driven by stem cell activity of CRC cells. A major regulator of stemness in CRC is the WNT signaling pathway and its cargo protein EVI/WLS, which regulates secretion of all WNT ligands. Identification and development of drugs targeting the WNT pathway or specifically EVI/WLS are thus of high interest to improve CRC therapy.

Using tumor biopsies of CRC patients, we have established a unique collection of patient-derived CRC organoid cultures. These cultures retain mutations, gene expression, and drug response of the original patient tumors. Our biobank comprises different CRC subtypes reflecting the heterogenous mutational and gene expression profiles seen in the clinic.

To identify drugs that specifically target stem cell activity in CRC, we have developed an organoid-based high-throughput drug screening platform. In this scalable, reproducible and standardized screening approach, we are able to screen CRC organoids with hundreds of drugs per assay. Using confocal imaging of fixed and stained organoids, our pipeline allows for the assessment of subtle phenotypic changes upon drug perturbation. In our cloud-based machine learning-assisted image analysis pipeline, preprocessing, segmentation, and feature extraction are performed to generate a unique atlas of phenotypic profiles described by hundreds of phenotypic features. To assess drug-induced changes in stemness, we have developed a WNT signaling reporter that allows for quantification of changes of endogenous EVI/WLS levels following drug treatment.

Phenotypic profiles and reporter readouts are then combined with the obtained mutational and gene expression profiles and fed into our drug discovery knowledgebase. Overall, through the unique combination of high-throughput organoid screening and our machine learning-based profiling pipeline, we present a novel drug discovery platform for identification of stemness targeting drugs against CRC.

Multicomponent Peptide Stapling and Miniaturization Driven Tool for the Development of Inhibitors of Protein–Protein Interactions

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Macrocycles offer an attractive format for drug development due to their good binding properties and potential to cross cell membranes. However, when it comes to selectivity and potency of the macrocyclic peptides, large libraries of diversified analogs are required, which is a synthetically lengthy, challenging, and resource-intensive process. To address this problem, we combined Ugi multicomponent reaction (UMCR) and miniaturized acoustic droplet ejection technology for the rapid assembly of diverse macrocyclic peptide libraries from simple precursors. Here, we mutated alanine by glutamic acid in a small P53-MDM2 peptide which serves as dual acid and amino component for the UMCR. Macrocyclization occurs smoothly when the peptide library is treated with diverse sets of oxo- and isocyanide components. Cyclization occurs in DMSO on 384 well microplates, in DMSO at nano scale, to facilitate the direct high-throughput screening of these compounds without purification in subsequent assays.

We demonstrate that these crude libraries showed highly potent dual antagonistic action of MDM2 and MDMX binding p53. Such a potent inhibitory activity of p53-

MDM2/X interactions was assessed by fluorescence polarization, microscale thermophoresis, and 2D NMR, while several cocrystal structures with MDM2 were obtained.

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Spirooxindole Scaffolds: Unlocking the Potential for Novel Therapeutics for Cancer Treatment

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Spirooxindoles have emerged as a highly valuable scaffold in targeted anticancer therapy due to their strong binding affinity and ability to interact with multiple molecular targets. These compounds exhibit significant anticancer effects against various cancers, driven by their selective action and lower toxicity. As multitarget agents, spirooxindoles work through diverse mechanisms, such as inhibiting kinases, blocking MDM2–p53 interactions, modulating PIM-1/TRKA/CDK2 and EGFR/PARP-1 pathways, altering DNA binding, and triggering apoptosis. Their broad-spectrum activity highlights their potential as advanced anticancer therapeutics, warranting further research to explore their full clinical potential.

A novel migrastatic compound: molecular mechanisms and anti-metastatic effects

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Metastases cause the highest mortality in patients with solid tumors, yet few therapies directly target tumor cell invasion. Our lab has proposed a new class of drugs called migrastatics, designed to block the invasive behavior of cancer cells and prevent metastatic spread. To identify migrastics, we screened a library of small-molecule compounds for their ability to inhibit the invasion of two phenotypically distinct cancer cell lines: mesenchymal melanoma BLM and amoeboid NSCLC H1299. We selected candidates that suppressed both invasion modes while remaining minimally cytotoxic at their anti-invasive concentrations. The most effective hit was further optimized to enhance solubility and potency, resulting in a migrastatic compound with low toxicity toward both tumor and normal cells. The resulting compound exhibits broad anti-invasive effects in a wide range of tumor cell lines and is capable of effectively reducing overall tumor burden and metastasis formation in both a human cancer cell line xenograft model in NSG mice and in an orthotopically implanted syngeneic mouse allograft model.

To explore the mechanism of action of the resulting migrastatic compound, we applied thermal proteome profiling and identified 13 thermally stabilized proteins, including eight kinases, suggesting the compound is a multikinase inhibitor. Comparative phosphokinome microarray profiling confirmed broad kinase network disruption. Kinase Enrichment Analysis and Kinase-Substrate Enrichment Analysis indicated coordinated downregulation of receptor tyrosine kinases, Src family kinases, and signaling regulators involved in adhesion, polarity, and cytoskeletal remodeling. Altogether, these data indicate that the compound acts as a context-dependent multikinase inhibitor that targets signaling networks critical for adhesion, migration, and invasion, highlighting its potential as a novel anti-metastatic therapeutic.

Colchicine-BODIPY probes: Evidence for the involvement of intracellular membranes in the targeting of colchicine to tubulin

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The natural product colchicine (Col) is a medication used to treat severe inflammatory conditions. Although its mechanism of action at the level of the cytoskeleton is known, its subcellular distribution has not yet been properly studied. In this work, we present the first rational approach to assess the intracellular localization and biological activity of this alkaloid. We conjugated Col to green-emitting BODIPY dyes (CBs) with alternative linkers of different lengths (CB1-CB12) via different types of linkages. Connections of Col with BODIPY generally reduced its cytotoxicity to different levels depending on the type of linker. From the analysis of CB effects on cytotoxicity, cell cycle, and tubulin polymerization, we selected the most potent substances for fluorescence microscopy. Treatment of cells with Col conjugates showed different effects on microtubule organization. Live-cell imaging revealed that CBs rapidly associated with cellular membranes. Double label experiments unveiled that the CB4, which was the most effective in inhibiting tubulin polymerization, binds to the endoplasmic reticulum (ER) and mitochondria. In silico modeling and SPR analyses confirmed the high potency of CB4 to bind to the colchicine site on tubulin.

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WEDNESDAY NOVEMBER 26, 2025

Chairs: Stjepan Uldrijan, Martin Mistrik

Uncovering the Role of Translational Control in Growth Signaling and Therapy Resistance in Malignant Melanoma

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Advanced malignant melanoma is an aggressive cancer, still with rather limited prospects for curative treatment. The rapid proliferation of melanoma cells is driven by oncogenic mutations in the BRAF and NRAS genes, which markedly enhance the activity of the RAS/RAF/MEK/ERK signaling pathway. This pathway is targeted by clinically approved BRAF and MEK kinase inhibitors, which can suppress tumor cell division and induce disease regression; however, their therapeutic benefit is usually only temporary. One of the molecular mechanisms implicated in resistance to these inhibitors is the elevated activity of the eIF4F translational initiation complex. We have recently described a novel molecular mechanism whereby eIF4F-dependent translation contributes to the regulation of oncogene-driven ERK signaling in melanoma by maintaining a key negative feedback loop. Our latest, yet unpublished, proteomics-based data further indicate that eIF4F-mediated translational control is directly involved in regulating the cell cycle and DNA metabolism. Moreover, eIF4F activity is crucial for regulating several additional signaling pathways that control the growth of malignant melanoma. Together, our findings provide new insights into the role of eIF4F-dependent translation in melanoma cell physiology and highlight novel opportunities for therapeutic targeting of eIF4F to suppress tumor growth resistant to current treatments. At the same time, they also suggest potential mechanisms that may contribute to the emergence of resistance to newly developed eIF4F-targeted therapies.

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Autophagy Inhibition: A Key to Overcoming Lung Cancer Resistance

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The inherent drug resistance of lung adenocarcinomas, combined with the further development of resistance during long-term treatment, poses a significant challenge in the treatment of non-small cell lung carcinoma (NSCLC). Colchicine (Col), a mitotic poison, shows promising potential in lung cancer therapy due to its cytostatic effects. However, its clinical application is limited by its ability to induce cytoprotective autophagy, a process that contributes to the chemoresistance. To inhibit this cytoprotective mechanism, thereby overcoming the resistance of NSCLC and enhancing the therapeutic efficacy of Col at non-toxic concentrations, we combined Col with autophagy inhibitor chloroquine (CQ). We identified Col/CQ combinations that acted synergically in the inherently Col-resistant lung adenocarcinoma cells (A549), as well as in A549-R cells, the in-house-developed variant with further increased Col resistance after long-term treatment. Fluorescent labeling of the autophagosomes, combined with stimulated emission depletion microscopy and RNA sequencing, revealed that Col/CQ acted synergistically in both A549 and A549-R cells by stabilizing microtubules and inhibiting Col-induced cytoprotective autophagy. The RNA sequencing further indicated that in A549-R cells, the synergistic cytotoxic effect of Col/CQ was also mediated by disruptions in cell cycle regulation and DNA repair pathways which further contributes to overcoming their resistance to the treatment. Thus, the combination of Col with autophagy modulators represents a potent strategy for overcoming drug resistance, improving the clinical applicability of Col in treating lung adenocarcinomas.

Global Interactome Mapping Reveals Pro-tumorigenic Interactions of NF-κB in Breast Cancer

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NF-κB pathway is involved in inflammation; however, recent data shows its role also in cancer

development and progression, including metastasis. To understand the role of NF-κB interactome dynamics in cancer, in this work (1) we study the complexity of breast cancer interactome in luminal A breast cancer model and its rearrangement associated with NF-κB modulation. Liquid chromatography-mass spectrometry measurement of 160 size-exclusion chromatography fractions identifies 5460 protein groups. Seven thousand five hundred sixty eight interactions among these proteins have been reconstructed by PrInCE algorithm, of which 2564 have been validated in independent datasets. NF-κB modulation leads to rearrangement of protein complexes involved in NF-κB signaling and immune response, cell cycle regulation, and DNA replication. Central NF-κB transcription regulator RELA co-elutes with interactors of NF-κB activator PRMT5, and these complexes are confirmed by AlphaPulldown prediction. A complementary immunoprecipitation experiment recapitulates RELA interactions with other NF-κB factors, associating NF-κB inhibition with lower binding of NF-κB activators to RELA. This study describes a network of pro-tumorigenic protein interactions and their rearrangement upon NF-κB inhibition with potential therapeutic implications in tumors with high NF-κB activity.

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(1) Lapcik, Petr, R- Greg Stacey, David Potesil, Petr Kulhanek, Leonard J. Foster, Pavel Bouchal, Global Interactome Mapping Reveals Pro-tumorigenic Interactions of NF-κB in Breast Cancer. Molecular & Cellular Proteomics 2024, 23, 4, 100744.

Spatiotemporal organization of biomolecules in cancer cells by phase separation

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Our research investigates the spatiotemporal organization of biomolecular materials within cells, with a particular focus on their roles in gene regulation and cytoskeletal remodeling during cancer progression. We study phosphatidylinositol 4,5-bisphosphate (PIP2), a phosphoinositide that regulates compartment formation in the nucleus and cytoplasm. In the nucleus, PIP2 forms membraneless condensates with RNA molecules through liquid-liquid phase separation. PIP2 exhibits RNA-dependent nuclear localization and is involved in the transcriptional activity of RNA polymerases I and II. These findings suggest that PIP2 plays a pivotal role in organizing the nuclear architecture necessary for gene expression. We demonstrate that increasing nuclear PIP2 levels promotes the condensation of transcriptional regulators and enhances the formation of distinct nuclear foci. To further explore this mechanism, we developed an inducible system that uses a specific PIP2-binding peptide to attenuate nuclear PIP2 levels and evaluate its impact on nuclear condensation and transcriptional activity. This system allows us to directly investigate how nuclear PIP2 influences the spatial organization and functionality of the transcriptional machinery in cancer cells. Additionally, we are investigating the role of PIP2 at the inner leaflet of the plasma membrane, where it contributes to the formation of invadosomes—dynamic, actin-rich structures that mediate cancer cell invasion. PIP2 regulates the recruitment and assembly of invadosomal components, likely through localized phase separation. This process controls the architecture and activity of these invasive structures. Together, these studies reveal that PIP2-regulated phase separation is a key mechanism governing nuclear gene expression and cytoplasmic dynamics involved in cancer progression. Our findings underscore the dual function of PIP2 in organizing cellular architecture and suggest that interfering with PIP2-mediated processes could be a promising therapeutic strategy.

This project is supported by the project National Institute for Cancer Research (Programme EXCELES, ID Project No. LX22NPO5102) by the European Union – Next Generation EU.

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Characterisation of Extracellular Vesicle Content from Cancer-Associated Fibroblasts with Different αSMA Expression in Head and Neck Squamous Cell Carcinoma

David Kalfert[1], Jan Balvan[2,3], Monika Kratochvilova[3], Kveta Pilarova[2], Klara Hanelova[2], Martina Raudenska[2,3], Jiri Navratil[2,3], Tomas Vicar[2], Jaromir Gumulec[2], Jan Plzak[1], Michal Masarik[2,3]

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Introduction

Cancer-associated fibroblasts (CAFs) are a major component of the tumour microenvironment (TME) in head and neck squamous cell carcinoma (HNSCC), where they contribute to tumour progression, immune modulation, and therapy resistance. CAFs are known for their heterogeneity, including subpopulations differing in α-smooth muscle actin (αSMA) expression, which may have distinct effects on tumour behaviour and immune responses.

Material and Methods

In this study, we established and characterised CAF cultures from primary HNSCC tissues. Samples were obtained from 15 patients treated at the Department of Otorhinolaryngology and Head and Neck Surgery, 1st Faculty of Medicine, Charles University and Motol University Hospital, Prague. Tumour samples were collected under general anaesthesia prior to any treatment, and histological diagnosis of squamous cell carcinoma was confirmed by a pathologist. Only patients with available TNM classification and histological grading were included. Patients with recurrent disease or other malignancies were excluded.

Results

We successfully established 10 CAF cell lines from these samples. All cell lines exhibited more than 90% fibroblast purity as determined by morphology and flow cytometry using CD90 staining. Conditioned media were collected from these CAF cultures for subsequent extracellular vesicle (EV) isolation. To confirm the activated phenotype of the isolated CAFs, we analysed the expression of fibroblast activation markers fibroblast activation protein-alpha (FAP) and podoplanin (PDPN) by Western blotting. All patient-derived CAFs expressed both FAP and PDPN, whereas these markers were weakly expressed or absent in human gingival fibroblasts (HGF), serving as controls.

Further analyses of the CAFs focused on comparing the content of EVs derived from CAF populations with high and low αSMA expression. The proteomic and RNA cargo of these EVs were subjected to detailed characterisation; however, the results of these analyses are currently being processed and will be presented.

Conclusion

Our findings confirm the establishment of activated CAF cultures from primary HNSCC and the presence of distinct αSMA-defined subpopulations suitable for further EV analyses. This model provides a platform to explore the role of CAF-derived EVs in tumour progression and immune modulation in HNSCC.

This work was supported by the Ministry of Health of the Czech Republic NU22-03–00202.

Creatine kinase B, a downstream effector of c-Myb, controls migration of osteosarcoma cells via regulation of N-cadherin

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Background We have recently identified transcription factor c-Myb as a negative prognostic factor in osteosarcoma (OSA) patients associated with metastatic disease. Transcriptomic analysis identified creatine kinase B (CKB) as one of the most deregulated genes in OSA cell lines with depleted MYB. CKB is a key component of the creatine/phosphocreatine system that plays a key role in maintaining cellular energy homeostasis and energy transport to sites with high demand. This study was therefore conducted to investigate the functional significance of CKB in OSA.

Methods Deregulation of CKB by c-Myb in OSA cells was analyzed using gain-of-function/loss-of-function experiments. Transactivation of the CKB promoter by c-Myb was assessed using a reporter assay. CRISPR/Cas, RNAi and cyclocreatine were used to inhibit the expression/activity of CKB in OSA cells. Cell growth, colony-forming capacity, cell migration, chemosensitivity *in vitro* and metastatic capacity *in vivo* was examined. CKB protein effectors were identified using liquid chromatography-mass spectrometry (LC-MS) in data-independent acquisition-parallel accumulation serial fragmentation mode.

Results CKB was validated as c-Myb target in OSA cell lines. Depletion of

CKB using CRISPR/Cas resulted in slower migration of OSA cells *in vitro* and reduced metastatic capacity in immunodeficient mice. siRNA and cyclocreatine inhibited OSA cell migration as well but in this case, cell proliferation was also reduced. A total of 8566 protein groups were quantified, with 147 upregulated and 143 downregulated protein groups associated with the CKB knockout phenotype. The deregulated proteins were enriched for those associated with cell migration and motility. N-cadherin, an established regulator of cell migration, was identified as a target of CKB signaling and its role in OSA cell migration and metastasis was confirmed.

Conclusion c-Myb – CKB – N-cadherin axis was identified as pathway regulating OSA cell migration and metastasis.

Overcoming P-glycoprotein-mediated multidrug resistance in cancer cells through micelle-forming copolymers for doxorubicin delivery

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Cardiovascular diseases and cancer still remain the leading causes of death worldwide. Cancer treatment often involves multiple therapy strategies, usually in various combination of surgery, chemotherapy, radiotherapy and immunotherapy. One of the most critical obstacles in cancer treatment is so-called multidrug resistance (MDR), often associated with the overexpression of ATP-binding cassette (ABC) transporters, particularly P-glycoprotein (P-gp). MDR, intrinsic (naturally present before treatment) or acquired (generated during or after treatment), contributes to approximately 50% of cases in which tumours become unresponsive to therapy. As a result, MDR is responsible for the majority of treatment failures and relapses, contributing to high mortality (in up to 90% of cases)1.

This study aims to improve the efficacy of pharmacological therapy of MDR solid tumours through inhibition of P-gp using polymer-doxorubicin conjugates containing poly(propylene oxide). Poly(propylene oxide) (PPO) is known as a high molecular weight inhibitor of ABC transporters, but its use in a biological system is impossible due to its high hydrophobicity. An attachment of PPO to hydrophilic polymer based on poly[N-(2-hydroxypropyl) methacrylamide] (PHPMA) increases the hydrophilicity of the system and enables use of this copolymer as a polymeric carrier and MDR inhibitor in one. In aqueous solutions, these copolymers self-assemble into supramolecular micellar structures with a hydrophilic shell and a hydrophobic core, enabling the loading of additional free PPO into the micelle core to increase its P-gp inhibitory activity. Due to self-assembling, the molecular weight of the whole system increases, which significantly contributes to prolongation of circulation time in bloodstream, protecting the drug from premature capture by the reticuloendothelial system (RES) and subsequent elimination from the body. As benefit, PHPMA(drug)-PPO conjugate can be due to high molecular weight passively targeted into solid tumours through Enhanced Permeability and Retention (EPR) effect.

We designed, synthesized and characterized two types of novel amphiphilic copolymers composed of a hydrophilic block based on PHPMA copolymer and the hydrophobic PPO block: 1) diblock copolymers with non-degradable covalent bond between blocks (DB), possibly loaded with additional PPO, and 2) graft copolymers with biodegradable linkers between PHPMA backbone and PPO side chains. Both types of copolymers carried the anticancer drug doxorubicin (Dox) attached to the PHPMA via biodegradable hydrazone bonds. The undisputed advantage of using hydrazone bonds is their pH sensitivity, which affects their stability under various environmental conditions (e.g. in bloodstream or tumour tissue).

Recently2, we published new results obtained with DB, DB-Dox conjugates and their PPO-loaded analogues confirming their potential as promising P-gp inhibitors. The DB copolymers and their Dox conjugates significantly increased Dox accumulation in P-gp positive cells, markedly sensitizing them to Dox cytotoxic activity. The underlying mechanisms included depletion of intracellular ATP with subsequent inhibition of P-gp mediated drug efflux, an altered mitochondrial membrane potential, and increased ROS production. Moreover, the DB-Dox conjugates inhibited tumour growth *in vivo* more effectively compared to the corresponding PHPMA-based drug delivery system. Copolymers with additionally loaded PPO in the micelle core demonstrated superior efficacy in terms of P-gp inhibition, ATP depletion, and chemo sensitizing effect *in vitro*, as well as antitumor activity *in vivo*. DB copolymers effectively depleted ATP levels both *in vitro* and *in vivo* using patient-derived xenograft (PDX) models, underscoring their capacity to enhance the effectiveness of standard chemotherapy and translational potential.

Based on these encouraging results with non-degradable DB derivatives, we continue with study

of graft copolymers, where PPO was attached to PHPMA backbone through 3 types of biodegradable linkers – LEV, IPL and PYR. *In vitro* results give us hope, that these copolymers could be more effective in inhibiting P-gp even more than non-degradable DB copolymers. Biological evaluation *in vivo* of graft copolymers is now underway.

Acknowledgement

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Uncovering the dual Impact of CuEt: T-Cell signaling and tumour immunogenicity

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The involvement of LCK (leukocyte-specific kinase) in T-cell receptor signaling is well established, particularly through the phosphorylation of tyrosine residues that regulate its activity. CuEt, a compound with emerging anti-cancer properties, has been shown to activate T-cells at nanomolar concentrations. However, the specific intracellular pathways underlying this effect remain to be clarified. In this study, we investigated the impact of CuEt at a 1 nM concentration on CD8+ T cells and the HCT116 cancer cell line, including both parental and p53-knockout (KO) variants. Our analysis focused on the phosphorylation status of LCK at tyrosine 394 (activating) and tyrosine 505 (inhibitory) residues. We further examined downstream signaling components such as Zap70, LAT, and ERK1/2. In addition, we evaluated the expression levels of NKG2D ligands—including ULBP1, ULBP2/5/6, MICA, and MICB—in HCT116 cells to explore potential immunogenic changes induced by CuEt treatment.

This work was supported by the project National Institute for Cancer Research (Programme EXCELES, ID Project No. LX22NPO5102) - Funded by the European Union – Next Generation EU.

Targeting Activated CD8+ T Cells with CD25-Biased IL-2 Complexes Enhances Anti-Tumor Immunity Despite Treg Expansion

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Background:

Interleukin-2 (IL-2) plays a central role in T cell expansion and effector differentiation, but its therapeutic application in oncology is hindered by systemic toxicity and the preferential activation of regulatory T cells (Tregs). To overcome these limitations, we investigated CD25-biased IL-2 agonists formed by complexing IL-2 with the monoclonal antibody JES6-1A12 (IL-2/JES6), which selectively target CD25+ cells. Importantly, activated tumor-specific CD8+ T cells upregulate CD25, making them susceptible to such selective stimulation. To further potentiate this effect, we combined IL-2/JES6 treatment with immune checkpoint inhibitors (anti-CTLA-4 and anti-PD-1 mAbs). These agents are known to drive re-expression of the high-affinity trimeric IL-2 receptor (CD25/CD122/CD132) on tumor-reactive T cells, thereby increasing their responsiveness to CD25-biased IL-2 agonists.

Methods:

We used an adoptive transfer model with OT-I CD8+ T cells in ovalbumin-primed congenic B6 mice. Flow cytometry was employed to monitor T cell expansion and expression of effector molecules (CD25, granzyme B, perforin). Treg suppression assays were conducted *in vitro* to dissect functional interactions between IL-2/JES6, CD8+ T cells, and Tregs. *In vivo* anti-tumor efficacy was tested in GL261 glioma and CT26 colon carcinoma models, with or without combination checkpoint blockade.

Results:

IL-2/JES6 complexes induced robust expansion of antigen-primed OT-I CD8+ T cells and markedly enhanced their effector phenotype, including upregulation of CD25, granzyme B, and perforin. Although Tregs were also expanded, their suppressive activity was functionally neutralized due to the saturating availability of IL-2/JES6 complexes. Mechanistically, CD25-biased IL-2 agonists saturated the IL-2 uptake capacity of Tregs and provided sufficient IL-2 to activate CD8+ T cells -counteracting one of the key suppressive mechanisms mediated by Tregs, namely IL-2 consumption. This finding was supported *in vivo*, where tumors in mice treated with IL-2/JES6 exhibited delayed growth despite the concurrent expansion of Tregs. Notably, the combination of IL-2/JES6 with checkpoint inhibitors significantly enhanced anti-tumor efficacy, likely due to the synergistic induction of CD25 expression on tumor-specific T cells, which further amplified their responsiveness to IL-2/JES6.

Conclusions:

CD25-biased IL-2/JES6 complexes selectively stimulate activated CD8+ T cells while overcoming Treg-mediated suppression through IL-2 competition. When combined with checkpoint blockade, this strategy leverages re-expression of high-affinity IL-2 receptors on tumor-reactive T cells, resulting in synergistic and durable anti-tumor responses. These findings support further development of CD25-biased

IL-2 agonists as part of combinatorial immunotherapy regimens.

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Temporal Optimization of CD25-Biased IL-2/anti-IL-2 mAb Complexes and Immune Checkpoint Blockade Enables Eradication of Large Established Tumors

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Introduction

Interleukin-2 (IL-2) is a cytokine with multifaceted roles in the immune system. It is essential for the proliferation, survival, and effector differentiation of T cells. Although IL-2 was among the first FDA-approved agents for cancer immunotherapy, its clinical use has remained limited due to its short half-life, high-dose toxicity, and preferential stimulation of immunosuppressive regulatory T cells (Tregs). To address these limitations, various strategies have been developed to alter the pharmacokinetic properties and receptor selectivity of IL-2, including its complexation with monoclonal antibodies (IL-2/anti-IL-2 complexes, or IL-2cx).

The IL-2/JES6 complex, formed by combining IL-2 with the JES6-1A12 monoclonal antibody, selectively targets the high-affinity IL-2 receptor (CD25), thereby strongly stimulating both Tregs and recently activated CD8+T cells. Due to its capacity to robustly expand Tregs, IL-2/JES6 has traditionally been associated with immunosuppressive effects and has primarily been studied in models of autoimmune disease. However, our findings challenge this conventional view and demonstrate that IL-2/JES6, when administered in combination with immune checkpoint inhibitors (ICIs), can exert potent antitumor effects.

Aim and Methods

This study aimed to evaluate the therapeutic efficacy of the CD25-biased IL-2/JES6 complex in combination with ICIs (anti-CTLA-4 and anti-PD-1 mAb) in a tumor setting. In particular, we focused on determining how the timing of IL-2/JES6 administration relative to ICI treatment influences therapeutic outcomes. Using the CT26 murine colorectal carcinoma model, we compared the effects of administering IL-2/JES6 either before or after ICIs. Tumor progression, survival, immune cell phenotypes, and treatment-related toxicity were systematically assessed.

Results

Administration of IL-2/JES6 following ICI therapy resulted in significant tumor regression and prolonged survival compared to ICI treatment alone. In contrast, administering IL-2/JES6 before ICIs conferred no additional benefit. Mechanistically, the synergistic effect observed with sequential ICI and then IL-2/JES6 treatment is attributed to enhanced expansion and cytotoxic activity of tumor-specific CD8+T cells, along with reduced Treg-mediated suppression due to saturation of IL-2 availability. Importantly, IL-2/JES6 exhibited markedly lower systemic toxicity compared to the CD25-blocking IL-2/S4B6 complex, allowing for the safer administration of higher, more effective doses. Moreover, mice that achieved complete tumor rejection developed strong, durable antitumor memory, as demonstrated by their resistance to subsequent tumor rechallenge.

Conclusion

Our findings suggest that CD25-biased IL-2 complex, IL-2/JES6, may represent a previously underestimated tool in cancer immunotherapy. When administered with optimal timing, specifically following immune checkpoint blockade, IL-2/JES6 can substantially enhance CD8+T cell-mediated antitumor responses without inducing high toxicity. This combination strategy presents a promising approach for the development of next-generation immunotherapies aimed at improving the efficacy and durability of immune checkpoint inhibitor treatments.

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Navigating Obstacles in Exosome and Cell-Free RNA Extraction Protocols

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Introduction: Cell-free circulating RNA (cfRNA), particularly when encapsulated in exosomes, represents a promising avenue for minimally invasive cancer biomarker discovery. Exosomes (30–150 nm) carry a diverse array of RNA molecules that mirror the physiological and pathological states of their cells of origin, underscoring their potential in cancer diagnostics and prognostics. However, the translation of exosome-derived cfRNA into clinical practice is contingent upon the reproducibility and standardization of isolation and extraction techniques.

Methods: We compared multiple exosome isolation approaches from cell culture conditioned media and plasma, focusing on ultracentrifugation and commercial kits (Norgen EXTRAClean, Qiagen

exoRNeasy, exoEasy). As a baseline, ultracentrifugation was performed at 100,000 ×g for 90 minutes at 4 °C in 38.5 ml open-top tubes using a swinging bucket rotor. We also evaluated a two-round spin protocol under the same conditions to assess potential yield improvements. Additionally, we tested ultracentrifugation in 1.5 ml tubes compatible with a fixed-angle rotor using adapters, following technical advice to use a shorter 21-minute spin at 100,000 ×g, accounting for the reduced separation path length in these tubes. RNA was extracted from isolated exosomes using the Qiagen miRNeasy kit, while direct cfRNA extraction from plasma was performed with complete protocols of EXTRAClean and exoRNeasy kits, with and without DNase treatment. RNA yield and quality were assessed using the Agilent Bioanalyzer RNA 6000 Pico Kit.

Results: All isolation methods produced low cfRNA concentrations, typically in the tens to low hundreds of picograms per microliter. Ultracentrifugation yielded the most consistent RNA recovery and accommodated larger sample volumes, with the best results at 100,000 ×g for 90 minutes in 38.5 ml tubes using a swinging bucket rotor. The swinging bucket configuration offers the advantage of forming the exosome pellet centrally at the bottom of the tube, making it easier to locate and thus minimizing sample loss. Commercial kits (Qiagen exoEasy, exoRNeasy) tended to recover predominantly short RNA fragments (~25 nucleotides), which may restrict their applicability for sequencing-based analyses. The Norgen EXTRAClean kit provided similar RNA concentrations, though the higher elution volume resulted in diluted RNA, and overall RNA quality remained suboptimal. DNase treatment further reduced RNA yields, likely due to increased sample handling and processing time.

Conclusion: Our comparative analysis highlights the technical challenges and trade-offs inherent in cfRNA extraction from exosome preparations. Ultracentrifugation remains the most robust method for consistent RNA recovery, while commercial kits may require further optimization for downstream applications.

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Gemcitabine nanotherapeutics with increased stability and different rate of hydrolytic release

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The treatment of cancer is a complex process that typically combines multiple therapeutic approaches, such as surgery, chemotherapy, radiotherapy, and biological therapy. In chemotherapy, low-molecular-weight cytostatics are used, such as cytarabine, doxorubicin, or gemcitabine, which is the subject of this research.

Gemcitabine is widely used in the treatment of pancreatic cancer. Pancreatic cancer is usually diagnosed very late, mainly due to its nonspecific symptoms and the need for multiple imaging methods. The vast majority of patients have an advanced form of the disease or metastases at the time of diagnosis. Gemcitabine is used as a less aggressive and supportive treatment for patients in very poor condition who can no longer undergo more intensive therapy.

However, the administration of cytostatics in their free form brings a number of disadvantages, such as low specificity, short circulation time in the body, fast degradation into inactive derivatives, the need for high doses, and increased toxicity to healthy tissues. One possible solution to these issues is the use of nanotherapeutics as drug delivery systems, which allow controlled release of the active compound. The release occurs either locally, where the nanomaterial is enhanced and retained at the tumor site (so-called Enhanced Permeability and Retention effect; EPR effect), or the drug is released systemically at a defined rate. Both systems extend the circulation time of the drug in the body; a system activated within tumor tissue also helps reduce its undesired toxicity.

Examples of such type of nanomaterials are copolymers based on poly[N-(2-hydroxypropyl) methacrylamide] (pHPMA), which serve as a biocompatible and water-soluble drug carriers. The drug can be attached to nanomaterials via a biodegradable linker that enables its controlled release. This work focuses on the design, synthesis, physicochemical characterization, and *in vitro* testing of new polymer-gemcitabine conjugates based on pHPMA. In the first step, a monomer containing gemcitabine bound via amide bond to methacryoled 8-aminooctanoic acid (Octan) and p-aminomethylbenzoic acid (PAMBA) was prepared. The comonomer was then copolymerized with HPMA using the RAFT mechanism. Two polymer conjugates with similar properties (molar mass and drug content) were prepared with the aim of achieving the low sustained release rate of the drug from the polymer conjugate.

The release study of the drug from the prepared polymer carriers was performed in PBS, mouse blood serum, and human blood serum to get the information about the release also in conditions closely mimicking real *in vivo* conditions. The *in vitro* cytotoxic and cytostatic activity was also studied on mouse pancreatic cancer cells Panc02 and human pancreatic cancer cells MiaPaca2.

The newly designed polymer systems are expected to not only prolong the circulation time of the drug in the body, but also to protect the drug from fast degradation in blood stream, thereby increase the effectiveness and quality of treatment for pancreatic cancer or breast cancer.

The role of cyclins in the development of prostate cancer

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Prostate cancer is a major cause of disease and mortality among males. Genetic, epigenetic and environmental factors contribute to the development of prostate cancer. The progression of this hormone-dependent cancer is driven by androgens. Androgen deprivation therapy (ADT) is the primary treatment for advanced prostate cancer. ADT is initially highly effective but the majority of patients relapse and develop castration-resistant prostate cancer over 2-3 years, characterized by a lack of response to ADT.

Cyclins are the regulatory subunits of cyclin dependent kinases (CDKs), that control progression through cell-cycle checkpoints by controlling the activation of CDKs. Their levels oscillate during different phases of the cell cycle. Cyclin D1 (CCND1) is dominant in phase G1, cyclins A1 and E1 (CCNA1 and CCNE1), in phase S, and cyclins A1 and B2 (CCNA1 and CCNB2), during mitosis. Dysregulation of cyclin expression/activity can lead to disruption of the normal regulation of cell growth and division and contribute to the development of cancer, including prostate cancer. Androgens are well-established drivers of prostate cancer progression. The androgen receptor-androgens axis induces cell cycle progression, partly through the upregulation of cyclin D1 expression. We aimed to estimate the contribution of selected polymorphisms of CCND1 rs9344, CCNE1 rs997669, CCNA1 rs3829944 and CCNB2 rs12907957 to prostate cancer risk. Additionally, we examined whether these selected polymorphisms influence the dynamic changes in gene expression of these cyclins in tumour tissue of patients with prostate cancer and thus to determine any important roles that they might play in prostate carcinogenesis.

We used TaqMan®SNP Assays and High-Resolution Melting (HRM) analysis to determine CCND1 rs9344, CCNE1 rs997669, CCNA1 rs3829944 and CCNB2 rs12907957 genotypes in subjects comprising 532 patients (median/range: 67/61-73 years) with histologically verified prostate cancer and 567 healthy individuals (median/range: 65/58-72 years) as control. Cases and controls were tested for total serum PSA levels. Forty-four radical prostatectomy specimens and 31 benign prostatic hyperplasia (BPH) specimens were analyzed for the relative mRNA expression levels of cyclins by using qRT-PCR. The median age of patients in the prostate cancer and BPH cohorts was 71.5 years (range: 55–88) and 73 years (range: 58–82), respectively.

We observed a significant association between the GA genotype of the CCND1 rs9344 polymorphism and an increased risk of prostate cancer (OR, 1.64; 95% CI, 1.23-2.20; $p<0.001$). However, no significant associations were found between the CCNE1 rs997669, CCNA1 rs3829944, and CCNB2 rs12907957 polymorphisms and prostate cancer risk. Moreover, the relative mRNA expression levels of CCND1, was significantly lower ($p<0.05$) in prostate tumor tissues compared to BPH tissues. The relative mRNA expression of remaining cyclins in prostate tumor tissues did not differ significantly from that in BPH tissues ($p>0.05$). Consequently, we observed no effect of selected polymorphisms on the relative mRNA expression levels of their corresponding genes.

Furthermore, we analyzed possible associations between CCND1 rs9344, CCNE1 rs997669, CCNA1 rs3829944 and CCNB2 rs12907957 polymorphisms, the relative mRNA expression of their corresponding genes and clinicopathological characteristics (serum PSA level and Gleason score. We categorized the cases into groups according to the PSA level (<10 and ≥10 ng/ml) and Gleason score (≤7 and >7). No significantly increased risk of prostate cancer was seen in association with CCND1 rs9344, CCNE1 rs997669, CCNA1 rs3829944 and CCNB2 rs12907957 mutant genotypes and clinicopathological parameters ($p>0.05$). Interestingly, we found that the relative CCNE1 mRNA expression was statistically significantly elevated in individuals with a Gleason score >7 in comparison with Gleason score ≤7 [median log 2 (FC) values: 1.09 and -1.47, respectively, p=0.014].

Our findings indicate that genetic alterations, including polymorphisms and/or gene expression changes in CCND1, are associated with prostate cancer risk. We hypothesize that changes in the expression of CCND1 may be regulated by different cytoplasmic/nuclear CCND1 expression and/or mechanisms associated with microRNA (miR) activity. It has been shown that cytoplasmic localization of CCND1 was predominant in low-grade prostate carcinomas, whereas nuclear CCND1 was associated with high-grade tumors (Comstock et al. 2007). Previous study has demonstrated that overexpressed miR-15a and miR-16-1 can directly bind to the 3'-untranslated region of CCND1, thereby reducing its expression levels (Boci et al. 2008). Taking together, genetic variants in cyclin D1 are good candidates to have its association with susceptibility to prostate cancer assessed. Future studies involving large, multiethnic populations are required to validate and further explore the clinical potential of these findings.

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Changes in expression of the genes involved in sphingolipid metabolism during EMT in HBEC-12KT cells

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The epithelial-to-mesenchymal transition (EMT) is a key mechanism in cancer progression, enabling cells to gain motility, resist apoptosis, and acquire stem-like properties. Understanding metabolic adaptations during EMT is essential for uncovering novel therapeutic targets. In this study, we developed a unique *in vitro* model of gradual EMT progression using immortalized normal human bronchial epithelial cells (HBEC-12KT) continuously exposed to the environmental carcinogen benzo[a]pyrene (BaP) for up to 12 weeks. This model captures the dynamics of EMT progression and allows simultaneous monitoring of cell phenotype, intracellular sphingolipid (SL) and glycosphingolipid (GSL) composition, and transcriptional changes in associated metabolic genes. We analyzed wild-type cells, cells at early (2 weeks), intermediate (8 weeks), and late (12 weeks) stages of BaP exposure, as well as fully transformed mesenchymal-like HBEC12KT-B1 cells. Using HPLC-MS/MS and qRT-PCR, we focused on changes involved in SL and GSL metabolism and we identified stage-specific transcriptional alterations across key genes, including SPHK2, SGPL1, ST8SIA1, A4GALT, B4GALNT1, and B3GNT5. These genes are key regulators of sphingolipid and glycosphingolipid biosynthesis and degradation pathways, and their altered expression during EMT reflects metabolic reprogramming linked to cancer cell plasticity and progression.

In early EMT stage (after 2 weeks), only subtle transcriptional changes were observed, despite phenotypic alterations and shifts in lipid composition, such as increased sphingosine and gangliosides GM3 and Lc3. A longer, 8-week exposure to BaP further increased cell migratory capacity, induced epithelial-to-mesenchymal transition (EMT) markers and EMT-related transcriptional regulators (SNAI1, ZEB1 and ZEB2) and it increased intracellular sphingosine, ceramide-1-phosphate, as well as a series of GSLs (glucosylceramide, lactosylceramide, GM1a, GD3, Lc3 and Gb3). In contrast, fully transformed mesenchymal-like cells exhibited significant upregulation of genes responsible for the synthesis of bioactive lipids (e.g., sphingosine-1-phosphate) and tumor-associated GSLs (e.g., GD3, Gb3, Lc3), accompanied by corresponding shifts in their lipid profiles.

Furthermore, siRNA knockdown of core EMT-related transcription factors SNAI1, ZEB1, ZEB2 indicated that they may contribute significantly to the deregulation of SL/GSL metabolism, especially in the late stages of EMT.

Altogether, our data suggest that transcriptional remodeling of SL/GSL metabolism occurs predominantly during the late phase of EMT and contributes mainly to the maintenance of the mesenchymal phenotype in BaP-transformed HBEC cells. The mechanisms underlying deregulation of SL/GSL levels and functions of individual SL/GSL species deserve further attention.

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Does biological material retain its integrity after long-term storage in biobanks?

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Biobanks play an irreplaceable role in biomedical research by providing access to high-quality and well-characterised biological samples, such as blood, tissue and DNA. However, the long-term integrity and usability of stored material remain a concern, particularly for studies requiring precise molecular or genetic analyses. We evaluated the impact of long-term storage on the quality of tissue specimens stored in biobank at Masaryk Memorial Cancer Institute. In our biobank we store tissue and serum samples in -80 °C since 2000 and in liquid nitrogen vapour since 2006. During that time, we have created an extensive collection of several tens of thousands of aliquots and therefore there was a need to verify whether the stored biological material is still of sufficient quality. In our study, we assessed both RNA and protein integrity, including phosphorylation status, in 120 fresh frozen tumour specimens from breast, colon, and ovarian carcinomas collected between 2000 and 2023 and maintained under monitored conditions for up to 23 years. RNA integrity was evaluated using the Agilent 2100 Bioanalyzer, while protein quality was examined by Western blotting and immunohistochemistry targeting total and phosphorylated p44/42 MAPK. Our results demonstrate that samples stored in liquid nitrogen vapor since 2006 retained high and consistent RIN values and also the overall protein stability. These findings support the use of long term

archived tissues in translational biomedical research, provided that specimens are processed correctly from the outset and stored at ultra low temperatures.

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Analysis of expression of selected long non-coding RNAs as potential diagnostic and prognostic markers of prostate cancer

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Long non-coding RNAs (lncRNAs) are a diverse class of RNA molecules longer than 200 nucleotides that do not code for proteins but play essential roles in regulating gene expression at the epigenetic, transcriptional, and post-transcriptional levels. Over the past decade, lncRNAs have gained significant attention for their involvement in various human diseases, including cancer. In prostate cancer, lncRNAs have emerged as key players in tumor initiation, progression, metastasis and therapy resistance. Several lncRNAs have been found to be differentially expressed in prostate cancer tissues compared to normal prostate tissue, often correlating with disease severity, resistance to therapy, and clinical outcomes. PCA3, for instance, could be utilized as a non-invasive biomarker for prostate cancer diagnosis due to its high specificity in urine samples. Furthermore, lncRNAs modulate key signaling pathways such as sex hormones signaling, which is central to prostate cancer biology. Their ability to interact with DNA, RNA, and proteins could make them promising therapeutic targets and diagnostic tools. However, the complex regulatory networks involving lncRNAs remain only partially understood, highlighting the need for further research. Understanding the specific roles and mechanisms of lncRNAs in prostate cancer and changes in their expression profiles could pave the way for novel therapeutic interventions and improved patient management and outcomes.

The aim of this study was to analyze the expression profiles of panel of 11 lncRNAs (CBR3-AS1, GAS5, HOTAIR, LINC00963, MALAT1, PCA3, PCAT1, PCGEM1, PRNCR1, PVT1 and TERC) in 37 prostate cancer tissues compared to 38 benign prostate hyperplasia tissues (BPH). All tissue samples were collected from patients during indicated surgical procedures at the Clinic of Urology of the Jessenius Faculty of Medicine and the University Hospital Martin. Total RNA, isolated from tissue samples using AllPrep DNA/RNA/miRNA Universal Kit (Qiagen) according to manufacturer´s protocol, was transcribed to cDNA using RT2 First Strand kit (Qiagen). The determination of the expression was performed using RT2 lncRNA qPCR Assays (Qiagen) specific for the analyzed lncRNAs and with beta-2 microglobulin (B2M) and ribosomal protein lateral stalk subunit P0 (RPLP0) as house-keeping genes.

For all analyzed lncRNAs, except GAS5, MALAT1 and PCA3, we found statistically significantly changed relative expression in tumor tissues. Almost all lncRNAs whose relative expression was significantly changed in prostate cancer tissues compared to BPH tissues had a significant increase in relative expression. Only in the case of PCGEM1 we found a significant decrease in relative expression in prostate cancer tissues. The ROC curve generated using a Random Forest classification model using the relative expression of all analyzed lncRNAs had an overall area under the curve (AUC) value of 0.792, indicating that the use of all tested lncRNAs had good classification ability in discriminating tumor tissues from BPH. The ROC curve generated using the relative expression of 5 most important lncRNA from previous model (PCA3, PCAT1, HOTAIR, TERC and MALAT1) had AUC value of 0.811, which points to the fact that expression profile of these 5 lncRNA is sufficient for effective discriminating of tumor and benign tissues. The results of the study point to the possible importance of lncRNA expression analysis as potential diagnostic and prognostic markers of prostate cancer.

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Genetic variability in progesterone receptor and its effect on prostate cancer

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According to the Global Cancer Observatory, prostate cancer is the third most common cause of cancer-related death in men in Slovakia, it is also the most diagnosed cancer in Slovak men and the second most common cancer in men worldwide. Progesterone is a sex steroid hormone synthesized from cholesterol that is mainly associated with the female reproductive system, pregnancy, and menstrual cycle regulation. However, it has complex physiological effects such as CNS and immune system modulation. In men, progesterone is synthesized by both the adrenal cortex and the testes, and it affects spermatogenesis. Progesterone acts via its nuclear and membrane receptors. Progesterone receptor (PR) is a transcriptional factor encoded by the PGR gene on chromosome 11 that ensures genomic progesterone signalling. Progesterone binds to PR in the cell cytoplasm, PR undergoes conformational changes, dimerizes, and translocates to the nucleus, where PR dimer binds to progesterone-responsive elements on DNA and regulates transcription of target genes. The prostate contains both PR isoforms, while high PR expression is associated with prostate cancer. Prostate carcinogenesis is mostly attributed to PR-B isoform of this receptor and PR expression in prostate cancer is viewed as a potential prognostic factor for this disease. Single nucleotide polymorphisms (SNP) can potentially change the expression and function of a receptor and there have been several SNPs identified in the PGR gene. PROGINS is a group of SNPs consisting of Alu element insertion, SNP rs1042838, and SNP rs1042839. These three SNPs are in complete linkage and therefore always occur together. The potential pathogenicity of PROGINS is attributed to Alu insertion and a missense mutation (Val660Leu) of SNP rs1042838. SNP rs3740753, a missense mutation (Ser344Thr), is in partial disequilibrium with PROGINS. It is also hypothesized that this SNP might influence protein structure however it has not been tested so far. PROGINS SNP rs1042838 has been associated with higher breast cancer risk, on the other hand, SNP rs3740753 was associated with lower breast cancer risk. In this study, we aim to evaluate the influence of PR SNP rs1042838 and rs3740753 on prostate cancer risk and the clinical parameters of the disease. The study population consisted of 637 men in the control group and 662 men in the patient group. The presence of SNP rs1042838 and rs3740753 was determined by TaqMan Genotyping Assay using DNA isolated from blood. PR expression was determined using semi-quantitative real-time PCR using RNA isolated from prostate tissue. For PR expression we used prostate cancer tissues from 33 patients and as a control, we used tissues from 33 men with benign prostate hyperplasia. Patients with prostate cancer had 0.72 times elevated PR expression. Although this increase wasn't statistically significant. SNP rs1042838 heterozygotes showed a decreased overall risk of developing prostate cancer and decreased risk of prostate cancer with lower prostate-specific antigen levels (<10ng/ml) and lower Gleason score (<7). Variant allele G of SNP rs3740753 showed a decreased risk of worse pT3/pT4 staging in prostate cancer patients and it should be evaluated as a potential prognostic marker.

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fastGEN MSI - fast NGS method for detection of microsatellite instability

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Microsatellite instability (MSI) serves as a crucial biomarker for the detection and monitoring of various tumor types. Depending on the proportion of unstable microsatellite regions, tumors can be classified into three categories: high instability (MSI-H), low instability (MSI-L), and microsatellite stable (MSS). MSI-H status is strongly linked to Lynch syndrome and is commonly observed in several cancer types, particularly colorectal cancer (CRC), endometrial cancer (EC), prostate cancer, and other malignancies.

To facilitate rapid and straightforward MSI detection, a highly sensitive NGS protocol has been developed using fastGEN technology. The fastGEN assay is based on the amplification of short DNA fragments through a single polymerase chain reaction (PCR) utilizing tagged hybrid primers, followed by high coverage sequencing.

The fastGEN MSI diagnostic kit allows the analysis and visualization of 12 microsatellite regions with 100% sensitivity and specificity, as validated using the cohort of 36 CRC and 15 EC samples.

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Brain Metastases-Derived Cancer-Associated Fibroblasts Enhance the Invasiveness of Lung Carcinoma Cells

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Cancer-associated fibroblasts (CAFs) are well-established drivers of tumor progression in primary lung carcinoma. More recently, CAFs have also been identified within brain metastases (BM-CAFs), but their role in the brain metastatic microenvironment remains poorly understood. Unlike CAFs from primary lung tumors, which typically originate from fibroblasts residing in lung tissue, BM-CAFs are thought to arise locally within the brain, suggesting they may possess distinct functional properties. In this study, we investigated how BM-CAFs, isolated from human lung carcinoma brain metastases, influence the invasiveness of the human A549 lung adenocarcinoma cell line.

Co-culture of A549 cells with BM-CAFs in 3D spheroids significantly enhanced tumor cell invasion compared to A549-only spheroids. In contrast, A549 cells exposed to BM-CAF-conditioned medium did not exhibit increased invasion, suggesting that transient exposure to soluble factors is insufficient to drive this effect. Notably, in the mixed spheroids, BM-CAFs tended to emigrate first, suggesting they may remodel the surrounding extracellular matrix (ECM) in a manner that facilitates subsequent tumor cell invasion. To distinguish between ECM-mediated effects and phenotypic changes in tumor cells, we pre-cultured A549 cells with BM-CAFs using a transwell system that allowed bidirectional signaling but only limited cell–cell interaction, without a shared ECM environment. These pretreated A549 cells also demonstrated increased invasion, supporting a role for CAF-induced pro-invasive reprogramming.

In summary, BM-CAFs enhance lung carcinoma cell invasiveness through at least two mechanisms: possible ECM remodeling and interactions that induce a pro-invasive phenotype in lung carcinoma cells. These findings highlight the role of metastasis-derived stromal cells in shaping tumor behavior and may inform therapeutic strategies targeting the brain metastatic niche.

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Tropomyosin isoform Tpm2.3 inhibits osteosarcoma progression and metastasis

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Osteosarcoma is the most common primary malignant bone tumor in children and adolescents with high risk of recurrence and metastasis. Although numerous genes are involved in osteosarcoma development, markers predicting osteosarcoma progression remain elusive.

Tropomyosin 2 (Tpm2) is a member of a large and diverse tropomyosin family of proteins that polymerize along actin filaments, control their organization and dynamics by regulation of interaction with actin-binding proteins. Tpm2 has been implicated in tumorigenesis and progression of various cancer types. Four Tpm2 isoforms has been described with a different roles in regulation of differentiation of osteogenic progenitors, presumed osteosarcoma cells of origin.

In this study, we analyzed the expression of various Tpm2 variants in non-metastatic and highly metastatic osteosarcoma cell lines and in patient tissue samples.

Significant inter-individual heterogeneity in Tpm2 protein staining was found in osteosarcomas, and high expression of TPM2 mRNA in tumors was associated with better prognosis in osteosarcoma patients. TPM2.1and TPM2.3 were the predominant isoforms expressed in osteosarcoma cell lines, with lower levels observed in cells exhibiting high metastatic capacity. Overexpression of Tpm2.3 isoform suppressed osteosarcoma cell growth and lung metastases in immunodeficient mice, as well as their growth and migration *in vitro*. Increased Tpm2.3 expression also slightly enhanced the sensitivity of osteosarcoma cells to doxorubicin.

These data demonstrate that Tpm2.3 isoform functions as a tumor and metastasis suppressor in osteosarcoma.

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Cancer Knowledge and Cancer Awareness in Adolescents: Key Findings from a Systematic Review

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In recent years, an increase in cancer incidence has been observed among younger age groups, including children, adolescents, and young adults. Prevention therefore plays a crucial role in reducing the cancer burden, with both awareness of risk factors and warning signs, as well as health-related behaviors, representing key intervention points. Adolescence is a developmental stage in which these habits and attitudes are shaped, yet evidence indicates that adolescents' awareness in this area remains limited.

Findings from studies included in a systematic review show that adolescents' knowledge about cancer is uneven. While risks associated with smoking and alcohol consumption are relatively well recognized, awareness is considerably lower regarding unhealthy dietary habits, physical inactivity, or overweight. Similarly, knowledge of warning signs—such as changes in the skin, non-healing wounds, unexplained bleeding, or persistent cough—remains limited. Psychological barriers, including fear, embarrassment, and uncertainty, also play an important role, as they may lead to delays in seeking medical help. A specific area of concern is awareness of HPV and vaccination, where results are highly variable; however, educational interventions have proven effective in increasing preventive intentions.

The analysis also highlights substantial variability in the research instruments used. The most frequently employed tool was the Cancer Awareness Measure (CAM), which was in some cases adapted for adolescent populations. Other studies relied on self-developed questionnaires based on existing literature, though information about their validity or reliability was often lacking. This methodological inconsistency reduces the comparability of findings and underscores the need for standardized, age-appropriate, and psychometrically validated instruments that can provide more accurate mapping of knowledge and support the design of effective prevention strategies.

At the conference, key findings from the reviewed studies will be presented, emphasizing the necessity of targeted and age-appropriate preventive interventions. Such programs should simultaneously promote healthy lifestyles, strengthen adolescents' health literacy, and address psychological barriers to seeking professional help. Furthermore, the results point to the importance of developing valid and reliable tools for assessing cancer awareness among adolescents, which would enable more precise monitoring of knowledge levels and facilitate the planning of preventive strategies with long-term benefits for the health of future generations.

Prognostic significance of POLE and TP53 gene expression in endometrial carcinoma

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Introduction: Endometrial carcinoma (EC) is the most prevalent gynecological malignancy affecting women globally, with its incidence steadily increasing in recent years. Among gynecological cancers, it generally carries the most favorable overall prognosis. Nonetheless, this outlook can vary significantly due to the existence of diverse molecular subtypes, some of which are associated with a high risk of recurrence and poor clinical outcomes. Current scientific efforts are largely devoted towards the in-depth characterization of these molecular subtypes at both genetic variation and expression levels. Our aim is to develop a more refined diagnostic framework, enhance prognostic accuracy, and ultimately enable more personalized therapeutic strategies—particularly for endometrial cancers lacking a well-defined molecular profile.

Methods: In this study, a total of 43 EC patients were included. Gene expression of target genes (ERBB2 - Hs01001580_m1, POLE - Hs00923952_m1 and TP53 - Hs01034253_m1 and Hs01034249_m1), and specifically for this cancer, selected housekeeping genes (PPIA - Hs99999904_m1, GADPH - Hs99999905_m1 and YWHAZ - Hs03044281_g1) was analyzed by qPCR with use of TaqMan Gene Expression Assays in ViiA7 Realtime PCR System (Applied Biosystems, USA), including reaction optimization.

Results: Expression of all transcript targets of interest was successfully detected in EC tissue and evaluated towards clinical data. Expression correlation analysis showed statistically significant correlation between two genes - POLE and ERBB2 ($p=0.03$, $R2=0.318$), further between both TP53 transcripts ($p<0.001$; $R2=0.895$) and between age at the time of diagnosis with invasion depth of tumor ($p=0.002$, $R2=0.478$), also expression of estrogen and progesterone receptors correlated ($p<0.001$, $R2=0.674$). Higher POLE expression significantly associated with pre-operative histological risk, based on histological characterization of tumor ($p=0.023$), but not with post-

operative one, and pre-operative tumor grade (p=0.028). TP53 transcripts expression associated with histological tumor type (p=0.014, respectively p=0.015), we observed higher TP53 expression in carcinosarcoma subtype in comparison to adenocarcinoma, serous and mixed subtypes.

Conclusion: The data gathered reinforce the prognostic relevance of the POLE gene, indicating that its dysregulated form is associated with a more favorable patient characteristics. In contrast, elevated expression of the TP53 gene is linked to more aggressive carcinosarcoma histological subtype of endometrial carcinoma. Supported by the Ministry of Health of the Czech Republic in cooperation with the Czech Health Research Council under project No. NW24-09-00505, Cooperatio program no. 207035, "Maternal and Childhood Care" by 3rd Faculty Medicine, Charles University and the project National Institute for Cancer Research – NICR (Programme EXCELES, ID Project No. LX22NPO5102) - Funded by the European Union - Next Generation EU.

Regulation of Src Kinase and Its Functional Role in Invasive Cellular Structures

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Src kinase is a key regulator of signaling pathways controlling survival, motility, proliferation, mechanosignaling, and differentiation. Its precise regulation is essential, as aberrant activation can drive cellular transformation and cancer progression. Metastasis, the deadliest aspect of cancer, involves mechanisms that enhance cell migration and invasion, including the formation of specialized adhesion-related invasive structures such as invadopodia and podosome belts.

In this study, we investigate the role of Src kinase in these structures - invadopodia in cancer cells and closely related podosome belts in osteoclasts. Src kinase is known to regulate invadopodia; for example, it phosphorylates the Tks5 protein, which enables invadopodia maturation. However, excessive Src activation blocks maturation, suggesting that tightly controlled Src activity is crucial. Despite its established role, the precise dynamics of Src activity within these adhesion structures remains unclear.

To address this, we use a FRET-based biosensor developed in our lab to monitor Src activity in living cells, allowing real-time analysis of its activation dynamics.

Our results show that Src activity spatiotemporally colocalizes with regions of podosome belts exhibiting high actin dynamics, suggesting a role in the dynamic remodeling of these adhesion-related structures. In invadopodia, Src localizes to the structures, but how its activity changes during their lifetime remains to be elucidated. This is the focus of our ongoing research.

Silencing long non-coding RNAs (lncRNAs): An *in vitro* approach for investigating their role in multidrug resistance

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Introduction: Multidrug resistance (MDR) remains a major barrier in successful cancer treatment. Long non-coding RNAs (lncRNAs) have emerged as key regulators in cancer biology, influencing processes such as gene expression, chromatin remodeling, and drug resistance. In this study, we focused on two lncRNAs with potential roles in MDR in ovarian cancer. MALAT1 was selected based on literature reports linking it to metastasis, proliferation, and chemoresistance, while ADAMTS9-AS1 was identified through our own transcriptomic data from ovarian cancer patients.

Methods: We optimized MALAT1 knockdown in the NCI/ADR-RES multidrug-resistant ovarian cancer cell line using antisense LNA GapmeRs. Various conditions were tested, including GapmeR concentrations (10, 30, 50 nM), transfection reagent volumes (0.4–7 μl), and incubation durations (48 or 72 h). RT-PCR was used to assess knockdown efficiency, and cell proliferation was monitored using the xCELLigence system. ADAMTS9-AS1 was targeted based on its differential expression in our patient cohort, and its functional effect was similarly evaluated.

Results: Optimal knockdown of MALAT1 was achieved with 50 nM GapmeR and 72-hour incubation, though 30 nM with 0.4–0.6 μl transfection reagent provided similar results at lower cost. MALAT1 silencing led to decreased proliferation of NCI/ADR-RES cells. Knockdown of ADAMTS9-AS1 also resulted in a significant reduction in cancer cell growth, supporting its potential role in MDR.

Conclusion: We developed an efficient approach for lncRNA silencing in MDR ovarian cancer models. MALAT1, supported by previous studies, and ADAMTS9-AS1, newly implicated in our dataset, both contribute to the proliferative capacity of drug-resistant cancer cells. These findings underscore the importance of lncRNAs in MDR and provide a foundation for further functional exploration.

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Dual role of miR-125b in progression and response to chemotherapy in breast carcinoma

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Background: miR-125b is recognized as a tumor suppressor gene, with its downregulation directly linked to breast carcinoma (BC) progression. However, the expression of miR-125b varies significantly among different molecular subtypes of breast carcinoma.

Aim: To investigate the potential prognostic and predictive role of miR-125b in patients with the luminal (estrogen receptor-positive, ER+) subtype of breast carcinoma treated with chemotherapy.

Methods: We analyzed miR-125b-5p expression in a cohort of 177 luminal BC cases treated with adjuvant chemotherapy using real-time PCR. To further explore the significance of miR-125b-5p in therapy response, we transfected miR-125b-5p mimics into two ER+ (MCF-7, T-47D) and one ER− (BT-20) BC cell lines, followed by treatment with doxorubicin. Changes in cell viability and gene expression were analyzed using the CellTiter-Blue Cell Viability Assay and Lexogen poly(A)-based RNA sequencing.

Results: miR-125b-5p was found to be significantly downregulated in breast tumors compared to non-malignant breast tissues, with its expression further reduced in the more aggressive luminal B subtype compared to luminal A, consistent with its function as a tumor suppressor gene. Moreover, low miR-125b-5p expression was associated with shorter relapse-free survival in patients with luminal A tumors. *In vitro*, pretreatment with miR-125b-5p significantly reduced the cytotoxic effect of doxorubicin in BT-20 and MCF-7 cells, but not in the T-47D cell line. Subsequent analysis of potential miR-125b targets revealed downregulation of genes involved in mitochondrial dynamics and apoptosis.

Conclusion: Our data suggest that miR-125b plays a differential role in prognosis and chemotherapy efficacy across various subtypes of luminal breast carcinoma.

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Cytotoxicity Profiling of IMTM Proprietary Library by Quantitative High-Throughput Screening

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Cytotoxicity profiling is a useful phenotypic and predictive tool for characterizing the toxic potential of new chemicals. Cytotoxic activity of compounds can provide information on how cell damage and death occurs, which can be valuable for the development of targeted therapies. It is therefore a key approach early in drug development process.

Using the IMTM high-throughput screening (HTS) platform, cytotoxicity of more than 12,000 compounds from the IMTM Proprietary Library was assessed against eight cell lines (six cancer cell lines and two non-cancer cell lines) in 384-well plate format by the MTS assay. In the primary screen, all compounds were tested at one concentration (50 μM) and the percentage of inhibition (PI value) was calculated. In the secondary quantitative high-throughput screening (qHTS), active compounds (PI > 50%) were screened for a concentration-dependent response which allows to calculate IC50 values and to assess the biological activity more precisely.

All data were analyzed by Dotmatics software. To quantify the suitability of cytotoxic assay in HTS, the Z-factor was determined for each plate and cell line. Presented cytotoxicity profiling was analyzed for overall screening outcomes, hit rates, pan-activity and selectivity. Separately we analyzed compounds having PAINS patterns to estimate how well these criteria align with MTS cell-based assays. We also demonstrated how the IMTM Proprietary Library overlap

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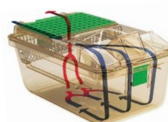

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with previously explored space of chemical compounds and drugs. Obtained results provided insight on the extent of cytotoxic compounds in IMTM Proprietary Library.

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The role of extracellular vesicles in cell-to-cell communication

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Extracellular vesicles (EVs) are small particles surrounded by a lipid bilayer that cannot replicate on their own. They are released from cells into the external environment and are typically about 100 nm in size. Recent research shows that EVs are secreted from most cell types. *In vitro* cell cultures release EVs and other factors into the culture medium, creating what is known as conditioned cell culture medium. In the first part of our study, we compared the expression of specific cancer markers—such as EGFR, PD-L1, c-MET, and E-cadherin—between cell lines and the EVs derived from their conditioned medium. In the second part, we examined paired patient tissue samples (tumor and non-tumor) and the EVs isolated from them.

EVs were extracted from the conditioned medium through ultracentrifugation, and the presence of these vesicles was confirmed using the CD63 marker. Subsequently, we measured the size, concentration, and polydispersity index (PI) of the isolated EVs using multi-angle dynamic light scattering (MADLS). The characterization of EVs was further analyzed using flow cytometry analysis of small particles.

Our results show that EGFR expression is consistent in samples from cell lines and EVs isolated from conditioned media. Both analyses indicate that EGFR levels are higher in tumor cell lines but lower in non-tumor cell lines, supporting the idea that EVs mirror the properties of the cells from which they originate. Elevated EGFR expression may suggest a tumor, but for accurate results, mutational analysis should be conducted to identify excessive or ongoing pathway stimulation.

The expression levels of the CDH1 gene, which encodes E-cadherin, were analyzed in tissue patient samples using RT-qPCR. Notably, higher CDH1 expression was observed in tumor samples compared to non-tumor samples. Similarly, tissue EVs isolated from tumor samples showed higher E-cadherin expression compared to EVs from non-tumor samples.

Our results showed that EVs isolated from conditioned media and tissue samples can contribute to intercellular communication, with the expression of examined proteins matching that of the originating cell sources.

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Novel taxane derivatives for ovarian cancer – *in vitro* and *in vivo* insights

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Taxanes are widely used anticancer drugs that have proven effective in treating many types of carcinomas, including ovarian cancer. However, a significant challenge in their use is the development of multidrug resistance, which limits their effectiveness. This resistance is a complex, multifactorial process involving various cellular mechanisms such as drug efflux, alterations in microtubule dynamics, and changes in pathways. To address this problem, newly synthesized taxanes developed at Stony Brook University, known as Stony Brook taxanes (SB-Ts), show promising potential for overcoming this resistance. These novel compounds include SB-T-1214, SB-T-1216, SB-T-121402, SB-T-121605, SB-T-121606, as well as their biotinylated conjugates (BLT-SB-Ts), which have been designed to improve drug delivery and efficacy and for lower toxicity due to specific targeting.

The aim of our study was to compare the efficacy of conventional paclitaxel (PTX) with SB-Ts and BLT-SB-Ts in ovarian cancer cell lines. In

addition to assessing their cytotoxic effects, we also investigated the impact of these compounds on the migration potential of cancer cells. Furthermore, we analyzed the effects of selected taxanes on the cell cycle. We also conducted *in vivo* studies using cell-derived xenograft models.

Cell viability in the presence of PTX and SB-Ts was assessed using the CellTiter-Blue® Cell Viability Assay following 72 hours of incubation with the tested compounds. To evaluate the impact of the taxanes on cancer cell migration, the xCELLigence real-time cell analysis system was employed. Cell cycle distribution was analyzed after 24 hours of treatment using the BD Cycletest Plus DNA Kit. For *in vivo* studies, immunodeficient nude (nu/nu) mice were used to establish xenografts derived from ovarian cancer cell line NCI/ADR-RES. Once measurable tumors had formed, the mice were treated intraperitoneally or intravenously with PTX and/or selected SB-Ts twice weekly. Tumor volumes were measured using a caliper after each treatment.

The efficacy of the novel experimental taxanes was up to 50 times higher than PTX, with SB-T-121605 and SB-T-121606 demonstrating the highest potency. Therefore, both compounds were selected for further evaluation *in vivo*. Treatment with SB-Ts significantly slowed down tumor progression, and the combination therapy consisting of 9 mg/kg PTX and 1 mg/kg SB-T-121606 proved to be the most effective regimen, achieving substantial antitumor activity while maintaining acceptable toxicity levels. To further reduce systemic toxicity and improve targeted delivery, BLT conjugates of the taxanes were tested *in vitro* and *in vivo*. Although these conjugates exhibited lower cytotoxicity compared to their unconjugated counterparts, they represent a promising approach for more selective drug delivery with low systemic toxicity.

Stony Brook taxanes have demonstrated a strong ability to overcome multidrug resistance in the NCI/ADR-RES ovarian cancer cell line model, both *in vitro* and *in vivo*. Their enhanced efficacy highlights their potential as promising candidates for the treatment of resistant tumors.

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MethAge performance in blood donors‘ samples, focusing on outliers

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Changes in methylation levels in specific CpG areas associated with the chronological age of an individual were used for the construction of epigenetic age prediction, MethAge. Samples from volunteered blood donors from the University Hospital Olomouc (the ENIGMA cohort) were used for its external validation.

The study aimed to investigate the individuals, who are predicted to be significantly older or younger than their chronological age (group A outside of the prediction interval; group B with absolute error > 7 years; group C with absolute error > 10 years) and look for specific qualities such as BMI status, reported medical history, smoking status or drinking habits these individuals might share.

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Analysis of circulating microRNAs and cell-free DNA in relation to RAS mutations in patients with metastatic colorectal carcinoma

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Background: Colorectal carcinoma (CRC) is among the most common malignancies worldwide, with a high incidence of metastatic progression (mCRC). Mutations in RAS genes, particularly in KRAS, represent important predictive biomarkers, although their prognostic value remains unclear. MicroRNAs (miRNAs) are small non-coding RNAs that regulate gene expression and modulate various signaling pathways, including those associated with RAS.

Aim: The aim of this study was to analyze the levels of selected miRNAs (miR-17-92a cluster and miR-143-3p), as well as the overall levels of cell-free DNA and RNA (cfDNA/cfRNA), in the blood of patients with mCRC in relation to RAS mutations. Additionally, a digital PCR (dPCR) method was optimized for the detection of selected KRAS mutations in plasma.

Methods: Plasma samples were collected from patients before the

initiation of systemic therapy (N = 30). For comparison, plasma samples from healthy individuals were used as controls (N = 10). The plasma levels of selected miRNAs were quantified using qPCR. RAS mutations were identified from tumor or metastatic tissue samples analyzed during the diagnostic process. For the optimization of KRAS mutation detection in plasma using dPCR, parameters such as amplification, sensitivity, and specificity were evaluated using the QIAcuity digital PCR system with QIAGEN's dPCR LNA mutation assays.

Results: Patients with mCRC had significantly higher pre-treatment plasma levels of cfDNA and cfRNA compared to the control group, while levels of the miR-17-92a cluster and miR-143 did not differ significantly between the groups. Higher cfDNA levels predicted poorer treatment outcomes and survival in patients with RAS mutation and were also associated with reduced therapy response in the overall cohort. Patients with wild-type RAS and poor treatment response showed elevated pre-treatment levels of miR-17-5p, miR-18a-5p, miR-19a-3p, and miR-143-3p. Increased levels of both strands of miR-18a were associated with shorter overall survival. The optimized dPCR method enabled sensitive and specific detection of KRAS mutant alleles (G12C, G13D).

Conclusion: Our study highlighted the importance of RAS mutation status in evaluating the clinical significance of plasma levels of the miR-17-92a cluster and miR-143-3p, as well as the overall cfDNA levels. The optimization and testing of the dPCR assay on selected plasma samples confirms the potential of non-invasive detection of KRAS mutations through liquid biopsy.

This study was supported by the Czech Health Research Council (Project No. NW25J-08-00050), the Charles University (GA UK, Project No. 183424), and the project National Institute for Cancer Research – NICR (Programme EXCELES, ID Project No. LX22NPO5102) - Funded by the European Union - Next Generation EU.

Cell-free DNA-based Comprehensive Profiling of Solid Tumors Using TruSight Oncology 500

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Background

Liquid biopsy (LB) is a modern, non-invasive approach for detecting and monitoring tumors through the analysis of tumor-derived biomarkers circulating in body fluids, particularly peripheral blood. Key components of LB include circulating tumor cells (CTCs) and circulating tumor DNA (ctDNA), which provide valuable insights into tumor characteristics and evolution. Although not yet standard in clinical practice, significant progress in the development and standardization of LB methods has advanced its potential for diagnostic, prognostic, and predictive applications. In this pilot implementation study, we focused on solid tumors, particularly colorectal cancer (CRC), and pancreatic cancer (PC).

Materials and Methods

Peripheral blood samples from 9 CRC patients were collected in Cell-Free DNA BCT® tubes (Streck, Inc.) and from 9 PC patients in K3EDTA (Vacuette®) tubes. Cell-free DNA (cfDNA) was extracted from plasma using the Qiagen Circulating Nucleic Acid Kit (Qiagen), followed by the quantification of 167bp fragment peak using the High Sensitivity DNA Kit on a Bioanalyzer (Agilent). Sequencing libraries were prepared using TruSight Oncology 500 v2 Kit (Illumina). Genomic DNA (gDNA) derived from matched tumor FFPE samples was processed with the TruSight Oncology 500 HT Kit (Illumina). As controls, reference panel-derived gDNA and cfDNA (Mimix™ OncoSpan, Horizon Discovery) were also analyzed. Sequencing was carried out on a NovaSeq 6000 platform (Illumina). The sequencing of libraries was performed on NovaSeq 6000 (Illumina). For tertiary data analyses, two approaches were compared: Illumina Connected Insights (Illumina) and Velsera Clinical Genomics Workspace (Velsera, Inc.).

Results and conclusions

The cfDNA yield corresponding to the 167 bp peak ranged from 5 to 100 ng per sample. Variant allele frequency (VAF) concordance between the reference gDNA and cfDNA profiling using TSO 500 kits versus stated VAFs was within ±2%. Illumina® Connected Insights was selected for tertiary analysis due to its streamlined and user-friendly workflow. Comprehensive DNA profiling using TSO 500 enabled assessment of tumor mutational burden and identification of clinically relevant variants present in both cfDNA and gDNA, as well as variants detectable exclusively in cfDNA. These preliminary findings support the feasibility of comprehensive cfDNA-based tumor profiling using TSO 500 in clinical research settings and underscore its potential for future clinical application.

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Biochemical characterization and high-throughput inhibitor profiling of CD73 for cancer metabolism targeting

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CD73 (ecto-5'-nucleotidase) plays a central role in generating extracellular adenosine, an immunosuppressive metabolite that promotes tumor progression. While CD73 inhibitors are under active investigation, their optimization has been limited by a lack of deep biochemical insight and the absence of sensitive high-throughput assays.

Here, we present a comprehensive biochemical characterization of soluble human and mouse CD73, as well as cell-associated human CD73. We focused on enzyme kinetics, substrate specificity, and inhibition by nucleotide analogues. We systematically investigated the effects of metal ions, pH, ionic strength, and solvent tolerance on CD73 catalytic activity. Our data further indicate that CD73 activity is glycosylation-dependent since a deglycosylated CD73 mutant commonly used for crystallographic studies exhibits markedly reduced enzymatic activity.

To facilitate the discovery of CD73 inhibitors, we adapted the DIANA assay for high-throughput screening of chemical libraries. Screening the IOCB compound collection yielded novel scaffolds, which we subsequently fine-tuned in SAR studies to obtain highly selective, picomolar inhibitors of both human and mouse CD73. These compounds also inhibit CD73 expressed on a breast cancer cell line, without inducing cellular toxicity. Instead, they enhance the activity of cytotoxic CD8+ T cells, suggesting immunomodulatory potential.

Our findings provide a detailed biochemical map of CD73 under diverse physiological and assay conditions, and establish a robust platform for the rapid identification and optimization of CD73-targeted therapeutics. These insights further underscore the role of CD73 as a critical metabolic checkpoint in the tumor microenvironment.

SYNTHETIC ANTIBODY MIMETICS AS A POTENTIAL THERAPEUTIC TOOL FOR TARGETING ACUTE MYELOID LEUKEMIA CELLS

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Acute myeloid leukemia (AML) is one of the most prevalent leukemic diseases. Current treatment often leads to initial remission; however, a significant proportion of patients develop chemoresistant clones, which lead to relapses and poor prognosis. Therefore, alternative strategies for AML treatment need to be developed involving novel therapeutic targets with CD64 emerging as a potential therapeutic target. CD64, a high-affinity IgG1 receptor that serves as a monocytic marker in AML emerges as such a target.

Here, we describe the development of synthetic polymer antibody mimetics, called iBodies. They are based on the N-(2-hydroxypropyl) methacrylamid (HPMA) polymer, which provides an adaptable and versatile platform for attachment of diverse types of ligands, such as fluorescent dyes, targeting peptides, or cytotoxic payloads. We have engineered anti-CD64 iBodies that bind to CD64-positive cells, including human monocytes and AML cells. We also decorated the iBodies with cytotoxic payloads linked via a cathepsin-cleavable linker to the HPMA polymer. These cytotoxic iBodies are internalized upon binding to the target cell and induce apoptosis by activating caspases 3 and 7. Our data suggest that CD64-positive AML cell lines show susceptibility to our iBodies and therefore could selectively target and eliminate human CD64-expressing tumors, providing a potential tool for the treatment of AML.

High-Throughput Identification of Kinase Inhibitors Using Acoustic Sample Ejection and Echo® MS

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Protein kinases are central to numerous signaling pathways and represent critical targets in early-phase drug discovery. To improve the efficiency and throughput of kinase inhibitor screening, we have implemented the SCIEX Echo® MS system, a chromatography-free mass spectrometry platform that utilizes acoustic droplet ejection

for rapid, contactless sample introduction. This setup enables direct electrospray ionization from standard well plates and supports automated dilution strategies. Coupled with the 6500+ Triple Quad mass spectrometer, the system allows sensitive and selective MS/MS detection across a mass range of 5–2000 Da, even in complex biological matrices. The Echo® MS assay has been optimized for kinases relevant to cancer and neurodegenerative diseases, supporting its application in diverse therapeutic research areas. This workflow significantly accelerates hit identification and supports robust, high-throughput analysis without the need for chromatographic separation.

Non-Invasive Detection of Circulating mRNA Markers in Colorectal Diseases

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Colorectal adenomas (CRA) are abnormal epithelial growths and key precursors to colorectal cancer (CRC). Their timely removal significantly reduces CRC risk, a major global health burden with nearly 1.9 million new cases annually. Early detection strategies, particularly non-invasive biomarkers, are critical for improving patient outcomes. This study aimed to identify and validate mRNA markers for CRA and CRC using a multi-phase approach.

In the discovery phase, next-generation sequencing (NGS) was used to analyze transcriptome profiles in CRA (n=16), incident CRC (n=10), and cancer-free individuals (CFI, n=10). We identified 10 genes deregulated between adenomas and adjacent tissues and 8 differentially expressed among tumor tissue, surrounding tissue, and healthy mucosa.

For validation, we used RT-qPCR in an expanded cohort (CRA n=48, CRC, and CFI n=51). CRC patients were stratified by stage (n=48 per stage). The most promising markers (CEMIP1, TACSTD2, SP5 and VWA2 specific to CRA, and KRT80, CLDN2, and CEMIP1 to CRC) were then analyzed in liquid biopsies (plasma and stool) from CRA (n=30), CRC (n=36), and CFI (n=51). In CRC patients, plasma markers were assessed at diagnosis and ~10 days post-surgery to evaluate their potential to reflect tumor burden reduction.

Multi-level validation confirmed differential expression of key mRNA markers in tissues and biofluids, supporting their non-invasive detection potential. Plasma and stool analyses showed significant expression changes, with certain transcripts decreasing post-surgery (CEMIP1), indicating responsiveness to tumor resection. These findings suggest that selected mRNA markers could serve as sensitive and specific biomarkers for early detection of CRA and CRC.

This study demonstrates the feasibility of transcriptomic biomarkers in liquid biopsies for non-invasive CRC screening. The integration of NGS-based discovery with RT-qPCR validation provides a robust framework for identifying clinically relevant markers. Future research should assess their diagnostic utility in larger, independent cohorts.

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Analysis of antimetastatic effects of Nilotinib and its structural derivatives

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While metastasis causes the majority of cancer related deaths in solid tumors, migrastatics, a proposed class of preventative anti-metastatic drugs, have yet to be introduced into clinical research. The trend of minimal research focus on metastasis prevention in cancer therapy has been changing in recent years and anti-metastatic drugs receive increasing attention, exemplified by improvements such as metastasis free-survival being recognised as a viable endpoint in clinical trials. These drugs should have potent and specific anti-invasive effects on cancer cells while having low cytostatic effects on both cancer and homeostatic cells to avoid drug resistance and allow for long-term therapy.

In the search for novel migrastatic compounds, we identified Niltoinib, a clinically approved tyrosine kinase inhibitor currently used for the treatment of chronic myeloid leukemia, as a promising candidate based on preliminary experiments. To redirect its activity towards inhibition of the Arp2/3 complex, we introduced structural modifications. In 3D invasion assays, both Nilotinib and its similar derivatives significantly reduced invasive behaviour in cancer cell lines without affecting cell viability. Using live-cell imaging, we observed that these compounds disrupt the actin cytoskeleton in the U2OS cell line. Subsequent *in vitro* analyses confirmed that these compounds inhibit Arp2/3 mediated actin polymerization rather than spontaneous F-actin assembly.

Overall, the modified compounds demonstrated even greater potential than the original Nilotinib

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Circulating HPV DNA and Inflammatory Markers as Predictors of Disease Course in HPV-Driven Oropharyngeal Tumours

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Introduction: Head and neck cancers, traditionally classified as HPV-negative or HPV-positive, are being researched for treatment optimisation and early relapse detection. New molecular biology techniques can measure circulating tumor DNA (cfDNA), including viral DNA (cfHPV DNA), without invasive procedures.

Material and methods: The study involved collecting blood samples from patients before treatment, analysing cfDNA using droplet digital PCR, and assessing haematological parameters like neutrophil to lymphocyte ratio (NLR) and platelet to lymphocyte ratio (PLR). Gross tumor volume was determined using CT scans. The group included 51 patients who underwent surgery, radiation therapy, combined radiation and chemotherapy, or systemic therapy.

Results: Higher initial cfHPV DNA levels are linked to larger tumor sizes in patients with advanced disease stages, while those with lower levels have early-stage disease. Patients with poor immune systems, such as high NLR and PLR levels, have lower overall survival rates and are significantly associated with higher plasma cfHPV DNA levels.

Conclusion: Our results suggest that higher baseline cfHPV DNA levels are associated with greater tumor burden and more advanced clinical stages of the disease. Elevated NLR and PLR levels are associated with poorer prognosis and could represent simple but useful prognostic biomarkers. The link between higher NLR and cfHPV DNA levels might indicate a more severe disease progression or a weaker immune response.

GLYCOLYTIC CONDENSATES AS A METABOLIC ADAPTATION IN HYPOXIC GLIOBLASTOMA CELLS

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Glioblastoma (GBM) is the most common and aggressive primary malignant brain tumour, characterised by rapid proliferation, invasiveness and limited treatment options. Hypoxic regions within the tumour microenvironment drive metabolic adaptations that sustain energy production. A key mechanism in this adaptation is the upregulation of glycolysis, which is regulated by hypoxia-inducible factor 1-alpha (HIF-1alpha). Under low-oxygen conditions, HIF-1α enhances the expression of glycolytic enzymes, ensuring ATP production despite oxygen limitation. However, the spatial organisation of these enzymes remains poorly understood.

Recent studies have highlighted the role of biomolecular condensates, membrane-less organelles formed by liquid-liquid phase separation (LLPS), in metabolic regulation. In yeast, glycolytic enzymes cluster into glycolytic bodies (G-bodies) to facilitate efficient metabolism. We hypothesise that GBM cells use a similar strategy, forming biomolecular condensates to optimise glycolysis under hypoxia.

To investigate this, we use the U251MG glioblastoma cell line and expose them to controlled hypoxic conditions (1% O_2, 37°C). Western blot was used to analyse protein levels and metabolic assays were performed to measure glucose uptake and lactate production. In addition, fluorescence microscopy will be used to study the spatial reorganisation of glycolytic enzymes under hypoxia.

Our preliminary results show that exposure to hypoxia leads to a significant increase in HIF-1α levels and upregulation of glycolytic enzymes, as confirmed by Western blot. Metabolic assays indicate increased lactate production, reflecting increased glycolytic activity. Ongoing research using fluorescence microscopy will investigate whether glycolytic enzymes reorganise into condensates in response to hypoxia. These findings suggest that glycolytic condensates may contribute to metabolic plasticity in GBM by optimising energy production under oxygen-limiting conditions.

Understanding the formation of glycolytic condensates in GBM will provide new insights into tumour metabolism and its adaptation to hypoxia. These condensates may enhance metabolic plasticity by optimising glycolytic flux and ATP production. Studying their formation may help to identify novel therapeutic targets to disrupt metabolic adaptation, potentially impairing tumour growth and complementing existing therapies.

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Novel series of antimycobacterial compounds

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Tuberculosis, a disease caused by bacterium Mycobacterium tuberculosis, is a serious global concern and a leading cause of death from infectious diseases in Post-COVID-19 era. In the infected humans, disease does not occur only as a primary disease, but it also occurs as a disease secondary to an immunocompromised status associated with inherited conditions, HIV infectious disease, cancer, autoimmune disorders and treatments like chemotherapy. Our study was focused to assess the *in vitro* antibacterial property of the 8100 novel compounds from proprietary library against three different mycobacterial strains, Mycobacterium bovisBCG strain (substrain Russia) and two different Mycobacterium tuberculosis strains, standard reference (H37Rv) and multidrug resistant (8666/2010) strain. The antimycobacterial property of these compounds was identified in the high-throughput screen for growth inhibition of M. bovis BCG strain and validated in dose response assays. The high -throughput screen showed that the 271 primary hits that at the 50 μM concentration inhibited the bacterial growth at the rate greater than 50%. These primary hits belong to the different classes of compounds. The dose-response analysis of their antibacterial activity showed that the 129 secondary hits that had the IC50 values smaller than 10 μM concentration. Analyses of cytotoxicity of these secondary hits showed that the 24 hits were toxic to human BJ fibroblast and/or mouse J774 cells, and the 105 hits were considered for further analyses. One batch of 23 hits consisting of different modified nucleosides and of amino benzoic acid derivatives was assessed for ADME (Absorption, Distribution, Metabolism, and Elimination) properties and the activity against intracellular M. bovisBCG strain in the infected mouse J774 cells. Eleven of these compounds had the activity against intracellular bacteria and their IC50 values were smaller than the 10 μM concentration. Their ADME properties were optimal. These eleven compounds were tested for bacterial growth inhibition of the two of M. tuberculosis strains, standard reference (H37Rv) and multidrug resistant (8666/2010) strain. Nine of these compounds had activity against M. tuberculosis standard reference (H37Rv) strain, while eight of these compounds had the activity against the M. tuberculosis multidrug resistant (8666/2010) strain. However, the analyses of the other batch of 82 secondary hits are ongoing, and they might show that the compounds from other classes of compounds might be effective against these two M. tuberculosis strains. Thus, our results showed the series of novel compounds with the antimicrobial property against M. bovisBCG strain and two different M. tuberculosis strains, standard reference (H37Rv) and multidrug resistant (8666/2010) strain.

Downregulation of Translation Sustains ERK–mTORC1 Signaling Under Amino Acid Deprivation in Malignant Melanoma

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Malignant melanoma is an extremely aggressive form of skin cancer that frequently develops resistance to existing therapies. Its rapid proliferation is driven by activating mutations in key signaling pathways that regulate cell growth and metabolism, including the ERK and mTORC1 pathways[1].

In this study, using BRAF- and NRAS-mutant melanoma cell lines, we show that the ERK pathway becomes further upregulated in response to metabolic stress induced by prolonged amino acid deprivation (specifically methionine, glutamine, or asparagine). This activation of ERK also stimulates mTORC1, a key regulator of cellular metabolism and protein synthesis. Interestingly, despite elevated mTORC1 activity, global protein translation is reduced under these conditions.

To explain the uncoupling between mTORC1 signaling and protein synthesis, we investigated DUSP6, a rapidly turned-over phosphatase that negatively regulates ERK[2], and found its protein level significantly reduced after amino acid withdrawal. Similarly, the expression of subunits of the GATOR1 – a negative regulator of mTORC1 responding to amino acid limitation[3] – was also decreased.

We propose that the ERK pathway upregulation under amino acid restriction results from impaired protein synthesis and reduced levels of DUSP6. This signaling response may represent a compensatory mechanism that helps melanoma cells adapt to nutrient stress and mitigate the detrimental effects of unfavorable metabolic conditions.

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eIF4F controls AMPK activity in BRAFV600E-mutant melanoma

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Introduction

Malignant melanoma is an aggressive cancer with poor prognosis, frequently driven by mutations in the MAPK/ERK signaling pathway. Although BRAF and MEK inhibitors improve survival, resistance develops rapidly. The eukaryotic translation initiation complex (eIF4F) has recently been implicated as a key mediator of this resistance, and combined inhibition of BRAF and eIF4F has shown synergistic anti-cancer effects. This study aimed to precisely characterize the crosstalk between the ERK and eIF4F pathways in melanoma.

Results

We used MS-based proteomic approach and small-molecule MEK/eIF4F inhibitors to identify shared targets of ERK and eIF4F in NRAS- and BRAFV600E-mutant melanoma cells. Proteomic analysis revealed substantial overlap between ERK and eIF4F targets, including regulators of the cell cycle, DNA repair, and the energy sensor AMP-activated protein kinase (AMPK). Components of the AMPK-activating complex (LKB1-STRAD-MO25) and the AMPK-inhibiting phosphatase PP2Aα were downregulated. Notably, eIF4F inhibition induced both ERK and AMPK activation, even in LKB1-deficient BRAFV600E cells, indicating a novel LKB1-independent mechanism of AMPK activation. PP2Aα downregulation appeared critical, as RNAi-mediated knockdown or pharmacological inhibition of PP2A strongly enhanced AMPK activity.

Conclusion

Previous studies reported negative feedback regulation between the ERK pathway and LKB1 in BRAFV600E-mutant melanoma cells. Active ERK and its target RSK both phosphorylate LKB1, preventing it from activating AMPK, which would suggest mutually exclusive activity of AMPK and ERK. Our findings provide new insights into the molecular mechanisms underlying melanoma resistance and demonstrate functional cooperation between the ERK and eIF4F pathways in regulating key cellular processes. Inhibition of eIF4F promoted non-canonical, LKB1-independent activation of AMPK while simultaneously enhancing ERK signaling. These results uncover a previously unrecognized mechanism of AMPK regulation in BRAFV600E-mutant melanoma cells.

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Unraveling the Role of Mitochondrial DNA in Colorectal Carcinogenesis: From Healthy Mucosa to Tumor Progression

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Mitochondrial DNA (mtDNA) plays a crucial role in cellular metabolism, and its stability is essential for maintaining homeostasis. During colorectal carcinogenesis, progressive changes occur in mtDNA copy number (mtDNA-CN), integrity, and repair mechanisms, potentially influencing energy metabolism and stress responses.

This study aimed to investigate alterations in mtDNA-CN, mtDNA damage, and DNA repair genes expression along the healthy-adenoma-carcinoma sequence to determine their role in tumor development and progression.

We measured mtDNA-CN and mtDNA damage using qPCR and assessed DNA repair gene expression through RNA sequencing and qPCR in colon tissues from healthy individuals, patients with colon adenomas, and colorectal cancer (CRC) patients.

Our findings revealed that mtDNA-CN was significantly elevated in colon adenomas compared to colon mucosa from healthy individuals (P = 0.002), adenoma-adjacent mucosa (P = 0.009), tumor tissue

(P < 0.001), and tumor-adjacent mucosa (P < 0.001). Moreover, mtDNA-CN was higher in adenoma-adjacent mucosa compared to colon mucosa from healthy individuals (P = 0.002), tumor tissue (P = 0.002), and tumor-adjacent mucosa (P < 0.001). MtDNA damage was significantly higher in tumor-adjacent mucosa than in tumor tissue (P < 0.0001), while no significant differences were observed among other tissue types. Additionally, most DNA repair genes were upregulated in both adenomas and tumors compared to tissues from healthy individuals.

These findings suggest that mtDNA-CN and damage levels change during the progression from healthy mucosa to adenomas and carcinomas, underscoring their role in colorectal carcinogenesis. The observed overexpression of DNA repair genes may represent a compensatory response to mtDNA damage.

In conclusion, our results highlight the importance of mtDNA alterations in CRC development and suggest their potential as biomarkers for CRC.

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Establishing a Reproducible Pipeline for High-Throughput Screening of 3D Tumor Spheroids

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Three-dimensional (3D) cell culture systems provide a more physiologically relevant platform compared to conventional two-dimensional (2D) models. Among these, tumor spheroids are particularly valuable as they better reproduce the native microenvironment, with enhanced cell–cell and cell–matrix interactions that are crucial for studying tumor biology, drug discovery, and toxicity testing.

In this study, spheroids derived from the HCT116 cell line were generated in 384-well plates to develop an optimized high-throughput screening (HTS) workflow. We first standardized spheroid formation to ensure uniformity and reproducibility. Drug compounds at multiple concentrations were then dispensed with high precision using the ECHO liquid handler, followed by imaging and structural analysis with the Cell Voyager CV8000 High-Content Screening System.

Finally, treatment responses were quantified using the MTS viability assay, providing robust data on compound-induced cytotoxicity. By integrating these optimized steps, we established a streamlined and reproducible workflow for spheroid-based HTS, offering improved precision and biological relevance for preclinical drug evaluation.

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Targeting the Mutant NPM1c+ for Therapeutic Intervention in AML

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Acute Myeloid Leukemia (AML) frequently harbors mutations in Nucleophosmin 1 (NPM1), with approximately 30% of patients carrying the NPM1c+ variant characterized by aberrant cytoplasmic localization due to mutations in the C-terminal domain (CTD). The CTD harbors a critical nucleolar localization signal (NoLS), primarily formed by two key tryptophan residues, W288 and W290. Both tryptophans, or only Trp288 in some unfrequent mutants, are replaced leading to loss of nucleolar retention and consequent pathological mislocalization of NPM1 [1,2]. Our project focuses on identifying covalent small molecule binders targeting the W288C-NPM1c+ mutant to restore proper folding and localization, offering a precision treatment strategy for AML. Through high-content screening of a 1,235-compound library in NPM1 reporter cells, we identified PHP5075 as a candidate compound capable of promoting nuclear relocalization of NPM1. Ongoing work involves comprehensive biophysical characterization and downstream functional assays including AML cellular viability, proliferation and

differentiation to investigate the binding modality and mechanism of PHP5075's activity. In parallel, leveraging the structural scaffold of PHP5075, we are designing and synthesizing a novel compound library for high-throughput screening via Echo-MS technology. This approach strategically exploits the unique biochemical and structural alterations in the mutant NPM1, specifically the loss of critical aromatic residues integral to nucleolar localization, to facilitate targeted drug discovery efforts against NPM1-mutant AML.

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High-Content Morphology Profiling Enables Discovery of Autophagy-Modulating Therapeutics

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INTRODUCTION

Autophagy is a central regulator of cancer biology, acting as a tumor suppressor in early stages by clearing damaged organelles and proteins, but also supporting tumor survival in advanced disease by recycling cellular components under stress. Autophagy modulators, activators or inhibitors, are valuable in oncology because they can either suppress tumor initiation or block tumor survival strategies, offering a way to both prevent cancer progression and improve responses to therapy. Because different cancers rely on autophagy in distinct ways, modulators offer a precision-medicine opportunity — either to activate or suppress autophagy depending on tumor type and treatment context. However, conventional assays based on single markers often fail to capture the full complexity of autophagy dynamics. Cell Painting, a high-content morphological profiling approach, detects organelle- and cell-wide changes induced by autophagy perturbation, enabling sensitive and unbiased identification of both activators and inhibitors. This scalable platform offers a powerful strategy for discovering and characterizing autophagy-targeting compounds with potential relevance for cancer therapy.

METHODS

Multiplexed fluorescent dyes were used to visualize nuclei, mitochondria, ER, Golgi, and the cytoskeleton, generating rich morphological fingerprints in HepG2 cells and imaged them using the high-throughput CellVoyager 8000 system across four channels. Cells were treated with known autophagy inhibitors and inducers with annotated modes of action in four replicates. Automated image acquisition and analysis extracted over 1,500 morphological features to generate detailed cellular profiles. Feature values were normalized against DMSO controls, and Pearson correlation assessed reproducibility between replicates. Visualization via UMAP and k-nearest neighbors analysis enabled clustering of compounds based on their modes of action. Known autophagy modulators produced reproducible, distinct profiles that served as references for comparison.

RESULTS AND CONLUSION

Unsupervised clustering of morphological features clearly separated activators and inhibitors into discrete groups, demonstrating the ability of Cell Painting to discriminate between different modes of autophagy modulation. This classification not only confirmed the expected profiles of well-characterized compounds but also provided a framework for screening novel molecules.

Cell Painting enables unbiased, high-throughput identification of autophagy modulators by capturing the multiparametric morphological consequences of pathway perturbation. The distinct clustering of known activators and inhibitors highlights the assay's utility as a scalable platform for discovering and characterizing autophagy-targeting compounds with direct relevance for cancer therapy.

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Profiling cellular senescence: insights into biomarkers and senolytic therapeutics

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Cellular senescence is defined as a state of permanent cell cycle arrest. Although senescent cells lose their proliferative capacity, they remain metabolically active and are characterized by their senescence-associated secretory phenotype (SASP). Senescence can be triggered by telomere shortening, DNA damage or oxidative stress. It is a natural hallmark of aging and serves as a protective mechanism of the organism against the propagation of damaged cells. However, the accumulation of senescent cells can lead to the development of chronic diseases. As a result the identification of senolytics - compounds selectively targeting senescent cells – have emerged as an attractive strategy

for the prevention and treatment of age-associated disorders. The key regulators of senescence are the tumor supressor genes CDKN2A (p16) and CDKN1A (p21), whose upregulation inhibits cyclin-dependent kinases essential for cell cycle progression, thus they represent promising biomarkers of human aging. In this work, we analysed the expression of p16 and p21 in blood samples from cohort of 1 100 healthy individuals and compared the results with their chronological age to assess potential correlations. Additionally, senescent cell lines were generated by irradiation and used for testing the effects of both established and candidate senolytic compounds including MCOPPB, Navitoclax and selected nucleoside analogs.

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High-Throughput SPR-Based Screening of Small Molecule–Tau Interactions Using Bruker Sierra-24 PRO: A Platform-Driven Approach to Mutation-Selective Drug Discovery

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Background: Small molecule modulation of protein aggregation is an emerging strategy in drug discovery, particularly for targets with conformational flexibility. Tau protein, known for its aggregation-prone regions, serves as a robust model system—especially when studied through the lens of pathogenic mutations such as P301L, N279K, and V287I. These mutations reveal structurally vulnerable domains that enable targeted screening of compounds capable of stabilizing or disrupting aggregation-prone conformations. However, conventional screening approaches often lack the resolution and throughput required to capture subtle kinetic differences across variants. Methods: To address this challenge, we employed the Bruker SPR Sierra-24 PRO, a high-throughput, label-free surface plasmon resonance (SPR) platform, to profile the binding kinetics of two small molecules, Purpurin and Oleocanthal, against synthetic tau peptides representing wild-type and mutant sequences. The platform's 24-channel parallel flow system enabled simultaneous kinetic analysis across multiple targets. Key interaction parameters (kon, koff, KD, Rmax) were extracted using global fitting models. Aggregation inhibition was further validated using Thioflavin T (ThT) fluorescence assays. Results: SPR analysis revealed distinct mutation-selective binding profiles. Purpurin showed moderate affinity for wild-type tau (KD = 4.35×10^{-6} M) and enhanced binding to N279K and V287I, but weaker interaction with P301L (KD = 1.48×10^{-5} M), attributed to faster dissociation kinetics. In contrast, Oleocanthal demonstrated broad-spectrum binding across all tested mutants, with significantly slower dissociation rates (koff = 8.26×10^{-5} s^{-1} for P301L), indicating enhanced complex stability. Functional assays confirmed selective inhibition by Purpurin and broad-spectrum disruption by Oleocanthal. Conclusion: The Bruker SPR Sierra-24 PRO platform enabled high-resolution kinetic profiling of small molecule–tau interactions, revealing both selective and broad-spectrum binding behaviours. By leveraging tau mutations as structural probes, this approach enhances the precision and efficiency of early-stage drug discovery. These findings underscore the value of advanced SPR-based screening in identifying and validating small molecule modulators of protein aggregation.

Key Words: Drug discovery, Purpurin, Surface Plasmon resonance, Oleocanthal, Tau aggregation

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HPV-Related Oropharyngeal Cancer: Applications of Liquid Biopsies in Screening, Early Detection, and Recurrence Monitoring

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Background: The incidence of oropharyngeal squamous cell carcinoma (OPSCC) continues

to rise in the Czech Republic and worldwide, with human papillomavirus (HPV) as the dominant etiological factor. Despite therapeutic advances, recurrence remains frequent, highlighting the need for reliable biomarkers for early diagnosis and treatment monitoring. Liquid biopsy approaches, including oral gargle lavage, oropharyngeal swabs, and blood sampling, allow non-invasive detection of oral and circulating HPV DNA. These strategies hold promise for establishing effective screening, supporting timely diagnosis, and enabling sensitive recurrence monitoring in HPV-related OPSCC.

Methods: HPV tumor status was evaluated in both newly diagnosed OPSCC patients and those in remission using HPV DNA analysis of primary tumor tissue alongside p16 immunohistochemistry. Only cases testing positive for both HPV DNA and p16 expression were classified as HPV-related OPSCC. Liquid biopsy samples (gargle lavage, oropharyngeal swabs, and plasma) were collected before and after treatment, with ongoing sampling during follow-up for HPV testing. HPV DNA detection was performed using AllPlex™ HPV28 Detection (Seegene) in oral samples, and AllPlex™ HPV HR Detection (Seegene) in primary tumor tissue.

Results: A total of 205 OPSCC patients were enrolled, with 77.2% classified as HPV-related—predominantly associated with the HPV16 genotype, detected in 97.1% of these cases. In total, 683 sample sets were collected during the study. Pre-treatment liquid biopsy analysis showed high sensitivity for HPV detection: 88.1% in gargle lavage, 80.4% in oropharyngeal swabs, and 90.3% for circulating tumor HPV DNA (ctHPV DNA) in plasma. In cases of recurrent HPV-related OPSCC, ctHPV DNA was detected in 62.5% (5 out of 8), with oral HPV DNA identified in 37.5% (3 out of 8) of those cases.

Conclusion: This study aims to validate the use of liquid biopsy and DNA-based biomarkers for early diagnosis and recurrence monitoring in HPV-related OPSCC. Preliminary findings are encouraging, indicating strong potential for early detection, including in early-stage disease, and highlighting the clinical utility of non-invasive monitoring strategies. Nevertheless, HPV DNA detection showed limitations in T0 stage OPSCCs, carcinomas of unknown primary, and tumors of the base of the tongue, resulting in reduced assay sensitivity.

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Characterization of TERT Enzymatic Activity and Expression in Primary Colorectal Cancer and Liver Metastatic Disease

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Introduction: TERT, a catalytic subunit of telomerase, is essential for maintaining telomere length and chromosomal integrity in highly proliferative cells. In most somatic cells, TERT is strictly repressed, leading to telomere shortening and cellular senescence that acts as a barrier to unlimited proliferation. Disruption of telomere biology is therefore associated with malignant transformation in many cancers, including colorectal carcinoma (CRC). However, the importance of telomere biology—particularly telomere length, TERT activity and expression—in distinct molecular subtypes of colorectal cancer and its metastases has not yet been fully elucidated

Methods: In this ongoing study, we analyzed telomerase activity (TAQ assay with digital PCR), TERT expression (digital PCR) and relative telomere length (RTL; RT-quantitative PCR). The cohorts included primary CRC tumors (n=91), liver metastases and non-cancerous adjacent tissues (n=61). Data were further stratified according to molecular features, including microsatellite stability/instability (MSS/MSI) status and KRAS mutation.

Results (preliminary): In liver metastases of CRC (n=61), the TERT expression was significantly higher (average 29 copies/μl) compared to paired non-cancerous liver tissue (P<0.001), where TERT copies were mostly close to the detection limit. Across all CRC patients, tumor tissues consistently showed elevated TERT levels compared with matched non-cancerous tissues (n=91, P=0.002). Concretely, higher TERT expression was detected in tumors with MSI (n=36, P=0.006) and tumors with KRAS mutation (n=38, P=0.026), while no significant differences were detected in tumors with MSS and KRAS wild type cancers (n=17, P=0,071).

Conclusions: Our preliminary results suggest that the TERT expression may have potential as a diagnostic biomarker for liver metastases of CRC and could help with distinguishing different molecular subtypes of CRC. This study is ongoing, sample cohorts are being expanded, and final results, including extended telomerase activity analyses, will be presented at the conference.

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Identification of miRNAs with diagnostic potential in extracellular vesicles circulating in cerebrospinal fluid of glioma patients

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Gliomas are highly heterogeneous tumors at both the molecular and histological levels and are associated with markedly different prognoses (1). Their biological behavior is largely shaped by the tumor microenvironment and its dynamic intercellular communication. Extracellular vesicles (EVs) and the molecules they carry constitute an important mechanism of such communication across both short and long distances (2). Among them, microRNAs (miRNAs) represent promising candidates for intercellular information transfer. Consequently, EV-associated miRNAs may serve as powerful diagnostic and prognostic biomarkers in glioma patients (3).

In our previous study analyzing cerebrospinal fluid (CSF) from patients with brain tumors, we identified miRNA profiles with diagnostic and prognostic potential (4). Building on these findings, we now investigate whether selected miRNAs are also present in EVs isolated from the CSF of glioblastoma patients. As an initial step, we optimized both EV isolation and miRNA extraction protocols. For method comparison, we used CSF samples from four hydrocephalus patients, which allowed for larger sample volumes.

Upon optimization, we proceeded with EV-miRNA profiling in glioma patients. The study cohort comprised 57 patients with glioblastoma (IDH-wild type), 28 with high-grade gliomas, and 16 with low-grade gliomas, totaling 101 patients. EVs were isolated from 1–4 ml CSF samples, and their purity, size distribution, and particle counts were assessed relative to the initial CSF volume. RNA was subsequently extracted from EVs, converted into cDNA libraries, and subjected to small RNA sequencing after rigorous quality control and data normalization.

Across all samples, the most abundant species included well-characterized oncomiRNAs such as miR-21-5p and miR-26a-5p, tumor-suppressive miRNAs including the let-7 family, miR-16, and miR-204, as well as dual-function miRNAs such as miR-125. These findings suggest that glioma cells may exploit EVs to deliver oncogenic miRNAs to recipient cells, or alternatively, to eliminate tumor-suppressive miRNAs.

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The inhibitory effect of tolcapone on fibril formation in wild-type and mutant variants of the tau peptide

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Protein aggregation followed by prion-like behaviour underlies several human diseases, including those connected to p53 misfolding forms of cancer and neurodegenerative pathologies. Alzheimer‘s disease and other tauopathies, such as frontotemporal dementia and FTD, are prominent examples of such pathologies. Their shared hallmark is the accumulation of tau-based neurofibrillary tangles, which disrupt neurons and drive cognitive decline. Nitrocatechol derivatives, such as tolcapone, drugs used in Parkinson’s therapy, can be used in a drug repurposing strategy. They not only give hope as potential inhibitors capable of interacting with the hexapeptide in the microtubule binding domain of tau, but can also reduce the time required for a potential drug to reach the market and help millions of patients.

One way to uncover the secret of the mechanism and/or find new inhibitors of tau aggregation is to compare mutant forms of the protein with mutations in sequences located near PHF or in regulatory regions. This work demonstrates the effectiveness of a nitrocatechol-type inhibitor and compares the aggregation kinetics of wild-type peptides and mutant forms in control and with treatment *in vitro* and *in vivo*. First, the THT assay was performed to find the most efficient inhibitor. The results were also confirmed by electron microscopy, demonstrating the difference in fibril density in control and in the case of treatment. During the second phase inhibitors were tested in different concentrations on a biosensor model using image analysis. As the final step immunological assays were performed on modified and differentiated neuroblastoma cells after seeding with preformed fibrils, as the closest to adult cholinergic neurons model.

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Elevated interleukin 6 expression in porcine melanoma and its effect on malignant melanocyte migration

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Interleukin 6 (IL-6) is mainly recognized as a proinflammatory cytokine with major effects in acute phase of immune response. Recently, it has been shown that IL-6 effects are rather pleiotropic, affecting also growth, metabolism and cell migration/invasiveness. Elevated IL-6 levels in plasma have been found in cancer patients, particularly in the advanced stages of the disease. In the tumor microenvironment, the IL-6 is produced by both malignant and stromal cells, such as malignant melanocytes and cancer associated fibroblasts in melanoma. Simultaneous blockade of IL-6 and IL-8 attenuated the invasiveness of human malignant cells *in vitro*. IL-6 signaling is triggered by IL-6 binding to IL6Rα/gp130 receptor in the plasma membrane, or by IL-6 complex with soluble IL6Rα binding to membraneous gp130.

Melanoma-bearing Libechov Minipig (MeLiM) is a model of hereditary melanoma. Piglets are born with the tumors or the tumors start to grow shortly after birth. At the age of approximately 10 weeks, two courses of the disease can be recognized, i.e. progression and spontaneous regression. In the spontaneous regression, tumors are heavily infiltrated by immune cells and the tumor is continuously replaced by fibrous tissue leading macroscopically to tumor flattering and depigmentation. In our previous study, we have analyzed cytokine expression in tumors and blood of piglets with progressive and regressive disease development and compared them to cytokines in plasma of tumor-free animals. IL-6 was one of the most significantly increasingly expressed cytokines in tumors compared to adjacent healthy skin and also in plasma of melanoma-bearing animals compared to melanoma-free piglets. As the IL-6 has been described to stimulate migration and invasiveness of human malignant cells *in vitro*, we aimed to monitor IL-6 effects on cell migration and JAK/STAT signaling pathway activation in primary porcine malignant melanocytes.

Methods: Malignant melanocytes were isolated from lymph node metastasis of a MeLiM animal. The cells were propagated *in vitro* in medium with 10% serum. Expression of melanocyte/melanoma markers has been monitored by immunocytochemistry and western blot. Cells (passage 4) were treated by 0, 1 or 10 ng/ml of recombinant IL-6 and the cell migration was monitored on wound healing assay, while the JAK/STAT3 signaling pathway activation was analyzed by western blots with phospho-site specific antibodies.

Results: Addition of recombinant IL-6 into serum-containing medium significantly stimulated migration of malignant melanocytes on wound healing assay. The melanocytes expressed gp130 receptor subunit. Activation of Jak1 (Tyr1034/1035)/Jak2 (Tyr1007/1008) and Stat3 (Tyr705) was confirmed as highly advanced phosphorylation of particular aminoacid residues detected after cell treatment by recombinant human IL-6 protein.

Conclusion: We have shown that recombinant human IL-6 stimulates migration of porcine malignant melanocytes *in vitro* and activates the JAK/STAT3 signaling pathway. IL-6 signaling is one of the candidate targets for migrastatic drugs.

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Bridging the Biomarker Gap: Targeted SureQuant Analysis of Neurodegeneration and Glioblastoma in Tears

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Despite extensive search, reliable biomarkers for neurodegeneration

and glioblastoma remain elusive, with minimal overlap between reported candidates across discovery studies. This variability hampers translation into clinical practice and underscores the need for targeted, reproducible methods capable of validating and monitoring defined panels of proteins across independent cohorts and sample types.

Here, we evaluate SureQuant, an internal standard–triggered parallel reaction monitoring workflow, for sensitive and precise quantification of protein biomarkers in human tear fluid. Tears are a promising but underexplored biofluid: they are accessible in a minimally invasive manner, suitable for repeated sampling, and may provide insights into both central nervous system pathologies and ocular conditions. By employing SureQuant, we aim to overcome the limitations of discovery proteomics and establish a robust platform for quantifying candidate biomarkers associated with neurodegenerative disorders and glioblastoma.

We optimized a targeted panel of candidate proteins, focusing on workflow parameters including sample preparation, peptide selection, and instrument settings. These optimized parameters ensured reproducibility and scalability, with initial results demonstrating consistent quantification across replicates and successful detection of multiple biomarker candidates in tear samples. These findings highlight the feasibility of implementing SureQuant-based tear proteomics and lay the foundation for subsequent large-scale validation. Ultimately, this targeted approach may help bridge the gap between the fragmented biomarker landscape and the urgent clinical need for reliable diagnostic and prognostic tools in neurodegeneration and glioblastoma.

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De novo design of CDK inhibitors based on 3D pharmacophore models

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Introduction

The cyclin-dependent kinase (CDK) family plays a central role in regulating cell cycle progression and transcription, processes that are frequently dysregulated in cancer. Aberrant CDK activity, often driven by overexpression of cyclins or loss of CDK inhibitors, promotes uncontrolled proliferation, genomic instability, and tumor survival. Because of this, CDKs have emerged as key therapeutic targets. Targeting CDKs offers a way to restore cell cycle control, enhance sensitivity to existing therapies, and potentially overcome resistance mechanisms, making the CDK kinase family a critical focus for the development of novel anticancer strategies.

Methods

Three-dimensional pharmacophore models were obtained from X-ray crystallographic complexes of the relevant CDK kinases available in the Protein Data Bank. These pharmacophore models served as templates for the generation of novel compounds using the previously developed CReM-pharm methodology. This approach enabled the design of structures that correspond to the pharmacophore features and are thus predicted to fit the binding sites of the respective kinases. The designed molecules were subsequently subjected to molecular docking using the EasyDock protocol, and their docking scores as well as protein-ligand interaction profiles were systematically evaluated.

Results

The generated compounds exhibited docking scores comparable to those of established inhibitors and displayed similar ligand-protein interaction patterns. Notably, the designed molecules featured novel scaffolds with small structural overlap relative to known kinase inhibitors. Their synthetic feasibility was supported by favorable predicted synthetic accessibility scores and confirmed through retrosynthetic analysis. Collectively, these findings suggest that the designed compounds represent promising candidates for further investigations.

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Investigating the impact of nuclear PIP2 manipulation on transcriptional regulation in cancer cells

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Mutations in PTEN and PIK3CA are among the most frequent in cancer. These mutations directly affect phosphoinositidemetabolismmaking these pathways important targets for cancer research and potential therapies. Phosphatidylinositol 4,5- bisphosphate (PIP2) is a well characterized lipid at plasma membrane. In contrast, its function in nuclear environment remains poorly understood. Recent evidence suggest that nuclear PIP2 plays a critical role in transcriptional

regulation by promoting phase separation of regulatory proteins and facilitating the formation of RNA polymerase II (Pol II) initiation condensates.

Understanding the role of nuclear PIP2 function has been limited by the lack of tools for its nucleus-specific manipulation. To overcome this challenge, we established a tetracycline-inducible system expressing a EGFP-tagged PIP2-binding peptide derived from gelsolin protein and introduce a nuclear localization signal. This system enables attenuation of PIP2 signal specifically in the cell nucleus and allows targeted investigation of nuclear PIP2 function.

In both U2OS and U251 cells, induction of expression from this construct reduces the signal of nuclear PIP2 levels as confirmed by confocal microscopy. This decrease is accompanied by a reduction in the number of BRD4 nuclear foci. BRD4 is a transcriptional coactivator that forms condensates, which were previously shown to be affected by manipulating PIP2 levels.

We hypothesize that nuclear PIP2 is required for phase separation of transcriptional regulators. Attenuation of nuclear PIP2 disrupts condensate formation and alters transcription, which in turn affects cancer cell proliferation, transformation and invasiveness. Testing this hypothesis will clarify how changes in nuclear PIP2 metabolism contribute to oncogenic processes.

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Neuroprotective Efficacy of N-Acetylcysteine and Ascorbic Acid Against Aluminium Chloride-Induced Cytotoxicity in U87 Glioblastoma Cells

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This study investigates the neuroprotective effects of N-acetylcysteine (NAC) and ascorbic acid (AA) against aluminum chloride ($AlCl_3$)-induced cytotoxicity in U87 glioblastoma cells. Utilising the DPPH and MTT assays, we evaluated the antioxidant and cytotoxic properties of these compounds. Our results showed that both NAC and AA exhibited significant free radical scavenging activities, with NAC demonstrating greater efficacy. $AlCl_3$ was found to induce concentration-dependent cytotoxicity, leading to increased apoptosis, primarily through the activation of caspases. Flow cytometric analysis revealed that NAC and AA effectively reduced overall apoptosis and enhanced cell viability when administered before $AlCl_3$ treatment. Real-time qPCR analysis indicated that $AlCl_3$ treatment significantly upregulated pro-apoptotic genes (caspase-3, caspase-8, caspase-9) and downregulated anti-apoptotic genes (Bcl-2, Bcl-xl). Notably, the combination of NAC and AA provided the maximum protective effect, normalising gene expression levels and maintaining cell viability at near control levels. This study underscores the potential of NAC and AA as therapeutic agents in mitigating the neurotoxic effects of $AlCl_3$, offering a molecular basis for developing neuroprotective strategies in neurodegenerative conditions.

Epigenetic Modifications in Recurrent Grade 1 Meningioma: DNA Methylation Patterns as Predictors of Tumor Behavior

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Meningiomas are the most common primary intracranial tumors, comprising approximately 40% of all central nervous system (CNS) tumors. According to the World Health Organization (WHO) classification, meningiomas are divided into three histopathological grades and 15 subtypes, with WHO grade 1 tumors accounting for approximately 80% of reported cases. While grade 1 meningiomas are typically considered benign, recurrence rates range from 7% to 25%, and recurrence can occur many years after initial resection. This underscores the biological heterogeneity of meningiomas and the need for prolonged surveillance and improved prognostic tools.

Selection of patients for adjuvant therapy after surgical resection remains challenging, as histological grading alone does not reliably capture tumor behavior. Advances in molecular diagnostics, including targeted gene panel sequencing and whole-genome DNA methylation profiling, have demonstrated significant potential in refining risk stratification and identifying

biologically distinct meningioma subgroups.

DNA methylation profiling, in particular, has emerged as a robust tool to classify tumors beyond histology, offering improved prediction of recurrence risk and long-term outcomes.

The aim of this study was to establish a reliable predictive framework for identifying patients at elevated risk of recurrence following surgical resection of WHO grade 1 meningiomas. By integrating DNA methylation profiling with clinicopathological data, this work seeks to create a preselection tool that will guide further molecular analyses and facilitate personalized therapeutic strategies, ultimately improving patient management and prognosis.

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Searching for CDK16 inhibitors in ultra-large chemical libraries

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Introduction

Cyclin-dependent kinase 16 (CDK16), a member of the atypical CDK family, plays key roles in protein trafficking, vesicle transport, and non-canonical regulation of the cell cycle. Overexpression and dysregulation of CDK16 have been associated with tumorigenesis, cancer cell survival, and resistance to therapies, including radiotherapy and antimitotic drugs. Preclinical studies demonstrate that inhibition of CDK16 reduces cancer cell proliferation and can sensitize tumors to treatment. These findings highlight CDK16 as a potential target in tumors and a promising candidate for expanding CDK-directed therapeutic strategies beyond CDK4/6.

Methods

To identify potential inhibitors of CDK16, we utilized the Enamine REAL Database, which contains approximately 10 billion enumerated, synthetically accessible compounds. Given the impracticality of exhaustive screening across this vastlibrary, we implemented a two-step strategy to enhance search efficiency. Initially, promising molecular structures were generated de novo using the CReM-dock approach, a fragment-based generative method guided by molecular docking. These generated compounds, selected based on superior docking scores and their predicted ability to interact with the hinge region of CDK16, served as query structures for a rapid similarity search across the entire database. Multiple types of molecular fingerprints were employed during this similarity search. Subsequently, compounds retrieved from the Enamine REAL Database were subjected to docking against CDK16 to identify the most promising candidates.

Results

We investigated the impact of various generation scenarios and parameter settings on the efficacy of the proposed approach in retrieving promising hits from the Enamine REAL Database. Our findings indicate that structures generated from Enamine fragments during the initial stage yielded molecules with improved docking scores in the subsequent screening phase. Furthermore, Morgan fingerprints demonstrated superior performance in the similarity search step, effectively retrieving compounds with high docking scores and predicted binding affinity to the CDK16 hinge region. The compounds identified through this methodology were further evaluated using consensus docking to select the most promising candidates. Notably, the selected molecules exhibited a high degree of structural novelty relative to previously reported inhibitors and are considered strong candidates for subsequent acquisition and experimental validation.

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Tumour-Associated Immune Cell Spatial Heterogeneity in Prostate Cancer

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Introduction: Prostate cancer remains the second most common cancer in men, with a diverse course. The lethality of metastatic castrate-resistant prostate cancer is powered by the lack of therapeutic approaches, including immunotherapy. Strategies to relieve immunosuppression mediated by myeloid-derived suppressor cells and tumour-associated macrophages (TAMs) might be effective in patients with these tumours. Interestingly, levels of TAM infiltration were predictive of malignancy grade, tumour size and disease recurrence and associated with extracapsular tumour extension in prostate cancer. Therefore, deep exploration of tumour-associated macrophages, study of their context-dependent and spatial heterogeneity, may uncover many aspects of this question.

Aim of the study: Our aim is to study macrophage spatial heterogeneity and intensity in prostate cancer tissues and their benign counterparts, and their association with pathological parameters.

Material and methods: 47 formalin-fixed paraffin-embedded prostate cancer samples were stained with CD163, CD68 and CD204 antibodies by immunohistochemistry and statistically processed to find associations of different populations of macrophages with clinical and pathological data. Web-based bioinformatics resources and tools were used to identify prostate cancer-associated macrophage profiles.

Results: Immunohistochemical analysis demonstrated higher density of CD68+ mononuclear phagocytes and CD163+ scavengers in prostate cancer adjacent stroma in comparison to benign prostate hyperplasia (both p<0,05). In previous experiments, we have shown that the number of CD204+ tumour-associated macrophages was also significantly higher in the malignant structure than in benign prostate hyperplasia (p<0,05). CD163+ M2 type macrophages were predominantly seen in the tumour core than in the tumour margin. Interestingly, the number of CD68+ and CD163+ macrophages was significantly higher in cases with positive lymph nodes. However, no significant correlation was found between pathological variables and selected proteins. Analysis of the TCGA prostate adenocarcinoma (PRAD) database also confirmed abundance and positive association of M2 type macrophages and negative or no association with macrophages with M1 and M0 phenotypes. An abundance of different macrophage and monocyte populations in the PRAD database was observed using the CIBERSORT deconvolution algorithm.

Discussion: Our results confirm a higher density of CD68+ macrophage population in prostate cancer and are in agreement with previous findings that M2-type, CD204+, and CD163+ macrophages are more characteristic of malignant structures. Our results were validated on the publicly available PRAD dataset. The absence of significant correlation with clinical and pathological parameters of our samples can be explained by the small sample size. A larger cohort and further multiplex immunostainings will uncover more macrophage populations and their prognostic/predictive potential in prostate cancer.

Functional Validation of PHKB and KIF24 as Molecular Targets of 7-Deazaadenine Derivatives PNH173, PNH192, EVH206, and EVH383

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7-Deazaadenine derivatives PNH173 and PNH192, along with their structural analogues EVH206 and EVH383, exhibit potent anticancer activity. To investigate their mechanisms of action, a genome-wide CRISPRi screen was conducted in K562 dCas9-KRAB cells, identifying phosphorylase kinase regulatory subunit beta (PHKB) and kinesin family member 24 (KIF24) as candidate molecular targets. Stable cell lines with altered expression of PHKB and KIF24 were generated to test the hypothesis that reduced expression of these targets would decrease cellular sensitivity to the compounds, providing functional validation of their involvement. While some changes in gene expression were achieved, the resulting shifts in cellular sensitivity to compound treatment were modest, suggesting partial target engagement.

To further assess target involvement, a gene silencing approach using siRNA was employed. Efficient knockdown was confirmed at both mRNA and protein levels. In siRNA-transfected cells, the compounds exhibited enhanced shifts in cytotoxicity, consistent with the hypothesis that reduced target levels decrease cellular responsiveness and supporting the role of PHKB and KIF24 in the mechanism of action of PNH173, PNH192, EVH206, and EVH383.

Ongoing studies are evaluating the effects of these compounds on PHKB-associated glycogen metabolism to connect metabolic alterations with cytotoxic outcomes. Together, these results integrate genome-wide screening, lentiviral and siRNA-based target modulation, and functional assays, providing mechanistic insight into how 7-Deazaadenine derivatives exert their anticancer effects and highlighting strategies to optimize target engagement in therapeutic development.

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Liquid Biopsy as a Diagnostic Tool for Early Detection of Pancreatic Ductal Adenocarcinoma

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Pancreatic ductal adenocarcinoma (PDAC) is one of the deadliest malignancies, typically diagnosed at a locally advanced or metastatic stage due to its long asymptomatic course. Chronic pancreatitis (CP), a major risk factor for PDAC, shares overlapping clinical and histopathological features, which further complicates differential diagnosis. Early detection of PDAC is therefore essential to improve patient outcomes.

This study investigates potential non-invasive biomarkers that could facilitate earlier diagnosis. We focused on leukocyte telomere length (LTL) and gut microbiome composition as candidate diagnostic tools. The aim was to assess whether these parameters can help to distinguish PDAC patients not only from healthy individuals but also from those affected by CP or type 2 diabetes mellitus (T2DM).

Altogether, 251 participants were enrolled and divided into four groups: PDAC (n = 41), CP (n = 58), T2DM (n = 100), and healthy controls (n = 52). Peripheral blood was analysed for LTL using the multiplex monochrome qPCR method. For microbiome analysis, stool samples were collected from a subset including CP (n = 13), PDAC (n = 12), and healthy controls (n = 7), and subjected to 16S rRNA sequencing.

Our findings demonstrated that LTL was significantly affected by age, sex, and smoking status, particularly in CP and T2DM groups. Following the LTL adjustment, PDAC patients exhibited longer telomeres compared with healthy individuals ($p = 0.012$), and especially relative to CP patients, who showed the shortest LTL ($p = 0.006$). Microbiome profiling indicated a trend toward reduced diversity and species richness in PDAC and CP compared with healthy controls; however, these differences did not reach statistical significance.

In conclusion, LTL appears to be a promising parameter for distinguishing PDAC not only from healthy individuals but also from CP. In contrast, microbiome profiling indicated non-significant trends toward altered diversity and community structure; further research on larger cohorts is required, as microbial composition did not differentiate PDAC from CP.

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INTEGRATING ISOTHERMAL AMPLIFICATION TECHNIQUES AND LNA-BASED AI-ASSISTED ELECTROCHEMICAL BIOASSAY FOR ANALYSIS OF KRAS G12V POINT MUTATION

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Point mutations in the KRAS gene have been identified in a variety of cancers, including colorectal (CRC), lung and pancreatic tumor. They are often associated with poorer prognosis and resistance to treatment1. The G12V mutation, which is present in around 8% of colorectal cancer patients, serves as a predictive biomarker for targeted T-cell receptor (TCR) therapy, which enables the development of TCRs that can recognize and target cells with this mutation[2,3].

Standard techniques for detecting point mutations in the KRAS gene typically include next-generation sequencing (NGS) and variations of PCR-based methods[4,5]. However, these methods are often costly and time consuming. They can be sensitive to reaction inhibitors and require expensive instruments as well as complex data analysis. Isothermal amplification techniques are an interesting option because they tend to be fast, simple, sensitive and inexpensive to equip[6].

In our study, we present a bioassay that enables rapid and reliable detection of the G12V mutation in the KRAS gene using isothermal rolling circle amplification (RCA) technique for probe-mediated selective amplification of either wild-type or G12V mutant targets[7]. This is then combined with electrochemical readout employing high-affinity LNA capture probes to bind the amplified product. Streptavidin-horseradish peroxidase polymer, which can bind biotin incorporated into the target DNA during the RCA reaction, can then catalyze an enzymatic reaction, which is detected amperometrically. Finally, we also present an artificial intelligence (AI) model using logistic regression classifier for human-free discrimination of the presence or absence of the G12V mutation. A major advantage of our study is that, in addition to synthetic targets, we also applied the bioassay to 7 cancer cell lines and 11 CRC patient samples, which is rarely demonstrated in electrochemical

studies. Our findings were entirely consistent with the previous NGS analysis of the patient samples.

Our proposed bioassay has good analytical sensitivity with the limit of detection of 61 pM, excellent selectivity towards the G12V mutation and the ability to detect < 1% of G12V sequence in the excess of wild-type sample, making it suitable for possible future liquid biopsy analysis.

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PKN3 kinase interaction with CARMIL1: A novel link in actin cytoskeleton regulation

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The potential of cancer cells to metastasise is dependent on their ability to escape from the original tissue, which is a crucial step in the progression of cancer. These processes are regulated by a multitude of proteins and signalling pathways. PKN3, an AGC-type Ser/Thr kinase, has emerged as a key regulator in the malignant progression of certain aggressive cancer types, particularly breast and prostate cancer. Interestingly, PKN3 is not physiologically expressed in most tissues. However, its expression has been detected in endothelial cells, osteoclasts, and trophoblast cells, where it exerts a positive impact on cell motility and functionality, likely through the regulation of actin cytoskeleton dynamics. Despite the well-documented effects of PKN3 on cancer progression, the underlying mechanism remains elusive. In the present study, we identified CARMIL1 (CP, Arp2/3, myosin-I linker 1) as a potential substrate of PKN3. CARMIL1 is known to regulate actin cytoskeleton dynamics and cell motility by interacting with actin capping protein, thereby diminishing its inhibitory effect on actin polymerisation. Importantly, our findings confirmed a direct interaction between PKN3 and CARMIL1. Furthermore, our recent results suggest an intriguing interplay between the PKN3–CARMIL1 protein complex and active RhoA, indicating a novel crosstalk within these signalling axes. We propose that CARMIL1 may serve as an unknown mediator of PKN3's effects on cell adhesion and motility. Further elucidation of this molecular interaction could provide new insights into the underlying mechanisms of cancer progression and unveil potential therapeutic targets for inhibiting metastasis.

Phospholipidation as a Novel Post-Translational Modification in Cancer Cells

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Post-translational modifications (PTMs) are essential regulators of protein function, localization, and stability in cancer biology. Recent studies suggest that proteins may be post-translationally modified by phosphoinositides. This newly proposed PTM, known as phospholipidation, is conceptually related to lipid-based modifications such as palmitoylation, but its biological role remains largely unexplored. We hypothesize that phospholipidation occurs preferentially on nuclear proteins and that its dysregulation contributes to tumor progression. The aim of this project is to identify proteins selectively modified by phospholipidation, define their consensus sequence motifs, and uncover the enzyme responsible for this modification.

In this study, we optimized pull-down assays using nuclear fractions to identify candidate proteins associated with PI(4,5)P_2-linked complexes in cancer cells. Nuclear and cytoplasmic extracts from multiple cancer cell lines (U2OS, U251, and Mia PaCa) were incubated with the phospholipase C delta (PLCδ) pleckstrin homology (PH) domain, a specific PI(4,5)P_2-interacting domain, used to capture PI(4,5)P_2-associated proteins. Obtained samples were analyzed by Western blotting with a PI(4,5)P_2-specific antibody, which showed higher signals in nuclear fractions compared with cytoplasmic fractions, indicating that PI(4,5)P_2-associated proteins are enriched in the nucleus.

Lipids from the pull-down material were further extracted and analyzed by thin-layer chromatography (TLC). Staining with the PLCδ-PH domain and an anti-GST antibody confirmed the presence of PI(4,5)P_2 in nuclear protein complexes

These results provide initial evidence that subsets of nuclear proteins in glioblastoma and other cancer cells are linked with PI(4,5)P_2 complexes. Ongoing work will thoroughly characterize these protein–lipid complexes and determine whether PI(4,5)P_2 binding occurs via covalent linkage, which would confirm phospholipidation as a post-translational modification. Additionally, we aim to identify the consensus motif for this modification and determine the enzyme responsible. These findings open new avenues for investigating how nuclear phosphoinositide metabolism influences carcinogenesis and suggest that phospholipidation may represent an overlooked regulatory mechanism in cancer.

Impact of adenocarcinoma subtypes on clinical outcomes

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Lung adenocarcinoma is categorized into subtypes defined by morphological characteristics with underlying molecular alterations. As the present classification framework has only been implemented recently and is of limited practice, comprehensive large-scale data validating its prognostic and clinical implications are still lacking. We present retrospective study using high-quality Swedish cancer registry covering years 2005-2021.

The clinical and demographic data on patients with specified adenocarcinoma subtype were obtained from the Swedish cancer registry, accounting for 2103 patients in the study period. The clinical outcomes were examined by survival analysis, stratified by sex and stage at diagnosis.

The specific subtypes differed in predominant stage at diagnosis, ranging from early stages (T1: acinar cell, lepidic; T1-T2: papillary, colloid) to advanced stages (T4: invasive mucinous, adenosquamous). Female sex was associated with better overall survival, in particular for early stage cancers. Female 5-year survival decreased in order: acinar (90%), lepidic (59%), invasive mucinous and papillary (both 50%), colloid (36%) and adenosquamous carcinoma (21%). Male 5-year survival for lepidic (46%) and colloid subtypes (22%) were significantly lower than female survival. The effect of cancer staging on prognosis differed across specific subtypes. In T1, 5-year overall survival for was 80% for lepidic, 64% for papillary, 56% for colloid and 46 % for adenosquamous carcinoma. In T4 patients, 1-year survival was 54% for lepidic and 56% for papillary types, compared with 37% in colloid and 26% in adenosquamous carcinoma.

In summary, we show that the subtype of adenocarcinoma has a strong effect on patient prognosis. Patients diagnosed with distinct subtypes present with different staging at diagnosis and effect of cancer advancement modulates clinical outcomes differently across individual subtypes. We thus validate clinical relevance of adenocarcinoma subclassification on one of the largest cohorts up-to-date, arguing for wider adoption of subtype classification in clinical settings.

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Case Studies in High-Resolution Cancer Analysis from GeneCore

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Single-cell and spatial transcriptomics provide unprecedented resolution for investigating the complex cellular ecosystems of tumors, which are a primary driver of disease progression and therapeutic failure. These technologies can reveal novel cell states, identify rare populations, and map the intricate communication networks that dictate tumor behavior and response to treatment.

This poster presents a range of cancer research projects from GeneCore, an academic facility specializing in advanced transcriptomics, that leverage these cutting-edge tools to address diverse biological questions. The projects span multiple cancer models, from investigating how cancer cell metabolism remodels the immune landscape in murine mammary tumors, to deciphering cellular adaptations to nucleotide synthesis blockade in the bone marrow niche and in lung carcinoma models. To enhance accessibility, we also demonstrate a cost-saving multiplexing strategy that combines disparate projects into a single experimental run. Complementing these single-cell approaches, a spatial transcriptomics analysis of tumors with heterogeneous MHC class I expression illustrates how gene expression can be mapped directly onto tissue architecture.

Together, these case studies demonstrate the power of single-cell and spatial technologies to provide deep mechanistic insights into cancer metabolism, tumor-stroma

interactions, and the immune response. The robust and cost-effective service portfolio offered at GeneCore empowers researchers to pursue ambitious projects that were previously intractable, thereby accelerating the pace of cancer discovery.

Significance of somatic variability in KRAS and TP53 for prediction of platinum sensitivity and prognosis in patients with epithelial ovarian cancer

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Epithelial ovarian cancer (EOC) is seriously fatal gynecological malignancy, with especially high mortality. Understanding the genetic mutations driving this cancer, such as those in TP53 and KRAS, is crucial for developing effective treatments. Concerning the dismal prognosis of chemoresistant patients with EOC, we aimed to follow up the findings of a previous whole-exome sequencing study using an orthogonal Sanger sequencing on the same patients and a separate set of 127 EOC patients (N = 177, all fresh frozen tumor samples). We focused on TP53 as a frequently mutated gene relevant for chemosensitivity, included KRAS as an additional therapeutically relevant target. We complemented somatic mutation screening with an assessment of both genes' transcript levels in tumor RNA and protein levels in selected cases. We compared the results with sensitivity to EOC therapy and patient survival for evaluation of the prognostic value of these biomarkers.

All variants in TP53 and KRAS detected by exome sequencing were confirmed. KRAS mutated patients had significantly more frequent FIGO stages I or II ($p = 0.002$) and other than high-grade serous tumor subtypes (nonHGSCs) ($p < 0.001$), which was connected with lower KRAS transcript levels ($p = 0.004$). Patients with nonHGSC subtypes had less frequent TP53 mutations ($p = 0.002$). Carriers of TP53 variants disrupting the DNA binding loop had significantly longer platinum-free intervals than the rest ($p = 0.037$). Tumors bearing nonsense, frameshift, or splice site TP53 variants had a significantly lower TP53 transcript level, while those with missense variants had significantly higher levels than wild types ($p < 0.001$). The normalized intratumoral TP53 and KRAS transcript levels were correlated, and patients with co-mutated genes had poorer overall survival than others ($p = 0.015$). Protein levels of both genes significantly correlated with their respective transcripts ($p = 0.028$ and $p = 0.001$, respectively).

Our study points to KRAS as a target for future therapy of nonHGSCs and reveals TP53 variants in the DNA binding loop as prognostic biomarkers of EOC patients.

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PRECLINICAL ANALYSIS OF FAP-TARGETING DUAL-MODALITY TRACERS FOR PET/CT AND NEAR-INFRARED FLUORESCENCE IMAGING

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PURPOSE

Fibroblast activation protein (FAP) is a surface glycoprotein overexpressed by cancer-activated fibroblasts present in various types, making it a promising target for imaging1. Dual-labelled FAP inhibitors hold strong potential for both nuclear medicine and surgical-guidance imaging2. This work focuses on the

in vivo characterization of several novel dimeric dual-modality imaging agents targeting FAP, based on Fusarinine C scaffold labelled with gallium-67/68.

METHODS

In vivo characterization of tested imaging agents targeting FAP began with ex vivo biodistribution studies in healthy mice. Compound stability was further tested in murine serum and urine. Additionally, the ex vivo biodistribution and multimodal imaging (PET/CT, SPECT/CT and near-infrared fluorescence-NIRF) were performed in FAP-positive and FAP-negative tumor-bearing mice. The results were compared to the clinically established [67Ga]Ga- or [68Ga]Ga-FAPi-46.

RESULTS

In healthy mice, [68Ga]Ga-s775z-FFAPi showed the most favorable pharmacokinetics, with rapid blood clearance and minimal non-target uptake. No *in vivo* degradation was detected in serum nor urine for [68Ga]Ga-s775z-FFAPi, but minor instability was noted for [68Ga]Ga-IRDye-FFAPi. All tested compounds demonstrated specific accumulation in FAP-positive tumors and high tumor retention up to 1-day post-injection. PET/CT imaging confirmed ex vivo biodistribution findings, with [68Ga]Ga-s775z-FFAPi yielding image quality comparable to [68Ga]Ga-FAPi-46. SPECT/CT and NIRF imaging with gallium-67 labelled compounds confirmed tumor uptake in FAP-positive tumors up to 1 day post injection by both modalities for all tested candidates. However, at 2 days post injection, the tumor visualization was achieved through NIRF imaging only.

CONCLUSION

Fusarinine C–based dual-modality FAP tracers showed excellent tumor specificity, favorable pharmacokinetics, and multimodal imaging performance *in vivo*. Among them, [68Ga]Ga-s775z-FFAPi showed the most promising profile, comparable to the clinically used [68Ga]Ga-FAPi-46. These results highlight the strong potential of dual-labelled FAP inhibitors as versatile tools for both nuclear and fluorescence-guided cancer imaging.

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Comparative Analysis of Protein Extraction from Saliva and GARGtest

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Saliva is a complex biofluid containing proteins, enzymes, mucopolysaccharides, electrolytes, and antimicrobial peptides. Its easy, non-invasive collection makes it an attractive source of biomarkers in oncology, neurodegeneration, infectious diseases, and oral health. However, the variability in collection methods may influence protein yield and composition, affecting downstream proteomic analysis.

We compared protein extraction from samples obtained by conventional non-stimulated saliva collection with those obtained using the GARGtest, a method that incorporates stabilizing agents to preserve proteins through gargling. Samples from healthy volunteers were processed for protein extraction, quantified, and analyzed by liquid chromatography–mass spectrometry (LC–MS).

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Targeting fibroblast activation protein (FAP) in the tumor microenvironment with novel alpha-ketoamide inhibitors for imaging and therapy

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Fibroblast activation protein (FAP) is a serine protease abundantly expressed by cancer-associated fibroblasts and, in some tumors,

by malignant cells. Although its biological role in cancer progression remains incompletely understood, its restricted expression in normal tissues and overexpression across a wide spectrum of tumors, including brain tumors, make it an attractive target for cancer imaging and therapy.

We are developing a novel class of FAP-targeting probes based on alpha-ketoamide inhibitors. *In vitro*, both fluorescently labeled and 99mTc labelled derivatives exhibited FAP-specific binding. *In vivo*, 99mTc-labelled compounds accumulated selectively in FAP-positive tumors with retention for several hours. Ongoing work includes evaluation of multimeric constructs to enhance affinity and tumor retention, as well as stability testing.

These studies demonstrate the potential of alpha-ketoamide-based probes for targeting FAP in the tumor microenvironment. Further *in vitro*, *in vivo* and stability data will be presented to support the development of FAP-directed strategies for cancer imaging and therapy.

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Circulating tumor cells-derived xenograft as a model of castration-resistant prostate cancer

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Prostate cancer remains one of the most common cancers in men worldwide. New models are needed to facilitate the study of the metastatic stage of this disease. The present study describes a unique model of advanced-stage prostate cancer derived from circulating tumor cells from a patient with metastatic castration-resistant prostate cancer (mCRPC). A detailed immunohistochemical analysis of the developed CTCs-derived xenograft and matched needle biopsy from the primary tumor revealed strong expression of the neuroendocrine marker synaptophysin, simultaneously with the expression of luminal markers (AR, PSA, NKX 3.1), indicating that the developed xenograft represents amphicrine prostate cancer. A multiparametric panel of selected surface markers was examined using spectral flow cytometry to investigate the phenotype of the established xenograft. The sensitivity of our model to surgical castration or to the AR inhibitor enzalutamide was verified *in vivo*. In addition, given the fact that the xenograft is positive for the surface marker Trop2, the effect of an antibody-drug conjugate, sacituzumab govitecan, was examined on tumor growth *in vivo*. In conclusion, our CTC-derived xenograft from a patient with mCRPC represents a unique model of advanced prostate cancer, which can be used for further studies of the metastatic stage of the disease.

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Characterization of circular RNAs as potential biomarkers for early detection of pancreatic ductal adenocarcinoma

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Circular RNAs (circRNAs), a novel class of non-coding RNAs, have emerged as promising biomarkers in cancer biology due to their structural stability, high abundance, and tissue- and developmental stage-specific expression patterns. Unlike linear RNAs, circRNAs form covalently closed-loop structures lacking free 5'-3' end polarity and a poly-A tail, rendering them resistant to exonuclease-mediated degradation. Increasing evidence from high-throughput transcriptomic analyses has revealed dysregulated circRNA expression in pancreatic ductal adenocarcinoma (PDAC) tissues and plasma, implicating their role in tumorigenesis, immune evasion, and therapy resistance.

A major clinical challenge in PDAC is that it is often diagnosed at advanced stages due to the absence of specific early symptoms and its rapid progression. Early detection could significantly reduce mortality and improve patient outcomes, underscoring the urgent need for reliable biomarkers. Current diagnostic tools, relying mainly on histopathology, cancer staging, and imaging, lack sensitivity and

specificity for early detection of PDAC.

Based on the available literature, this study is designed to investigate the diagnostic potential of two circRNA candidates, circ-RNF13 and circ-HIPK3, in PDAC. Both circRNAs have been implicated in cancer biology, but their roles in PDAC remain incompletely elucidated. To address this, we will quantitatively assess their expression levels in paired tumor and adjacent normal tissues from PDAC patients using digital PCR. Validation will then be extended to an independent cohort including tissue samples from both PDAC patients and healthy controls. The experimental work is currently ongoing, and the results will be presented and discussed at the Czech Annual Cancer Research meeting, 2025.

Keywords:

Pancreatic ductal adenocarcinoma; circular RNA; early detection; digital PCR; diagnostic biomarker.

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Testing of the Laser-driven protons therapy potential on 3D models of human colorectal cancer

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New technologies are helping us to find more effective solutions for new therapeutic procedures in the treatment of the increasingly frequent occurrence of patients with cancer. Radiation therapy (accelerated electrons or proton, photon, neutron new opportunities) is in growing interest for its possibilities to directly affect the tumor cells but also to promote valuable and specific immune response. Advances in these new approaches include the EU Infrastructure ELI Beamlines User Facility (Dolní Břežany, CZ) novel technologies which explore application of advanced laser-driven accelerators for anticancer treatments as an alternative to traditional accelerator types. The laser-driven proton beams are produced with specific temporal and dose-rate characteristics (very high dose-rate beams pulsed in nanosecond delivery time) that may potentially offer novel and increased treatment benefits including elicitation of immune responses. The laser acceleration system is powered by the L3 HAPLS petawatt laser, integrated with the ELIMAIA (ELI Multidisciplinary Applications of laser-Ion Acceleration) laser-plasma accelerator and the ELIMED (ELI MEDical application) beam transport and dosimetry line, enabling multi-shot of laser-driven proton irradiation. In our experiments, we irradiated a three-dimensional cell model (spheroids) of human colorectal cancer. Spheroids have a more complex structure than cell monolayers and are more suitable for studying the tumor microenvironment responses. In the study, we monitored changes in 6-day-old spheroids 24 hours after irradiation using the Live-Dead Assay on confocal microscopy. The results showed damage to the nuclei after irradiation and the beginning of spheroid disintegration. New methods of generating protons have the potential to be used in very focused and healthy tissue sparing, enhancing the radiotherapy results in the treatment of cancer, including the induction immunologically useful damage of the tumor cells. Immunogenic cell death and stress molecules expression are under evaluation.

Keywords: three-dimensional cell models, human colorectal carcinoma, laser-driven proton

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Investigating the role of phase separation in p130Cas protein signallisation

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The p130Cas protein is an important adaptor, mechanosensor, and mechanotransducer of cell adhesive structures such as focal adhesions and podosomes. Despite lacking its own enzymatic activity, p130Cas forms a key node for multiple signalling pathways and thus exerts a profound effect on overall cellular behaviour including proliferation, differentiation and cell motility. Its intrinsically disordered substrate domain is essential for p130Cas multivalency, since it allows to undergo phase separation and form various multiprotein complexes. Recent studies indicate the specific involvement of different parts of substrate domain in different phase

separation processes affecting integrin signalisations dynamics. This specific engagement of either YxxP motifs or regulatory loops is dependent on presence of mechanical tension applied on p130Cas protein and subsequent YxxP motifs phosphorylation. However, the precise mechanism remains elusive.

In our research, we aim to determine how the spatial arrangement of the substrate domain responds to mechanical stimuli, modulates the accessibility of YxxP motifs for kinases through smoothening of regulatory loops, and influences phase separation processes. Our results with a truncated mutant of p130Cas, wherein YxxP motifs are accessible due to deletions of regulatory loops, revealed elevated tyrosine phosphorylation within the substrate domain correlating with an increased binding capacity of Crk and c-Abl proteins – potential phase separation partners. Findings from this project may provide insight into the regulation of the processes behind cancer cell metastasis, which could be used to improve the targeting of migrastatic drugs.

Optimization of Novel Diagnostic Solutions for the Detection of Somatic Variants in BCR::ABL1

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Abstract: Chronic myeloid leukemia (CML) is a clonal myeloproliferative disorder of hematopoietic tissue that arises from the malignant transformation of bone marrow stem cells, followed by their clonal expansion and accumulation in the peripheral blood. It is caused by a reciprocal translocation, known as the Philadelphia chromosome. CML can be effectively treated with tyrosine kinase inhibitors (TKIs). However, secondary molecular alterations in the kinase domain of ABL1 that confer resistance to TKIs may emerge during disease progression. These secondary changes, typically non-synonymous single nucleotide variants (SNVs), occur in up to 90% of patients. Our aim was to detect these aberrations using a user-friendly approach based on the reliable, time-tested fastGEN technology. Accurate detection of ABL1 variants can support CML diagnostics and guide treatment adjustments.

Methods: Twenty-six RNA samples containing the BCR::ABL1 fusion transcript were used for cDNA synthesis with two reverse transcriptase (RT) approaches: SuperScript IV (SSIV) and/or the High-Capacity RNA-to-cDNA kit (HC). We implemented a qPCR-based method for BCR::ABL1 pre-amplification and ABL1 genotyping using the fastGEN method, followed by next-generation sequencing (NGS) on the SurfSeq Genemind 5000 platform. Sequencing data were processed using a dedicated workflow in the GENOVESA software. Sanger sequencing of fresh samples was used as the gold standard for comparison.

Results: The SSIV-based approach, using a mixture of random primers and oligo(dT) primers, produced cDNA of higher molecular weight and yielded pre-amplification products with greater intensity compared to HC-based approach containing only random primers. For fresh RNA samples (<2 years), both RT approaches achieved 100% sensitivity and 100% specificity in NGS assay compared with Sanger sequencing. For long-term stored samples (>7 years), we observed a decrease in analysability of NGS assay, which could be mitigated by the SSIV protocol. NGS assay allowed the detection of variants with VAF below 15% that are hard to detect using Sanger sequencing.

Conclusion: We developed and validated a novel kit for genotyping the kinase domain of BCR::ABL1. Use of SSIV RT with a mixture of oligo(dT) and random primers is currently recommended. It remains to be determined whether adding oligo(dT) primers improves efficiency with other RT enzymes. Long term storage of samples can impair the quality of results. Incorporating a validated RT step into the fastGEN BCR::ABL1 procedure is advisable. The developed methods can be readily implemented in clinical practice, particularly as a replacement for Sanger sequencing-based assays.

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^{161}Tb-Pertuzumab as a Theragnostic Antibody for HER2-Positive Breast Cancer

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Introduction

Breast cancer remains one of the most common malignancies in women worldwide. HER2 overexpression, present in about 15–20% of breast cancers, is associated with aggressive disease and poor clinical outcomes. Pertuzumab, an FDA-approved monoclonal antibody, targets HER2 by binding to a distinct epitope of the extracellular domain. Given its established clinical utility, pertuzumab provides an attractive platform for radiolabeling with therapeutic radionuclides.

Terbium-161 is a promising isotope for theragnostic use. In addition to γ-radiation suitable for SPECT imaging, 161Tb emits β– particles and abundant Auger electrons, offering superior therapeutic potential compared to conventional β– emitters such as 177Lu, particularly for treating small lesions or disseminated disease. The aim of this study was to evaluate 161Tb-pertuzumab in dual model of HER2-positive and HER2-negative tumour-bearing mice, focusing on tumour uptake, biodistribution, and imaging performance.

Methods

Pertuzumab was radiolabelled with 161Tb using standard procedures. Radiochemical purity exceeded 95%, ensuring antibody integrity. Female SCID mice were xenografted with HER2-positive SKOV-3 or HER2-negative MDA-MB-231 tumours. Following intravenous administration of 161Tb-pertuzumab, animals underwent SPECT/CT imaging and biodistribution analysis at 1 h, 1-, 2-, 3-, and 7-days post injection. Organs and tumours were collected, weighed, and measured in a gamma counter to determine % injected dose per gram (%ID/g).

Results

In SKOV-3 tumours, uptake was high and sustained, peaking at 21.33 %ID/g on day 2 and remaining 11.67 %ID/g at day 7. In contrast, MDA-MB-231 tumours showed minimal accumulation, with a maximum of 4.86 %ID/g at day 2, confirming HER2 specificity.

Blood activity was initially high (49.06 %ID/g at 1 h) but decreased gradually over time. Clearance occurred mainly via the hepatobiliary pathway, consistent with antibody pharmacokinetics. Tumor-to-blood ratios improved steadily, from 0.07 at 1 h to 2.88 at day 7, enabling excellent imaging contrast at later time points.

SPECT/CT imaging confirmed these findings: SKOV-3 tumours were clearly visualized from day 1, with contrast improving through day 7. HER2-negative tumours remained faint or undetectable, highlighting the specificity of 161Tb-pertuzumab.

Discussion

The results demonstrate that 161Tb-pertuzumab selectively targets HER2-positive tumours, with high and persistent uptake and minimal accumulation in HER2-negative xenografts. The long circulation half-life resulted in elevated blood levels at early time points; however, gradual clearance and persistent tumour retention led to favourable tumour-to-blood ratios by later stages.

The emission properties of 161Tb provide unique advantages. In addition to its SPECT-compatible γ-emission, the β– particles and short-range Auger/conversion electrons may enhance therapeutic efficacy, particularly in small tumour deposits. Compared to 177Lu, 161Tb offers the potential for greater therapeutic impact while retaining imaging capability.

These findings align with the growing body of evidence supporting 161Tb as a promising theragnostic radionuclide. The strong HER2-specific uptake of 161Tb-pertuzumab provides a compelling rationale for further studies, including dosimetry, therapy experiments, and potential translation into clinical settings.

Conclusion

161Tb-pertuzumab demonstrated high, specific, and sustained uptake in HER2-positive tumours with favourable biodistribution and excellent imaging properties. These results support the continued development of 161Tb-pertuzumab as a theragnostic agent for HER2-positive breast cancer.

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Enhancing Chemotherapeutic Efficacy via PD-L1-Targeted Ferritin-based Nanocarrier Functionalized with Bioactive Peptides

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Introduction:

Cancer remains the second leading cause of mortality in developed nations, underscoring the need for continued innovation in therapeutic strategies. Despite recent advancements in oncology, challenges persist in enhancing treatment efficacy, particularly due to insufficient drug accumulation and limited penetration within solid tumor tissues. One promising approach to addressing these barriers might be a combination of treatments.

For this purpose, we investigated a recombinant human ferritin-based nanocarrier, designated THE-10, engineered to improve the delivery and therapeutic effects of co-administered drugs. THE-10 was functionalized with 24 copies of two genetically fused bioactive peptides (BAPs), specifically designed to enhance the effects of anti-cancer therapies. These peptides target programmed death-ligand 1 (PD-L1) and integrins αVβ3/αVβ5 in conjunction with Neuropilin-1, thereby integrating immune checkpoint inhibition with improved tumor permeability and drug internalization. Additionally, the nanocarrier was modified through PASylation - a polypeptide sequence enriched in proline (P), alanine (A), serine (S), and glutamic acid (E) residues (PASE) - to prolong systemic circulation and facilitate better tumor accumulation.

Methods:

To evaluate the synergistic potential of THE-10, we employed a syngeneic Balb/c murine model bearing subcutaneous colorectal adenocarcinoma (CT26). Irinotecan, a clinically approved chemotherapeutic agent for colorectal cancer, was administered as a monotherapy or in combination with THE-10. Treatments were delivered four times over two weeks, with THE-10 administered 24 hours before Irinotecan to allow sufficient nanocarrier accumulation based on its extended half-life in circulation. Tumor progression and body weight of animals were monitored throughout the study. To further investigate immunological responses, three additional mice treated exclusively with THE-10 were sacrificed one day after the final administration. Tumor tissues were analyzed via confocal microscopy and immunohistochemistry to assess immune cell infiltration.

Results:

Monotherapy with either Irinotecan or THE-10 yielded modest anti-tumor effects. However, their co-administration resulted in a statistically significant enhancement of their therapeutic efficacy. Immunohistochemical and confocal analyses revealed increased infiltration of $CD8^{+}$ T cells within the tumor microenvironment following treatment with THE-10, suggesting an immunomodulatory role of the nanocarrier.

Conclusion:

These findings demonstrate that co-administration of the PD-L1-targeting, peptide-functionalized nanocarrier THE-10 significantly enhances the efficacy of conventional chemotherapy. The observed improvement is likely attributable to increased tumor permeability and immune cell infiltration, facilitated by the multifunctional design of THE-10. This approach offers a promising avenue for augmenting current cancer treatment modalities.

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Shining a Light on Viral Detection: A Comparative Study of Electrochemical and Electrochemiluminescence Techniques for Human Cytomegalovirus

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Human cytomegalovirus (HCMV) is a widespread herpesvirus with a prevalence of approximately 70 %. After primary infection, it establishes lifelong latency in the host organism and can reactivate under conditions of immunosuppression. In recent years, it has attracted increasing attention due to its potential oncomodulatory properties. Several studies have reported the presence of HCMV in glioblastoma samples; therefore, reliable detection of HCMV in tumor tissues is of growing importance for the development of targeted therapeutic approaches.

Currently, detection of HCMV in clinical samples relies primarily on immunohistochemistry, quantitative PCR (qPCR), and next-generation sequencing, all requiring expensive instrumentation and relatively time-consuming workflows. As an alternative, isothermal amplification techniques (IAT), such as loop-mediated isothermal amplification (LAMP) and recombinase-polymerase amplification (RPA), have recently emerged as powerful methods. These techniques enable a fast and straightforward workflow by eliminating the need for thermal cycling. When coupled with electrochemical (EC) or electrochemiluminescence (ECL) detection methods, they offer low-cost and rapid diagnostics.

EC detection is widely used in biosensor development due to its high sensitivity, specificity, affordability, and rapid response. ECL presents a specialized subclass of EC, in which electrochemically

generated species undergo light-emitting reactions, enabling extremely low limits of detection.

In this work, we developed and compared PCR and IATs combined with EC and ECL detection for HCMV analysis. Multiple assay parameters were optimized and compared to achieve the lowest possible detection limits. Importantly, the EC bioassay successfully distinguished between HCMV-positive and HCMV-negative cancer cell lines. These results demonstrate the potential of EC-based bioassays as sensitive, cost-effective, and rapid tools for HCMV detection in clinical tumor tissue samples.

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Advanced Stony Brook Taxanes as a Promising Strategy Against Resistant Ovarian Cancer

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In the field of cancer pharmacotherapy, taxanes play a crucial role as frontline agents in the management of ovarian carcinoma. Despite their widespread use and initial effectiveness, their therapeutic success is frequently hindered by the emergence of multidrug resistance, which significantly reduces their efficacy over time. This research focused on evaluating a group of novel taxanes, referred to as Stony Brook taxanes (SB-Ts), in order to investigate their potential anticancer properties specifically against ovarian carcinoma.

The viability of various ovarian carcinoma cell lines, including NCI-ADR/RES, SKOV-3, and its resistant counterpart SKOV-3/R, was evaluated using the CellTiter Blue Cell Viability Assay following 72-hour incubation with paclitaxel (PTX), as well as with selected second-generation (SB-T-1214, SB-T-1216) and third-generation (SB-T-121402, SB-T-121605, SB-T-121606) Stony Brook taxanes. To investigate the influence of these taxanes on cancer cell migration, the xCELLigence real-time cell analysis system was utilized, allowing continuous monitoring of migratory behavior. Additionally, cell cycle distribution was examined after 24 hours of treatment using the BD Cycletest Plus DNA Kit, providing insights into the mechanisms underlying the antiproliferative effects of the tested agents. To further explore their antitumor activity *in vivo*, xenograft models were generated by subcutaneously injecting the cancer cells into immunodeficient mice. Once tumors had developed, the animals were treated twice per week with either PTX, SB-T compounds, or a combination of both therapies.

Initial *in vitro* experiments focused on assessing the cytotoxic potential of both second- and third-generation Stony Brook taxanes (SB-Ts) in ovarian carcinoma cell lines exhibiting sensitivity or resistance to paclitaxel. Among the tested compounds, SB-T-121605 and SB-T-121606 emerged as particularly effective, exhibiting strong antiproliferative activity. These two derivatives were subsequently selected for *in vivo* evaluation using xenograft models established in immunocompromised mice. In this context, both agents demonstrated superior tumor-suppressive effects in comparison to standard paclitaxel treatment.

Building upon these findings, further research was directed toward the development of biotin-conjugated SB-T derivatives, with the aim of improving drug delivery specificity to tumor tissues and minimizing systemic toxicity. When tested *in vitro*, these biotinylated compounds showed enhanced cytotoxic efficacy against ovarian carcinoma cells compared to paclitaxel alone, indicating promise for targeted therapeutic applications.

The study confirmed the strong anticancer activity of third-generation SB-T compounds in preclinical ovarian carcinoma models, although a dose-dependent toxicity was observed at concentrations equal to or exceeding 5 mg/kg. Current efforts are centered on the continued evaluation of tumor-targeted biotin-conjugates of SB-Ts to further optimize their therapeutic index and clinical applicability.

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Patterns of Chromosomal Instability and Epigenetic Alterations in Colorectal Cancer Progression: From High-Grade Dysplasia to Liver Metastases

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Introduction: Chromosomal instability (CIN) and aberrant DNA methylation are implicated in colorectal cancer (CRC) development. Tracking these alterations from precancerous lesions to tumors and metastases may help identify biomarkers of CRC initiation and progression.

Study Overview: Fresh-frozen tissue from 44 patients with either high-grade colorectal dysplasia (HGA) or advanced metastatic CRC was analyzed. CIN was assessed using low-coverage whole-genome sequencing or comparative genomic hybridization, and a subset also underwent whole-methylome profiling.

Findings: CIN was present in 85% of HGA patients, ranging from focal CNVs in MALAT1 (46%) to recurrent alterations on chromosomes 11, 13, and 20, with the proto-oncogene PTK6 the most frequently amplified (61%). Additional common CNVs in HGA tissues affected genes linked to intestinal differentiation, ERBB, PI3K/AKT, TP53, SMAD/TGF-β, and DNA repair pathways. Metachronous metastases showed significantly more DNA amplifications than primary tumors, while the proto-oncogene DEK was consistently amplified in metastases but absent in primaries, irrespective of chronicity. The degree of CIN did not correlate with patient survival. Global methylation profiling distinguished HGA from both normal and tumor tissues, whereas primary tumors and metastases were nearly identical, differing at only four CpGs.

Conclusion: PTK6 amplification may represent an early biomarker detectable at the HGA stage, while DEK amplification appears important to metastatic progression and a potential therapeutic target. DNA methylation can differentiate HGA from cancer but not cancer from metastasis.

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Comprehensive transcriptome and miRNome profiling in metachronous colorectal liver metastasis: A comprehensive Transcriptomic and miRNomic characterization

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Colorectal cancer is the second most deadly cancer in the word. Metachronous colorectal liver metastasis (mCLM) represents distinct clinical and molecular characteristics compared to synchronous metastases; however, the mechanism underlying its appearance remains unknown. This study integrates transcriptomic and miRNomic analyses to uncover molecular pathways and regulatory networks driving mCLM progression.

Differential expression analysis of RNA-seq data on 36 mCLM and adjacent non-malignant liver tissue pairs was used to identify deregulated genes and miRNAs. Gene set enrichment analysis and consensus molecular subtypes (CMS) classification helped to explore dysregulated pathways and tumor subtypes. miRNA-mRNA interactions were investigated through correlation analysis, with prognostic relevance assessed by survival analysis and validated using the multiMiR database.

The transcriptomic and miRNomic

analysis revealed 1809 upregulated and 1639 downregulated genes, and 108 upregulated and 92 downregulated miRNAs in mCLM compared to the adjacent non-malignant liver. The genes were associated with epithelial-mesenchymal transition, cell-cycle, and cell signaling pathways (e.g. PMEPA1, ITGA2, CDK1, CCND2, and S100A6).The CMS classification showed 47% CMS2 (canonical pathway) and 22% CMS4 (mesenchymal subtype), with distinct mutations (e.g., BRAF, KRAS, APC, TP53) and copy number variations. Tumor samples were stratified by their KRAS mutation status. Predicted miRNA-mRNA interactions included PEA15 with hsa-miR-320b/c, TEX2/CTSO with hsa-miR-103a-3p, and PHLDA3 with hsa-miR-1304, along with TEX2, CTSO, EID1, FAM217B, VGLL3, and ISLR with several miRNAs. Together, these results highlight the main regulatory mechanisms in mCLM progression.

This study provides an insight into the molecular landscape of mCLM and reveals dysregulated pathways, novel miRNA-mRNA networks and potential prognostic implications. This work may guide future targeted therapies and improve patient lives.

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Genomic Profiling of Metachronous Colorectal Cancer Liver Metastases Reveals Prognostic and Therapeutic Insights

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Colorectal cancer is a leading cause of cancer-related mortality, with the liver being the most common site of metastasis. To better understand the genetic landscape of the liver metastases, we performed whole exome sequencing on a series of metachronous colorectal cancer liver metastases (mCLM) and matched non-malignant liver tissues. Our goal was to investigate the genomic profile and identify genetic markers associated with prognosis and treatment response. We also aimed to classify these tumors using the established consensus molecular subtypes (CMS) to correlate them with genomic features.

We analyzed DNA samples from 41 paired mCLM and non-malignant liver tissues using whole exome sequencing. We assessed somatic and germline genetic variability, copy number variations, and mutational signatures to determine their associations with patient relapse-free survival (RFS) and overall survival (OS). Additionally, we performed RNA sequencing to classify tumors into CMSs.

Our analysis revealed that commonly mutated genes in mCLM were TP53, APC, KRAS, and PIK3CA, along with several passenger genes. We discovered that somatic alterations in homologous recombination repair, Notch, and Hedgehog pathways were associated with significantly prolonged RFS. Conversely, alterations in the MYC pathway and the KRAS-12D variant were linked to poor survival outcomes. Patients with genetic changes in the JAK-STAT pathway showed a prognostic benefit for OS, while alterations in VIPR2 were associated with shorter OS and MUC16 with prolonged OS. Interestingly, common markers such as tumor mutation burden, mismatch repair deficiency, and microsatellite instability did not show prognostic value in this cohort. Additionaly, about 47% of samples were classified as CMS2 (canonical pathway), a subtype generally linked to a better prognosis and a favorable response to standard therapies, while 22% were CMS4 (mesenchymal), a subtype known for its association with poor survival outcomes, extensive stromal infiltration, and resistance to standard treatment.

Our findings underscore the importance of in-depth molecular profiling for personalizing the treatment of mCLM. The identified genetic alterations and pathways provide valuable prognostic information that could help stratify patients for existing targeted therapeutic regimens. The results further suggest that distinguishing metachronous from synchronous liver metastasis scenarios might be beneficial for precision medicine approaches. More analyses, including the study of non-coding RNA and CMSs, will follow.

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Age-related clonal hematopoiesis in healthy blood donors

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Introduction: Clonal Hematopoiesis of Indeterminate Potential (CHIP) is an age-related condition characterized by the accumulation of somatic mutations in hematopoietic cells. While often asymptomatic, CHIP is recognized as a potential precursor to hematologic cancers and has also been linked to an elevated risk of cardiovascular diseases, such as atherosclerosis and stroke. Despite growing interest in CHIP, the prevalence of low-frequency clonal mutations in young, healthy individuals remains underexplored.

Methods: The study included 1,104 healthy individuals from the ENIGMA cohort, aged 18 to 63 years. Variants in 38 CHIP-related genes were identified from blood samples using the highly sensitive method of massively parallel sequencing. To increase specificity, novel bioinformatic approaches incorporating unique molecular identifiers and an artifact-correcting database were applied, enabling detection of variants with an allelic fraction as low as 0.5%.

Results and conclusions: Clonal hematopoiesis of indeterminate potential (CHIP) was identified in the healthy population, including individuals younger than 40 years. Its prevalence increased significantly with age, with most mutations occurring in DNMT3A and TET2. CHIP detection may represent a valuable tool for preventive medicine and personalized health care, although the clinical and biological relevance of low-frequency CHIP variants requires further investigation.

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Revealing the Role of p130cas in Mechanosensing

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The protein p130cas (human ortholog BCAR1) is a major substrate for phosphorylation by kinases from the Src family and plays a central role in oncogenic transformation and cancer cell invasivity. Localized to focal adhesion, p130cas serves as a mechanosensor and mediates key interactions with the extracellular environment. Its structural integrity, governed by anchoring domains SH3 and CCH, together with the substrate domain (SD), crucially contributes to its mechanosensory function, capable of elongation under tension. To elucidate the mechanosensory function of p130cas with emphasis on its SD domain, we introduced mutations into the SD inhibiting either its ability to stretch or to transmit the signals. Using traction force microscopy and cell stretching techniques, we evaluatedthe impact of these mutations on mechanosignaling and traction force generation. Additionally, we developed several variants of FRET-based tension biosensors using p130cas anchoring domains and SD to analyze the effect of various signals on SD structure and to monitor the dynamics of mechanical tension in cells. Furthermore, our investigation highlights p130cas role in cell invasiveness mediated by invadopodia structures, uncovering its novel mechanosensory function.

Portable Raman Spectroscopy for the Analysis of Gliomas: A Diagnostic Perspective

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Gliomas remain among the most prevalent and aggressive primary brain tumors, posing significant diagnostic and therapeutic challenges. Current diagnostic protocols rely heavily on histopathological examination, which, while essential, offer limited insight into intraoperative decision-making and long-term prognosis. Surgical resection remains the cornerstone of glioma management; however, accurately delineating tumor margins intraoperatively is a persistent challenge. Residual tumor tissue or undetected satellite lesions are major contributors to disease recurrence and poor clinical outcomes.

In this study, we present a novel intraoperative approach utilizing portable Raman spectroscopy for real-time spectral characterization of gliomas and adjacent brain tissue. The system, equipped with an optical fiber probe, enables neurosurgeons to spectrally interrogate tissue prior to excision, facilitating more precise

resection. Raman spectral data were processed through a pipeline involving background subtraction, signal smoothing, and classification via a machine learning model based on support vector machine.The methodology was validated on over 200 Raman spectra collected from 35 patients, demonstrating statistically significant discrimination between neoplastic and healthy tissue. These findings underscore the potential of portable Raman spectroscopy as a valuable adjunct in neurosurgical oncology, enhancing intraoperative accuracy and potentially improving patient outcomes.

Molecular dynamics of HSP proteins after targeted heat shock in cancer cells

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Understanding how cancer cells respond to heat-induced protein damage in real time requires advanced methods. Here we present an upgraded tool able to inflict defined, subcellular thermal damage that leads to protein unfolding and aggregation – processes that are implicated in diverse pathologies including cancer. Moreover, cancer cells display reliance on HSPs and insight into the functions of the involved proteins and the response pathways could be exploited as potential pharmacological strategy. We adapted a recently developed methodology that integrates laser-scanning microscopy and live-cell imaging with plasmon resonance principles using a titanium nitride nanoparticle layer. The targeted induction of dose-defined heat then causes protein damage in specified subcellular compartments, leading to rapid heat shock chaperone recruitment, and engages the ubiquitin-proteasome system. Using this method, we are able to measure the precise kinetics of the involved proteins within time range from less than a second to tens of minutes, providing unprecedented insights into spatiotemporal cellular response to damaged proteins. We report the dynamic recruitment kinetics of HSP70, HSP90, E3 ubiquitin ligase CHIP, and different types of ubiquitin. We also measured the effects of different HSP70 inhibitors on the activity of the protein. Overall, this modern approach not only advances our understanding of heat shock response and ubiquitination but also paves the way for new insights into cellular stress-response pathways that have emerged as potential therapeutic targets.

In Vitro Three-Dimensional Cell Culture Tools for Spheroid Models in Pancreatic Adenocarcinoma

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In the last decade, three-dimensional (3D) cell culture technology has gained a lot of interest due to its ability to better recapitulate the *in vivo* organization and microenvironment of *in vitro* cultured cancer cells. In particular, 3D tumour models have demonstrated several different characteristics compared with traditional two-dimensional (2D) cultures and have provided an interesting link between the latter and animal experiments. Indeed, 3D cell cultures represent a useful platform for the identification of the biological features of cancer cells as well as for the screening of novel antitumor agents. 3D cell structures better reflect the *in vivo* distribution of metabolites, nutrients, oxygen, and signalling molecules, while also more accurately mimicking 3D tissue architecture, cell proliferation, motility, and migration through an artificial extracellular matrix. These natural interactions enable cells to acquire morphological and other cellular characteristics like those found in solid tumours *in vivo*. The present study is aimed at investigation of the effects of selective steroid derivatives on cell proliferation, cell cycle progression, and apoptosis in both 2D and 3D models of pancreatic cancer cells. Study of the biological properties of 3D spheroid cultures and their responses to drug treatment reveals significant differences compared to conventional 2D cultures. Therefore, assessment of potential anti-tumour drugs in 3D cell models may represent an important standard component of preclinical testing.

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Multiplex immunohistochemistry in the evaluation of biomarker candidates in colorectal cancer

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Colorectal cancer (CRC) is the second leading cause of cancer-related death worldwide, highlighting the need for biomarkers integrating tumor biology, metabolism, and immune contexture. Increased expression of GLUT1, a key mediator of glucose transport across cell membranes, is frequently observed in tumors and correlates with enhanced glucose metabolism, supporting tumor growth and aggressiveness. It also contributes to increased glycosylation of selected lipid species, e.g. glycosphingolipids.

We applied high-content multiplex immunohistochemistry (mIHC) using the Orion™ platform (RareCyte) to a cohort of 36 CRC patients, enabling simultaneous spatial profiling of epithelial and immune markers in FFPE samples. The panel included E-cadherin, CD3ε, CD8α, FOXP3, GLUT1, B4GALT6, and CD15, while chromogenic IHC for p53 was performed in parallel. Cell and tissue segmentation was based on nuclear staining (Hoechst) and E-cadherin, respectively. Fluorescent assessment of B4GALT6 and CD15 in tumor regions was limited due to technical constraints and nonspecific staining. Microsatellite instability status and RAS/BRAF mutations were also assessed.

The analysis revealed several consistent and biologically meaningful trends: As expected, larger tumors were associated with reduced lymphocyte infiltration and diminished E-cadherin expression. Ascending colon tumors demonstrated higher infiltration by lymphocytes and neutrophils, yet lower GLUT1 expression. Positive lymph node status correlated with increased GLUT1 intensity and, unexpectedly, elevated E-cadherin levels. Mutant p53 status was associated with higher GLUT1 expression and lower immune infiltration. Microsatellite instability was linked to enhanced lymphocyte and neutrophil infiltration, whereas disease relapse was associated with reduced E-cadherin expression and decreased immune scores in both epithelial and stromal compartments.

These results highlight the utility of Orion-based mIHC for spatially resolved profiling of metabolic, epithelial, and immune features in CRC, supporting identification of clinically relevant biomarkers for prognosis and patient stratification.

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miRNA Profile of the Luminal Subtypes of Breast Cancer as Assessed by Microarray Approach

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Introduction: Breast cancer (BC) is the most common female neoplasm, with more than 2 million women diagnosed worldwide each year. Approximately 650,000 women die annually from this disease, representing as much as 6.9% of all cancer-related deaths. Despite its very high incidence and mortality, the molecular and genetic background of breast cancer remains insufficiently elucidated and explored. Moreover, the treatment of this disease is not optimal and represents a tremendous financial burden. The improvement and optimisation of therapy, especially treatment responses in specific subtypes, is closely linked to the understanding and identification of novel molecular mechanisms and markers in the tissues of these tumours. One type of molecular markers suitable for analyses are non-coding RNAs, which accounts for up to 80% of the total human transcriptome. By comparison, mRNAs, which are translated into protein sequences, represent only about 3% of transcriptome. A specific type of non-coding RNA – microRNA (miRNA) is currently the main focus of scientific interest. This fact is highlighted by the awarding of the 2024 Nobel Prize in Physiology or Medicine for the description of the mechanisms of action of these molecules. Analysing the effect of changes in miRNA activity and expression in native

tumours may reveal the molecular mechanisms underlying the onset and progression of this disease but also can provide a suitable basis for the design of non-invasive analyses. In this regard, the main goal is to overcome the limitations of mammographic screening and to make these analyses sufficiently practical and financially accessible for routine application. Assessing the role of miRNAs in BC tumorigenesis may also be used to accelerate the implementation of gene therapy in the future. In our study, we therefore focused on establishing a comprehensive microRNA profile using the microarray method, verified by qPCR, in luminal BC subtypes, which remain less analysed and investigated compared with the more aggressive triple negative BC (TNBC) subtypes.

Material and methods: In our study, a total of 16 luminal BC subtype and 21 control samples were analysed. Their expression levels were determined using the microarray method, which allows the simultaneous assessment of up to 2,549 microRNAs in a single run. Microarray analyses were performed using the Agilent SureScan DX system. The scan output in the form of TIFF image files was extracted and converted into text format using the Feature Extraction software. Provided the data met the qualitative and quantitative criteria, they were statistically analysed using Genespring Software. Data normality was assessed using the Shapiro-Wilk test and QQ plots. As normality was not achieved, the data were analysed using the Mann-Whitney U test with Benjamini-Hochberg FDR correction. Results were considered statistically significant at $p < 0.05$ and a fold change > ±2. Microarray analyses results were verified using qPCR, for which LNA technology was employed. Fold change was calculated using the standard ΔΔCt formula. The qPCR analyses were performed on samples from 16 BC patients and 16 controls, which were selected from the original set of samples used for the microarray analysis. The microarray analysis results were further processed under in silico conditions. Specifically, the identification of target mRNAs for miRNAs with different expression levels was performed using the miRTargetLink 2.0 tool. Subsequently, the protein products translated from these target mRNAs were analysed using the STRING tool to determine how they may be involved in interaction networks associated with the onset and progression of BC. The study was approved by the Regional Ethics Committee of Jessenius Faculty of Medicine in Martin (1866/2016).

Results: Our analyses revealed altered expression of a total of 17 miRNAs. Interestingly, 16 of these showed downregulation, ranging from -2.75 to -11.02-fold, while only one miRNA, the well-known oncomiR miR-21a, was upregulated, specifically by 3.10-fold. Verification by qPCR was performed on a total of five miRNAs, and the results were almost identical to the microarray findings, thereby confirming them. Using the online tool miRTargetLink 2.0 with the “strong validated” settings, the 17 miRNAs with aberrant expression were assigned a total of 454 target mRNAs. Due to this high number, we further focused only on mRNAs that were targets for at least three different miRNAs. A total of 14 such mRNAs were identified. In addition, the highest number of analysed miRNAs – 8– regulate the expression of the mRNA transcribed from the IGF1R gene. Six miRNAs were assigned to the mRNA transcribed from the AKT1 gene, and three of the aberrantly expressed miRNAs identified in our study are established to regulate the expression of mRNAs transcribed from the BCL2, CD44, and MYC genes. The protein products translated from all 14 mRNAs were subjected to STRING analysis. It was noted that they are involved in multiple interaction networks directly associated with the onset and progression of BC, as well as in pathways typically dysregulated in BC.

Conclusion: Changes in miRNA activity and expression have a significant impact on the onset and progression of oncological diseases, making them a subject of scientific interest. To date, these alterations in BC tissues have been studied primarily in the most aggressive TNBC subtypes. Our research therefore provides valuable insights into changes in miRNA expression in the development and progression of the less explored luminal subtypes of BC. Although these are considered less aggressive, they can still substantially reduce patients’ quality of life and, if treated improperly or belatedly, may be lethal. Our study demonstrated that multiple miRNAs exhibit altered expression in luminal subtypes. Interestingly, with the except of one, all miRNAs showed reduced expression, suggesting their potential tumour-suppressive character. Therefore, it can be hypothesised that loss of regulatory function may be a typical pattern in luminal subtypes, while upregulation of oncomiRs is likely to have a much smaller impact on their development. It should be noted, that abnormally expressed miRNAs have a broad spectrum of target mRNAs, which in turn affects protein levels, resulting in abnormalities in signalling pathways and interaction networks associated with BC. This study was funded by VEGA 1/0145/22.

Discovery of Novel Selective Inhibitors Targeting Carbonic Anhydrase IX

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The acidic and hypoxic tumor microenvironment is a major driver of cancer progression, metastasis, and resistance to therapy. Among the molecular mediators of this process, carbonic anhydrase IX (CA IX) plays a key role. As a hypoxia-inducible transmembrane

enzyme, CA IX catalyzes the reversible hydration of carbon dioxide, producing bicarbonate and protons to maintain intracellular pH homeostasis under acidic stress. Its pronounced overexpression in many solid tumors, combined with its limited presence in normal tissues, makes CA IX an appealing biomarker and a promising target for therapeutic intervention.

To identify new small-molecule inhibitors of CA IX, we established a high-throughput screening platform based on a pyranine fluorescence assay capable of monitoring pH shifts in real time. More than 10,000 compounds from the IMTM Proprietary Library were screened, yielding five selective hits. Their binding profiles were subsequently validated using surface plasmon resonance (SPR), confirming high affinity and isoform selectivity.

Guided by these results, a focused series of 46 structural analogues was synthesized to refine potency, specificity, and physicochemical properties. Here, we present their biological evaluation and structure–activity relationship (SAR) analysis, which revealed key molecular features responsible for efficient CA IX inhibition. These insights provide a foundation for rational optimization and the development of next-generation CA IX–targeted anticancer agents.

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Optimisation and High-Throughput Screening of potential modulators of P2Y11 purinergic receptor

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The human $P2Y_{11}$ receptor is a unique purinergic receptor that couples to Gq and Gs proteins and plays roles in immune regulation, neuroprotection, and cellular metabolism. However, its absence in rodent models and the lack of selective ligands have limited its study. Here, we present a functional assay using ES-293-A cells (Revity), a 1321N1-derived line stably expressing human $P2Y_{11}$, combined with the aequorin luminescence system for real-time calcium mobilisation measurement. This sensitive and robust platform enables reliable pharmacological profiling of candidate $P2Y_{11}$ agonists and antagonists. Our results demonstrate its suitability for screening novel modulators, supporting drug discovery efforts targeting $P2Y_{11}$ in inflammatory and neurodegenerative diseases.

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Optimisation and High-Throughput Screening of potential modulators of P2Y11 purinergic receptor

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Restoration of HLA genes expression in multiple myeloma cell lines following treatment with demethylating agents

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Background: The human major histocompatibility complex (MHC), also known as the human leukocyte antigen (HLA) system, is a group of molecules that play a central role in regulating the immune response. The HLA genes are located on the

short arm of chromosome 6 and represent the most polymorphic genetic system in humans. The biological function of HLA class I and class II molecules is to present processed peptide antigens to T cells, thereby initiating an immune response. Levels of class I MHC molecules (MHC I) can be downregulated either by irreversible gene-disabling mutations or by reversible inactivating methylation patterns.

Objective: AZA and DAC are prodrug cytosine analogues —a ribonucleoside and a deoxyribonucleoside, respectively. They are incorporated into nucleic acids and inhibit the activity of DNMT enzymes, preventing the addition of methyl groups to DNA. This process can reactivate genes that were previously silenced by hypermethylation. Recent evidence suggests that inhibition of DNMTs also produces cancer cell-extrinsic immunomodulatory effects. Using DNMT inhibitors (DNMTi) can increase tumor immunogenicity by raising levels of antigens displayed in MHC I. The study aimed to determine whether demethylation could affect the expression of genes from MHC class I (HLA-A, HLA-B, and HLA-C genes).

Material & Methods: RNA was isolated from three myeloma cell lines – KMS12-PE, KMS12-BM, and U266 affected by the demethylation agents 0.5 μM 5-Azacytidine (AZA) and 0.2 μM or 0.5 μM 5-Aza-2´-deoxycitidine (DAC). The expression of HLA genes was quantified by RT-PCR using primers specific for both promoter or coding regions of the respective HLA.

Results: Treatment of multiple myeloma cell lines significantly increased the expression of MHC class I genes. In the KMS12-PE cell line, HLA-B expression was upregulated after treatment with both demethylating agents, reaching statistical significance ($p < 0.05$; AZA: $p = 0.0087$, DAC 0.2 μM: $p = 0.001$, DAC 0.5 μM: $p = 0.0002$). In the KMS12-BM cell line, HLA-A expression was significantly increased following treatment with both agents ($p < 0.05$; AZA: $p = 0.001$, DAC 0.5 μM: $p = 0.005$). Additionally, HLA-C expression was elevated after treatment with decitabine at both 0.2 μM ($p = 0.002$) and 0.5 μM ($p = 0.0008$). In the U266 cell line, expression of all three genes was upregulated, with the most pronounced effect observed for HLA-C, which was significantly increased after treatment with 0.2 μM 5-aza-2′-deoxycytidine ($p = 0.0027$).

Conclusion: Demethylating agents AZA and DAC significantly restored MHC class I gene expression in multiple myeloma cell lines. Such reactivation may enhance tumor immunogenicity and represents a promising therapeutic approach to improve immune recognition in multiple myeloma.

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Selective stabilization of HDAC6 by HSP90 in cancer cell models

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Heat shock protein 90 (HSP90) plays a central role in maintaining proteostasis and supporting oncogenic signaling in cancer. Histone deacetylase 6 (HDAC6) implicated in tumor progression and drug resistance has been proposed as both a regulator and a potential client of HSP90. However, the extent of its chaperone dependency across cancer types remains unclear.

We investigated the impact of HSP90 inhibition on HDAC6 and other zinc-dependent HDACs in seven human cancer cell lines of different tissue origins using pimitespib (TAS-116), a clinically approved HSP90 inhibitor. Canonical HSP90 clients Akt and c-Raf were uniformly degraded upon treatment. In contrast, HDAC6 displayed cell-type-specific response: protein level was significantly reduced in A549, PC-3, U-2 OS, and HEK 293T, but not in MCF7, K-562, and RPMI 8226. HDAC6 mRNA levels were unchanged, indicating a posttranscriptional destabilization mechanism. Other zinc-dependent HDACs did not show consistent degradation, highlighting HDAC6 as a unique client of HSP90. Notably, HSP90 inhibition led to increased HSP90α protein levels, while mRNA levels declined post-treatment, highlighting potential feedback regulation and translational control mechanisms. These findings emphasize HDAC6's selective dependence on HSP90 in certain tumor contexts and support biomarker-driven strategies for dual targeting of HSP90 and HDAC6 in cancer therapy.

Simultaneous, high-throughput detection of RNA and protein biomarkers using the OrionTM imaging system and HCR Gold for comprehensive analysis of tumor samples in clinical trials

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Understanding the tumor microenvironment (TME) during cancer progression and treatment has been aided by multiomic analysis of tissues collected at initial patient screening and after treatment. Cyclic staining methods have recently demonstrated multiomic capabilities on a single tissue, but with drawbacks that can include tissue degradation and processing times up to several days or weeks. The Orion imaging system overcomes these limitations through single round staining and

scanning of whole tissue samples of up to 18 biomarkers simultaneously. When paired with Molecular Instruments' HCR Gold RNA-FISH, a novel product featuring a truly protease-free workflow with next-generation amplification technology, transcriptomic and proteomic multiplex immunofluorescence (mIF) analysis can be carried out in parallel on a single tissue section in a straightforward, high-throughput approach to provide comprehensive insight into tumor biology and immune response.

Separation of Blood-derived Extracellular Vesicles using droplet-based Spectral Sorting

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Extracellular vesicles (EVs) are cell-derived, membrane-bound, small particles measuring less than a micron in size, that demonstrate potential as valuable biomarkers and therapeutic agents in various disease environments. Due to their small size and heterogeneity, EVs can be difficult to characterize; however, single vesicle flow cytometry is a proven method to detect and analyze EVs. Growing interest exists for using high-speed, droplet-based sorting to isolate specific EV populations for further characterization in both functional assays and to explore their use in therapeutics. The work described herein outlines a proof-of-concept assay that demonstrates the ability to effectively enrich specific EV populations using a Cytek Aurora CS, droplet-based Spectral Cell Sorter.

Detection of epigenetic changes in the development of cervical cancer

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Introduction: Cervical cancer is the fourth most common malignancy and the fourth leading cause of cancer-related death among women worldwide. DNA methylation plays a critical role in the progression of cervical intraepithelial neoplasia (CIN) to invasive cancer. Assessing methylation status in the genome has therefore emerged as a promising screening marker in cervical cancer.

Aim: The main aim of the project is to elucidate methylation changes in relation to HPV infection in precancerous cervical lesions. We analysed epigenetic biomarkers in adhesion/ tumour-suppressor genes in cervical cytology samples, with the potential for clinical application.

Material and Methods: Cervical specimens were collected from 81 patients with cervical lesions. DNA was extracted from cervical cells, and subjected to conversion by sodium bisulphite. In total, 20ng of bisulphite-converted DNA was amplified in PCR reaction and subsequently analysed using pyrosequencing.

Results: The average age of women was 39 years. HPV infection was detected in 49% of women (40/81). Positive methylation status was detected in immune genes as BCL2, CD8A and ALCAM in CIN 3 lesions or worse.

Conclusion: The methylation assays applied in this study are under development and show promise as valuable tools for cervical cancer screening. The detection of methylation in immune-related genes such as BCL2, CD8A, and ALCAM in CIN3 lesions or worse suggests their possible role as biomarkers of advanced disease. Aberrant methylation patterns are closely linked to HPV infection and may contribute to dysregulated gene expression driving lesion progression. In the future, integrating methylation profiling with genomic and microbiome data may further improve risk stratification and support the development of personalized strategies for cervical cancer prevention and early intervention.

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Thank you!

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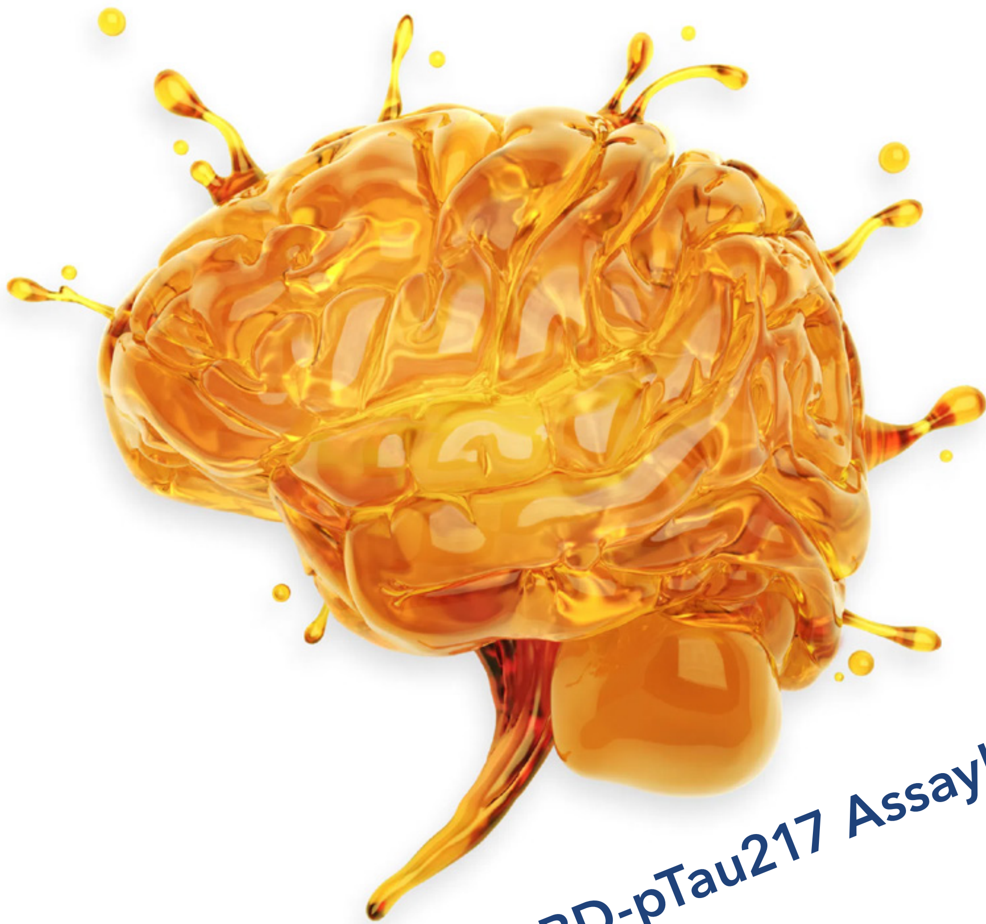

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