
9th Annual IMTM Retreat

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INSTITUTE OF MOLECULAR AND
TRANSLATIONAL MEDICINE

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## Scientific comitee

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# PROGRAM

## Wednesday, June 2

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| **09:33** | | **Departure from Olomouc Train station (train M5/Os3543)** | |
| 10:02 | | Arrival to Hrubá Voda | |

### Chair: Pavel Moudrý

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|---|---|---|---|
| 10:30 | 10:45 | **Juan Bautista de Sanctis** | The effect of CuEt and phosphatase inhibitors on the activation of Lck and Syk in lymphocytes. |
| 10:45 | 11:00 | **Martin Löffelmann** | Copper ionophores trigger proteotoxic stress in the context of a copper-dependent type of cell death – cuproptosis |
| 11:00 | 11:15 | **Adam Kiška** | Advancing the methodology of targeted subcellular heat |
| 11:15 | 11:30 | **Matthew Lacey** | Identification of Senolytic Compounds via Cell Painting Assay |
| 11:30 | 11:45 | **Jiří Hodoň** | Uncovering mechanism of action of active triterpenes |
| 12:00 | 13:00 | LUNCH | |

### Chair: Jiří Drábek

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|---|---|---|---|
| 13:00 | 13:15 | **Ondřej Bouška** | DNA Biomarkers in HPV-Related Oropharyngeal Cancer: Applications in Screening, Early Detection, and Recurrence Monitoring |
| 13:15 | 13:30 | **Monika Vidlařová** | Effect of multimodal therapy on the incidence of circulating tumor cells in the blood of patients with rectal cancer |
| 13:30 | 13:45 | **Pavel Stejskal** | Liquid biopsy as a tool for monitoring and molecular profiling of solid tumors |
| 13:45 | 14:00 | **Anna Sekyrová** | Cell-free circulating RNA extraction: technical hurdles and practical solutions |
| 14:00 | 14:15 | **Barbora Kalousová** | Age-related clonal hematopoiesis in healthy blood donors and in ischemic stroke patients |
| 14:15 | 14:30 | **Lucie Kotková** | MethAge performance in ENIGMA samples with focus on outliers |
| 14:30 | 14:45 | **Tatiana Mečiarová** | Gene Expression of p16 and p21 as Biomarkers of Senescence and Biological Aging |
| 14:45 | 15:00 | **Rastislav Slavkovský** | Optimization of novel diagnostic solutions for genotyping of somatic variants of BCR::ABL1 |
| 15:00 | 15:30 | COFFEE BREAK | |

### Chair: Martin Ondra

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|---|---|---|---|
| 15:30 | 15:45 | **Chariza Witters** | Repositioning yesterday's drug for tomorrow's vision: Drug repurposing to promote corneal endothelial regeneration |
| 15:45 | 16:00 | **Matěj Šamaj** | Elucidating the Mechanisms of PNH173, PNH192, EVH206, and EVH383: Functional Validation of PHKB and KIF24 as Molecular Targets |
| 16:00 | 16:15 | **Kateřina Ječmeňová** | Optimisation and High-Throughput Screening of potential modulators of P2Y11 purinergic receptor |
| 16:15 | 16:30 | **Lukáš Lenart** | The only pipeline for development of monoclonal cell lines you will ever need |
| 16:30 | 16:45 | **Nikta Ziaei** | Optimizing high-throughput screening: optimized 3D spheroid models for cutting-edge drug discovery |
| 16:45 | 17:00 | **Lenka Hrubá** | Metabolic and functional adaptations in nucleoside-resistant leukemia cells |
| 17:00 | 17:15 | COFFEE BREAK | |

### Chair: Lukáš Najdekr

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|---|---|---|---|
| 17:15 | 17:30 | **Martina Kintlová** | Targeting the Cell Cycle Kinome: Echo® MS Screening and Methodological Challenge |
| 17:30 | 17:45 | **Denisa Kroupová** | SPR and SureQuant for Targeted Protein Analysis |
| 17:45 | 18:00 | **Jana Václavková** | Identification of Bordetella pertussis infection biomarkers in exhaled breath condensate using mass spectrometry-based proteomics |
| 18:00 | 18:15 | **Miroslav Hruška** | Claire: A Cloud-Enabled Software for Detection of Rare Peptides with Enhanced Sensitivity and Efficiency |
| 18:15 | 18:30 | **Lukáš Najdekr** | Development and validation of a liquid chromatography and mass spectrometry method for the quantification of hepcidin levels in human plasma |
| 18:30 | 18:45 | **Martina Navrátilová** | Amyloidosis, state-of-the-art |
| 18:45 | 19:00 | **Patrik Flodr** | Amyloidosis Associated with Localised B-cell Neoplasia of Undetermined Significance |
| 19:00 | | DINNER | |

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# Thursday, June 3

### Chair: Václav Ranc

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| 9:00 | 9:15 | **Kateřina Dvořáková Bendová** | Ferrioxamine derivates for PET/CT imaging of bacterial infections |
| 9:15 | 9:30 | **Katarína Hajduová** | Radiopharmaceuticals for Oncological Theranostic Applications: Comparative Evaluation of $^{161}$Tb-Labeled PSMA Ligands and a $^{161}$Tb-Labeled HER2-Targeting Antibody |
| 9:30 | 9:45 | **Barbora Neužilová** | 68Ga/89Zr-labelled Desferrioxamine-B Analogues: Evaluation for Molecular Imaging with PET/CT |
| 9:45 | 10:00 | **Sunčica Sukur** | Surface-Functionalised Magnetic Nanoparticles for Multifunctional Biomedical Applications |
| 10:00 | 10:15 | **Aleksandra Ivanova** | GenCReM: de novo design guided by explainable docking |
| 10:15 | 10:30 | **Guzel Minibaeva** | Searching for new CDK16 inhibitors in ultra-large libraries guided by de novo design |
| 10:30 | 11:00 | COFFEE BREAK | |

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### Chair: Pravin Patil

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| 11:00 | 11:15 | **Ihor Kozlov** | Translational Modeling of Tauopathy Using Differentiated SH-SY5Y Cells: Validation with Prion-like Tau Aggregates and Oleocanthal Modulation |
| 11:15 | 11:30 | **Alladi Charanraj Goud** | Purpurin and Oleocanthal Inhibit MAPT Mutant Tau Aggregation with Strain Selectivity |
| 11:30 | 11:45 | **Jiří Řehulka** | Focused siRNA screen for regulators involved in neurodegeneration |
| 11:45 | 12:00 | **Soňa Gurská** | A high-throughput binding assay for screening novel inhibitors of coronavirus infections |
| 12:00 | 12:15 | **Jan Sebastian Novotný** | Identifying the onset of cognitive changes in adulthood using four longitudinal studies |
| 12:15 | 12:30 | **Jana Zelinková** | Investigating the role of amyloid precursor protein in reactive astrogliosis during Alzheimer´s disease |
| 12:30 | 12:45 | **Kaoud Salama** | Revolutionizing Aminoglycosides: Potent, Safe, and Orally Bioavailable Antibiotics Against AMR |
| 12:45 | 13:00 | **Vijayendar Venepally** | One-Pot Access to Amenamevir: Process Development and Optimization in Aqueous Conditions |

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|---|---|---|---|
| 13:00 | 14:00 | LUNCH | |
| 14:00 | - | COMMON TRIP | |
| 19:00 | | DINNER | |

# Friday, June 4

## Chair: Ivo Frydrych

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| 9:00 | 9:15 | **Anna Ligasová** | A Rapid Approach for Identifying Cell Lines Lacking Functional Cytidine Deaminase |
| 9:15 | 9:30 | **Jiří Voller** | Evaluation of biologic activity of organoselenium and organotellurium derivatives |
| 9:30 | 9:45 | **Dominik Vítek** | Response to diverse stress modalities in Tardigrades |
| 9:45 | 10:00 | **Ermin Schadich** | Antimycobacterial property of compounds from proprietary library |
| 10:00 | 10:15 | **Riccardo Fusco** | Multi-Platform Analytical Validation and Computational Analysis of High-Throughput Covalent Library Quality Control |
| 10:15 | 10:30 | **Samantha Masineni** | CDK PROTACs: Advancing Targeted Protein Degradation for Pediatric Neuroblastoma |
| 10:30 | 11:00 | COFFEE BREAK | |

## Chair: Alexander Dömling

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|---|---|---|---|
| 11:00 | 11:15 | **Antonio Conte** | Multicomponent Synthesis of Covalent DYRK1A Inhibitors: Integrating Triazolopiperazine and Aminoquinoline Strategies |
| 11:15 | 11:30 | **Elisabetta La Scola** | Triazolopiperazines Enabled By High Throughput Experimentation |
| 11:30 | 11:45 | **Mayur Mukim** | Innovative Design of K-Ras G13C Covalent Inhibitors. |
| 11:45 | 12:00 | **Foteini Efetzi** | Exploring KRas inhibition: A biophysical perspective |
| 12:00 | 12:15 | **Atilio Reyes Romero** | Accelerated Access to RNA-Aided Small Molecule Discovery |
| 12:15 | 12:30 | **Dinesh Kumar Sriramulu** | Structure standardization and curation pipeline |
| 12:30 | 13:30 | LUNCH | |
| **13:51** | | **Train departure from Hrubá voda (M5/Os 3552)** | |

# The effect of CuEt and phosphatase inhibitors on the activation of Lck and Syk in lymphocytes.

*Juan Bautista De Sanctis, Viktor Valentini, Helena Besta Smrčková, Jenny Garmendia, Hana Duchová, Marián Hajdúch*

*Institute of Molecular and Translational Medicine. Faculty of Medicine. Palacky University*

## Abstract

Introduction:
SHP phosphatases are activated through the inhibitory receptors PD-1, PDL1, and L2. CuEt induces T and NK cytotoxic response (Dumunt et al Front Immunol. 2025 Feb 12;16:1491450), and the effect of CuEt is inhibited by SHP phosphatases. The aim is to block the impact of phosphatases and enhance the activation of T and NK cells.

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 analysis of cell activation was performed by Wetern blot using anti-phospho Lck Tyrosine 394 (active)/505 (close) ratio and activated Syk by anti-phospho Syk Tyr 525.

Results:
CuEt was able to activate the cells, and the phosphatase inhibitors partially activated the cells (Lck and Syk). There is little additive effect of the phosphatase inhibitors when added with CuEt.

Conclusions:
The phosphatase inhibitors may help maintain primed cell activity, but they had a minor contribution to the effect of CuEt. Further research is needed to enhance the effectiveness of these inhibitors.

## Acknowledgment

This research was supported by SALVAGE (OP JAK)—Saving Lives through Research in the Field of Early Detection and Prevention of Cancer: Molecular, Genomic, and Social Factors. Registration number: Czech Ministry of Education, Youth, and Sports CZ.02.01.01/00/22_008/0004644. and PerMed Personalised Medicine: From Translational Research into Biomedical Applications from the Technology Agency of the Czech Republic (PerMed, project number TN02000109).

# Copper ionophores trigger proteotoxic stress in the context of a copper-dependent type of cell death – cuproptosis

*Martin Loffelmann*[1], *Zdeněk Škrott*[1,2], *Lucie Béresová*[1], *Martin Mistrík*[1]

## Abstract

Copper ionophores are small molecules that transport copper ions across the plasma membrane. Excess copper has a detrimental effect on cancer cells and can trigger a recently discovered type of cell death called cuproptosis. Typical markers of the cuproptotic type of cell death were described as oligomerization of lipoylated DLAT (dihydrolipoyl transacetylase) proteins, loss of iron-sulphur cluster proteins, and proteotoxic stress. We tested several copper ionophores in the form of their complexes with copper to see their effectiveness in activating cuproptosis: bis(diethyldithiocarbamate)-copper (CuET), bis(dimethyldithiocarbamate)-copper (CuMT), elesclomol (Ele-Cu), pyrithione (Pyrt-Cu), and NSC319726 (NSC-Cu). We observed that these ionophores cause aggregation and immobilisation of the NPL4 protein (Nuclear protein localization protein 4), as does CuET, as previously described in the literature. The NPL4 protein is a crucial cofactor involved in the p97/proteasome degradation pathway, so this result provides new insight into the cuproptotic mechanism connected with proteotoxic stress. We then focused on Ele-Cu, which showed the highest toxicity, and CuET, and tried to detect typical cuproptotic phenotypes in cancer cells. We observed that both CuET and Ele-Cu cause Fe-S cluster protein loss. But, more importantly, they trigger typical proteotoxic stress pathways, such as the unfolded protein response, the heat shock response, and the accumulation of polyubiquitinated proteins. This indicates that cuproptosis may be more associated with the proteotoxic stress pathway than expected.

## Acknowledgment

The Palacky University Internal Grant Agency in Olomouc (IGA_LF_2024_039), EATRIS-CZ, BBMRI-CZ, and the National Institute for Cancer Research EXCELES (LX22NPO5102) financially supported this work.

# Advancing the methodology of targeted subcellular heat shock

*Adam Kiska[1], Bui Thanh Lam[2], Stepan Kment[3], Martin Mistrik[4], Zdenek Skrott[5]*

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*[4]Affiliation: Institute of Molecular and Translational Medicine, Faculty of Medicine and Dentistry, Palacky University, Olomouc, Czech Republic.*

*[5]Affiliation: Institute of Molecular and Translational Medicine, Faculty of Medicine and Dentistry, Palacky University, Olomouc, Czech Republic, Czech Advanced Technology and Research Institute (CATRIN), Palacky University Olomouc, Olomouc, Czech Republic.*

## Abstract

Protein unfolding and aggregation are processes implicated in various pathologies, including neurodegenerative and systemic diseases. Currently, the combination of laser-scanning microscopy with plasmon resonance principles is the only published methodology capable of inducing targeted subcellular thermal damage while simultaneously enabling real-time quantification of aggregation-related heat-shock protein kinetics. Here we present an upgraded tool able to inflict defined, subcellular thermal damage that leads to protein aggregation. 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 various proteins related to heat shock. Overall, this modern approach not only advances our understanding of response to thermal damage and subsequent ubiquitination, but also paves the way for new insights into cellular stress-response pathways that have emerged as potential therapeutic targets.

## Acknowledgment

The study was supported by Palacky University Olomouc Young Researcher Grant (JG_2023_33) and the project National Institute for Cancer Research (Programme EXCELES, ID Project No. LX22NPO5102) - Funded by the European Union – Next Generation EU., and Large RI Project LM2018129 - Czech-BioImaging).

# Identification of Senolytic Compounds via Cell Painting Assay

*Matthew Lacey [(1)], Lucie Beresova [(1)], Alzbeta Srovnalova [(1)], Pavlo Polishchuk [(1)], Zdenek Skrott [(1,2)], Marian Hajduch [(1,2)], Petr Dzubak [(1,2)], Anna Siskova [(1)], Martin Mistrík [(1)]*

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*[(2)] Institute of Molecular and Translational Medicine Czech Advanced Technologies and Research Institute, Palacky University, 77147 Olomouc, Czech Republic.*

## Abstract

MCOPPB is a senolytic compound which was originally identified via chemical library screening. Our recent work revealed that it's mechanism of action was tied to autophagy inhibition. Additionally, Cell Painting Assay analysis revealed that AZ191, a DYRK1B inhibitor, co-clustered with other autophagy inhibitors which displayed senolytic activity. While DYRK1B is known to promote autophagy, AZ191 is not known to be an autophagy inhibitor. The appearance of AZ191 within the cluster of autophagy inhibitors with senolytic activity was unexpected and warranted further investigation.

## Acknowledgment

This work was supported by infrastructural projects (CZ-OPENSCREEN – LM2023052; EATRIS-CZ – LM2023053; Czech-BioImaging - LM2023050), and the project National Institute for Cancer Research (Program EXCELES, ID Project No. LX22NPO5102) - Funded by the European Union - Next Generation EU from the Ministry of Education, Youth and Sports of the Czech Republic (MEYS) and an integral grant of Palacky University in Olomouc (IGA-UP-2021).

# Uncovering mechanism of action of active triterpenes

*Jiří Hodoň, Anna Ligasová, Karel Koberna, Petr Džubák, Marián Hajdúch, Milan Urban*

*Institute of Molecular and Translational Medicine Czech Advanced Technologies and Research Institute, Palacky University, Olomouc, Czech Republic.*

## Abstract

Triterpenoids are natural compounds with various biological activities. Our research group primarily focuses on their cytotoxicity. [1] Triterpenoid pyridines and pyrazines had high cytotoxicity of IC50 0.5 – 1.5 μM in leukemic cell lines (CCRF-CEM, K-562). [2] Medoxomil-type prodrugs surprised us with an extreme selective cytotoxicity against K-562 cells with IC50 26–43 nM. [2] The mechanism of action of active derivatives is still unknown. To get more insight into the mechanism of action, it is essential to identify the target proteins of the studied compounds. For these purposes, conjugates of active triterpenes with a bifunctional linker containing diazirine and an alkyne moiety for photocrosslinking with interacting proteins were prepared. New methods for investigating the mechanism of action of active triterpenes will be introduced along with synthesis and future goals.

## Acknowledgment

Authors are grateful to EXCELES, ID: LX22NPO5102 and SALVAGE project, ID: CZ.02.01.01/00/22_008/0004644, sup. by OP JAK, with co-financing from the EU and the State Budget.

1. Borkova, L., Hodon, J., Urban, M. (2018). Asian J. Org. Chem., 7.8, 1542.
2. Hodoň, J., Frydrych, I., Urban, M. (2022). Eur. J. Med. Chem., 243, 114777.

# DNA Biomarkers in HPV-Related Oropharyngeal Cancer: Applications in Screening, Early Detection, and Recurrence Monitoring

*Ondřej Bouška[1], Vladimíra Koudeláková[1, 2], Zuzana Horáková[3,4], Marian Hajdúch[1,2]*

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*[2] Laboratory of Experimental Medicine, Institute of Molecular and Translational Medicine, Czech Advanced Technology and Research Institute, Palacky University Olomouc*

*[3] Department of Otorhinolaryngology and Head and Neck Surgery, University Hospital Olomouc*

*[4] Faculty of Medicine and Dentistry, Palacky University and University Hospital Olomouc*

## Abstract

Background: The incidence of oropharyngeal squamous cell carcinoma (OPSCC) has increased markedly worldwide over recent decades. In the Czech Republic, annual cases have more than tripled in the last 30 years, reaching 800 new diagnoses in 2022 - surpassing cervical cancer in incidence. The majority of OPSCC cases are now associated with human papillomavirus (HPV) infection, and approximately 20% of patients experience disease recurrence within five years. Liquid biopsy approaches, including analysis of plasma and saliva, are being actively explored for their potential to aid in diagnosis, monitor treatment efficacy, and enable early detection of recurrence through DNA-based biomarkers

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.

Results: A total of 192 patients with OPSCC were enrolled, with 77.2% classified as HPV-related—predominantly associated with the HPV16 genotype, detected in 98.9% of these cases. Pre-treatment liquid biopsy analysis demonstrated high sensitivity for HPV detection: 84.8% in gargle lavage, 81.6% in oropharyngeal swabs, and 93.2% 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.

## Acknowledgment

Supported by the Internal Grant Agency of Palacky University (IGA LF UP 2025_006), the project National Institute for Cancer Research (Programme EXCELES, ID Project No. LX22NPO5102) - Funded by the European Union – Next Generation EU, EATRIS-CZ (LM2023053), MH CZ (NW25J-09-00118), MH CZ – DRO (FNOL, 00098892), and and by the Cancer Research Czech Republic.

# Effect of multimodal therapy on the incidence of circulating tumor cells in the blood of patients with rectal cancer

[1] *Monika Vidlarova*, [1] *Pavel Stejskal*, [2] *Peter Ihnát*, [1] *Josef Srovnal*, [1] *Pavla Kourilova*, [1] *Marian Hajduch*

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[2] *Department of Surgery at the University Hospital Ostrava, Czech Republic*

## Abstract

Introduction: Adjuvant and neoadjuvant therapies are essential in the treatment of rectal cancer, particularly in intermediate to advanced stages. These approaches aim to improve prognosis, reduce recurrence, and increase the chance of sphincter preservation. To date, there is no clear consensus on whether adjuvant and neoadjuvant therapies have a fundamentally different effect on the presence of circulating tumor cells (CTCs) in the peripheral blood of patients with rectal cancer. This study aimed to detect CTCs in the peripheral blood of rectal cancer patients undergoing either adjuvant or neoadjuvant treatment.

Methods: CTCs were analyzed in the peripheral blood of 70 patients with rectal cancer using two methods: (1) quantitative real-time PCR (qPCR) targeting epithelial gene expression (carcinoembryonic antigen [CEA] and cytokeratin 20 [CK20]), and (2) CytoTrack CT11 (2/C, Denmark), a semi-automated immunofluorescence microscopy system detecting pan-cytokeratin and EpCAM signals. Patients receiving neoadjuvant therapy had six serial blood samples collected monthly. Patients treated with adjuvant therapy provided three blood samples over the course of treatment.

Results: Of the 70 patients, 16 received neoadjuvant therapy and 54 received adjuvant therapy. In the neoadjuvant group, 9 of 16 patients (56%) tested positive for CTCs in at least one of six blood samples using CytoTrack, while 13 of 16 (81%) were positive by qPCR. In the adjuvant group, CTCs were detected in 17 of 54 patients (31%) by CytoTrack and in 30 of 54 (55%) by qPCR.

Conclusion: Neoadjuvant therapy is typically indicated for patients with locally advanced rectal cancer or features associated with a high risk of recurrence. Monitoring CTCs may provide prognostic information, as a reduction or absence of CTCs after therapy could suggest a favorable treatment response.

## Acknowledgment

This study was supported by the European Union – Next Generation EU (LX22NPO5102), the Ministry of Education, Youth and Sports (SALVAGE – CZ.02.01.01/00/22_008/0004644), and Palacky University Olomouc (IGA LF UP 2025_006).

# Liquid biopsy as a tool for monitoring and molecular profiling of solid tumors

*Pavel Stejskal*[1, 2], *Josef Srovnal*[1, 2], *Monika Vidlařová*[1], *Alona Řehulková*[1, 2], *Marián Hajdúch*[1]

[1] *Laboratory of Experimental Medicine, Institute of Molecular and Translational Medicine, Faculty of Medicine and Dentistry, Czech Advanced Technology and Research Institute, Palacky University and University Hospital in Olomouc, Czech Republic*

[2] *University Hospital in Olomouc, Olomouc, Czech Republic*

## Abstract

Unlike anatomically restricted and invasive tissue biopsies, liquid biopsy (LB) offers a minimally invasive, repeatable approach to assessing tumor dynamics through biomarkers circulating in body fluids, especially peripheral blood. Key analytes include circulating tumor cells (CTCs), circulating tumor DNA (ctDNA), and RNA (ctRNA), which together provide complementary insights into tumor biology. This study builds on recent methodological advances to support the integration and clinical standardization of LB in solid tumors. We emphasize combining multiple biomarkers to enhance molecular resolution and improve understanding of tumor progression.

We demonstrate direct detection of CTCs using the CytoTrack CT11™ platform, which enables semi-automated, immunofluorescent identification of CTCs from whole blood without pre-enrichment. In parallel, we optimized ctDNA/ctRNA workflows for cancer plasma samples, focusing on nucleic acid preservation and high analytical sensitivity. These protocols are being applied in a prospective patient cohort currently under recruitment. Multi-analyte LB profiling shows promise for detecting minimal residual disease, monitoring treatment response, and identifying resistance mechanisms earlier than conventional imaging.

## Acknowledgment

This study was supported by European Union - Next Generation EU (LX22NPO5102), Palacky University Olomouc (IGA LF 2025_006), and SALVAGE - CZ.02.01.01/00/22_008/0004644.

# Cell-Free Circulating RNA Extraction: Technical Hurdles and Practical Solutions

[1] *Anna Sekyrova*, [1] *Pavel Stejskal*, [1] *Josef Srovnal*, [1] *Marian Hajduch*

[1] *Laboratory of Experimental Medicine, Institute of Molecular and Translational Medicine, Faculty of Medicine and Dentistry, Palacky University and University Hospital in Olomouc, Czech Republic*

## Abstract

Background: Actively released cell-free circulating RNA (cfRNA) within exosomes holds great promise as a source of minimally invasive cancer biomarkers. These extracellular vesicles (30-150 nm) encapsulate diverse RNA species, reflecting cellular states and making them highly relevant for cancer diagnostics and prognostics. However, the clinical utility of exosome-derived biomarkers depends on the standardization and reproducibility of exosome isolation and RNA characterization methods.

Methods: Exosomes were isolated from cell culture conditioned media and plasma samples using ultracentrifugation in various configurations, as well as the initial steps of commercial kits (Norgen EXTRAClean, Qiagen exoRNeasy) and Qiagen exoEasy. RNA was subsequently extracted from these exosome preparations using the Qiagen miRNeasy kit. For comparison, cell-free RNA (cfRNA) was also isolated directly from the same samples using the complete protocols of EXTRAClean and exoRNeasy with and without DNAse treatment. RNA yield and quality were assessed with the Agilent Bioanalyzer RNA 6000 Pico Kit.

Results: All methods yielded low RNA concentrations, ranging from tens to low hundreds of picograms per microliter, as expected. Ultracentrifugation provided the most consistent RNA recovery and allowed processing of larger sample volumes. The optimal protocol involved centrifugation at 100,000 ×g for 90 minutes using 38.5 ml tubes with a swinging bucket rotor. In contrast, Qiagen exoEasy and exoRNeasy kits predominantly isolated short RNA fragments (~25 nucleotides), which may limit their utility for downstream sequencing applications. The Norgen EXTRAClean kit produced comparable RNA concentrations; however, its larger elution volume diluted the RNA, but the overall RNA quality was still not optimal. DNase treatment substantially reduced RNA yield, likely due to additional handling and processing time.

## Acknowledgment

Acknowledgements: This study was supported by the European Union – Next Generation EU (LX22NPO5102), the Ministry of Education, Youth and Sports (SALVAGE – CZ.02.01.01/00/22_008/0004644), and Palacky University Olomouc (IGA LF UP 2025_006).

# Age-related clonal hematopoiesis in healthy blood donors and in ischemic stroke patients

*Barbora Kalousová[1], Rastislav Slavkovský[1], Pavla Kouřilová[1], Ondřej Blaťák[1], Monika Škopová[1], Jiří Drábek[1,2], Marián Hajdúch[1,2,3], Veronika Kunešová[6], Michal Haršány[5,6] and Robert Mikulík[4,6]*

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*[6] The International Clinical Research Centre, St. Anne's University Hospital Brno, Brno, Czech Republic*

## Abstract

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 analysis included 453 healthy individuals from the ENIGMA cohort, aged 19 to 64 years. Mutations in 38 CHIP-related genes were identified from blood samples using the highly sensitive method of massively parallel sequencing (MPS). The results were compared with findings from the study "CHIP in ischemic stroke patients".

Results and conclusions: CHIP was observed in the healthy population, including individuals under 40 years of age. Its occurrence correlated with age, with most mutations detected in the DNMT3A and TET2 genes. Compared to DNMT3A, TET2 mutations tended to emerge later in life and were more common among individuals in the stroke study. These findings suggest that TET2 mutations may be more closely associated with aging and/or disease-related processes than DNMT3A mutations.

## Acknowledgment

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# MethAge performance in ENIGMA samples with focus on outliers

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## Abstract

Changes in methylation levels in specific CpG areas associated with the chronological age of an individual were used for the construction of the epigenetic age prediction model MethAge (https://shiny.imtm.cz/apps/vrr/methage/). Samples from the ENIGMA cohort (https://www.iabio.eu/enigma/) were used for its external validation.

Moreover, the study aimed to investigate outliers, individuals who are predicted to be significantly older or younger than their chronological age (> 5 years). Clinical data parameters such as BMI status, smoking status, and drinking habits were investigated. Preliminary results are presented here.

## Acknowledgment

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# Gene expression of p16 and p21 as biomarkers of senescence and biological aging

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## Abstract

Senescence is a biological process in which cells permanently cease to divide in response to various forms of stress, serving as a critical tumor-suppressive mechanism. However, the accumulation of senescent cells contributes to tissue dysfunction and the development of age-related diseases. The main genes regulating this process are the cyclin-dependent kinase inhibitors CDKN2A (p16) and CDKN1A (p21). Both contribute to permanent cell cycle arrest and serve as established biomarkers of senescence and biological aging. In this study, we analyzed the expression of p16 and p21 in blood samples from a cohort of healthy individuals. The detected expression levels were evaluated in relation to the individuals' chronological age to explore potential correlations. In parallel, senescent cell line models were prepared and treated with selected compounds to assess their potential senolytic effects. Post-treatment analysis revealed changes in CDKN2A and CDKN1A expression, suggesting that some compounds may modulate senescence-associated pathways and merit further investigation as senolytic agents.

## Acknowledgment

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# Optimization of novel diagnostic solutions for detection of somatic variants of BCR::ABL1

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## Abstract

Chronic myeloid leukemia (CML) is a clonal myeloproliferative disease of hematopoietic tissue that arises from the malignant transformation of bone marrow stem cells and their clonal expansion and accumulation in the peripheral blood. It is caused by a reciprocal translocation also known as Philadelphia chromosome. Disease can be treated with tyrosine kinase inhibitors (TKIs). Secondary molecular changes in kinase domain of ABL1 that cause resistance to TKIs could arise during the progress of disease. These secondary changes, typically non-synonymous SNV, occur in up to 90% of patients, and our goal was to detect these aberrations using user friendly approach based on reliable time-tested fastGEN technology. Successful ABL1 detection can be used to CML diagnostics and adjust the patient's treatment.

Methods: Ten RNA samples containing fused BCR::ABL1 were used for cDNA synthesis using two different reverse transcriptase (RT) approaches based on SuperScript IV or High-Capacity RNA-to-cDNA kits. We implemented qPCR method for BCR::ABL1 pre-amplification and ABL1 genotyping using fastGEN method followed NGS sequencing using Surfseq Genemind 5000. Sequencing data were processed using dedicated workflow in GENOVESA software.

Results: Approach based on SuperScript IV using random primers and oligo(dT) primers resulted with cDNA of higher molecular weight and higher intensity pre-amplification products. Both RT approaches provided correct sequencing results giving 100 % sensitivity and 100% specificity compared to Sanger sequencing.

Conclusion: We developed and validated novel kit for genotyping kinase domain of BCL::ABL1. Utilization of RT using Superscript IV RT with mixture of oligo(dT) and random primers is recommended for the moment. It is yet unknown if the addition of oligo(dT) primers would improve the efficiency in other RTs. Further implementation of dedicated RT to the kit is advisable. Developed method can be implemented easily in clinical practice especially to replace Sanger sequencing-based methods.

## Acknowledgment

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# Repositioning yesterday's drug for tomorrow’s vision: Drug repurposing to promote corneal endothelial regeneration

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## Abstract

The corneal endothelium is a single layer of hexagonal cells and plays a crucial role in maintaining the cornea's hydration levels through a complex "pump-and-leak" mechanism. In that regard, a dysfunctional endothelial layer leads to fluid accumulation, resulting in corneal opacification and known as one of the most common causes of corneal blindness worldwide. Patients with corneal endothelial dysfunction are in need of an endothelial transplantation. Despite the high success rates, a global donor shortage poses a considerable challenge and therefore stresses the critical need for alternative treatment strategies. A pharmacological approach is a potential solution to complement or replace an endothelial transplantation. Therefore, we explore the repositioning, i.e. repurposing of existing drugs to promote corneal endothelial regeneration. Specifically, we have accomplished a high-throughput screening (HTS) of 2 chemical drug repurposing libraries (ENZO®, Prestwick®) covering 2066 compounds in total. A live cell imaging proliferation assay was performed on the HCEnC-B4G12 immortalized corneal endothelial cell line and nuclear counts were captured on day 5 (endpoint - Hoechst fluorescent staining). Using our HTS methodology, we obtained a hit rate of 1.6% (= 33 compounds). This hit list is tested in a follow-up confirmation screening and growth curves of the compound conditions are plotted based on the time-lapse digital phase contrast (DPC) images obtained by the Yokogawa CellVoyager CV8000 high-content screening system. DPC masking is executed by a customized Cellprofiler pipeline. In addition, a secondary scratch wound healing assay is performed to investigate the potential stimulatory effect on the migration of the HCEnC-B4G12 cells. Our findings show promising repurposing results for the stimulation of corneal endothelial regeneration, showing trends similar to Y-27632 (25 µM) and chroman-1 (10 nM), 2 ROCK inhibitors used as evidence-based, positive controls for boosting the regenerative capacity of corneal endothelial cells. (1) Moreover, our results highlight the relevance for future compound characterization and downstream pathway identification as well as the need to test the confirmed hits on primary corneal endothelial cell culture.

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# Elucidating the mechanisms of PNH173, PNH192, EVH206, and EVH383: Functional validation of PHKB and KIF24 as molecular targets

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## Abstract

7-Deazaadenine derivatives PNH173, PNH192, and their more recent structural analogues EVH206 and EVH383 have been shown to possess potent anticancer activity. A genome-wide CRISPRi screen was performed with PNH173 and PNH192 in K562 dCas9-KRAB cells to uncover their mechanisms of action. From this screen, two genes – phosphorylase kinase regulatory subunit beta (PHKB) and kinesin family member 24 (KIF24) – were identified as putative molecular targets common to both compounds. To elucidate the involvement of these candidate targets in the compounds' molecular mechanism, lentivirally transduced stable cell lines with overexpression or knockdown of PHKB and KIF24 were generated. Expression levels of PHKB and KIF24 were evaluated in these modified cell lines before their use in cytotoxicity assays with PNH173, PNH192, EVH206, and EVH383. These assays were designed to test the hypothesis that altered expression of each gene would directly impact the cell's sensitivity to treatment – thus demonstrating functional target engagement. As PHKB is known to participate in glycogen metabolism, the effects of PNH173, PNH192, EVH206 and EVH383 on glycogenolysis are currently being assessed. These investigations aim to determine whether changes in gene expression or metabolic activity support a functional role for PHKB in mediating the nucleoside-based compounds' anticancer effects.

## Acknowledgment

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# Optimisation and high-throughput screening of potential modulators of P2Y11 purinergic receptor

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## Abstract

The human $P2Y_{11}$ receptor is a unique member of the P2Y family of purinergic receptors, exhibiting dual coupling to Gq and Gs proteins. Despite its potential involvement in immune modulation, neuroprotection, and cellular metabolism, research on $P2Y_{11}$ has been limited due to its absence in rodent models and lack of selective ligands. In this study, we utilise the ES-293-A cell line (Revity), a 1321N1-derived stable cell line expressing the human $P2Y_{11}$ receptor, for functional calcium mobilisation assay using the aequorin luminescence system. This platform allows for real-time, high-sensitivity quantification of ligand-induced responses and provides a reliable tool for pharmacological profiling of potential $P2Y_{11}$ agonists and antagonists. Our approach enables the screening and characterisation of novel compounds targeting $P2Y_{11}$, with implications for drug discovery in inflammatory and neurodegenerative disorders.

## Acknowledgment

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# The only pipeline for development of monoclonal cell lines you will ever need

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## Abstract

As of now, up to 85% of cystic fibrosis (CF) patients are eligible for modulator-based treatments, which utilise combinations of cystic fibrosis transmembrane conductance regulator (CFTR) correctors and potentiators. However, aberrant CFTR variants that arise from splice site mutations of the CFTR gene remain unresponsive towards these therapies. As the splicing variants compose 12% of the described CF-causing mutations (Deletang et Taulan-Cadars, 2022), there is a need for the identification of novel compounds that would remedy the CFTR defects connected to its aberrant RNA splicing. Therefore, we aim to develop a CF model cell line that bears one of the most common splice site mutations, 621+1G>T, and meets the requirements for high-throughput screening of compounds. As a basic building block, we have utilised our validated reporter cell line, which enables a high-throughput detection of endogenous CFTR levels as it expresses a HiBiT-tagged WT-CFTR (Ondra et al. 2023). For the introduction of the 621+1G>T mutation into the WT-CFTR-HiBiT, we have performed a CRISPR/Cas9-mediated knock-in. Subsequently, we have applied our optimised genotyping pipeline to select and further validate monoclonal cell lines with the desired modification.

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## Acknowledgment

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# Optimizing high-throughput screening: optimized 3D spheroid models for cutting-edge drug discovery

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## Abstract

Three-dimensional (3D) cell culture systems bridge the gap between conventional two-dimensional (2D) models and the complexity of in vivo tissues, providing a more physiologically relevant environment for studying cellular functions. Among these systems, tumor spheroids—compact aggregates of cancer cells—are particularly notable for closely mimicking the native tumor microenvironment. Their improved representation of cell-cell and cell-matrix interactions makes them powerful tools for evaluating drug efficacy and toxicity in preclinical research.

In this work, we employed HCT116-derived spheroids cultured in 384-well plates as three-dimensional models to streamline high-throughput screening workflows across different spheroid formats. We first established a robust protocol for generating uniformly sized spheroids, then optimized the ECHO liquid handler settings to deliver drug compounds at defined concentration gradients with high precision and reproducibility. Treated plates were imaged on the Cell Voyager CV8000 High-Content Screening System to monitor treatment-induced shifts in growth dynamics, structural organization, and morphological characteristics. Finally, we used an MTS assay to quantify cell viability, yielding reliable, quantitative insights into the cytotoxic effects of each compound.

By systematically optimizing each step, this work delivers an efficient high-throughput drug screening pipeline in 3D spheroid systems, elevating both accuracy and physiological relevance in preclinical evaluations.

## Acknowledgment

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# Metabolic and functional adaptations in nucleoside-resistant leukemia cells

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## Abstract

Resistance to nucleoside analogs remains a key obstacle in leukemia therapy. While reduced activation by nucleoside kinases accounts for the primary resistance mechanism in our CCRF-CEM and K562 models, we now show that resistant cells undergo broader metabolic and functional adaptations. Metabolic profiling revealed altered glycolytic activity and variable glycogen storage, indicating shifts in energy metabolism. We also observed nutrient-dependent changes in signaling pathways, including phosphorylation of ribosomal S6 protein. In addition, resistant cells displayed altered proliferation dynamics. Preliminary data suggest potential modulation of immune-related markers such as PD-L1. Together, these findings underscore that nucleoside resistance is associated with complex cellular reprogramming, offering new potential targets for therapeutic intervention.

## Acknowledgment

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# Targeting the cell cycle Kinome: Echo® MS screening and methodological challenges

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## Abstract

Kinases remain essential therapeutic targets due to their regulatory roles in cell signaling and proliferation. In our current study, we employed the SCIEX Echo® MS system to perform high-throughput, label-free inhibition screening of a new set of synthetically derived compounds across a panel of 22 kinases. The screening specifically focused on 16 kinases implicated in cell cycle regulation, enabling rapid identification of inhibitory profiles relevant to cancer biology. The Echo® MS platform allowed for direct, chromatography-free MS/MS analysis with high sample throughput, leveraging acoustic droplet ejection and electrospray ionization. While the platform demonstrated robust performance for the majority of targets, we encountered a notable limitation in detecting activity against PLK2 and PLK3 kinases. This observation highlights the importance of understanding the technical constraints of mass spectrometry-based screening approaches, particularly in the context of structurally similar kinase subfamilies. In parallel, we are exploring the feasibility of applying this workflow to screen covalent ligands on intact proteins, although this approach is currently in a very early, exploratory stage. Overall, our findings contribute to the refinement of compound selectivity and support the integration of Echo® MS for early-stage drug discovery workflows.

## Acknowledgment

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# SPR-Based characterization of CAIX–ligand interactions and targeted MS analysis of tear fluid in neurodegenerative disease

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## Abstract

Characterizing drug binding to intact proteins is enabled by cutting-edge technologies such as surface plasmon resonance (SPR), which provides real-time insights into molecular interactions. This method allows precise analysis of binding kinetics, affinity, and specificity between proteins and their ligands—key factors for understanding enzymatic inhibition and developing targeted therapies. In our current work, carbonic anhydrase IX (CAIX), a tumor-associated isoform, is tested with eight small-molecule compounds including acetazolamide. Preliminary results demonstrate reproducible immobilization of CAIX on the SPR sensor surface and stable binding profiles for several inhibitors, with dissociation constants (KD) in the low micromolar to nanomolar range.

In parallel, a large-scale targeted proteomic study of tear fluid is being conducted within the Kardiovize project, focusing on patients with neurodegenerative diseases. Tear samples from 180 individuals (360 samples total, from both eyes) are analyzed using the SureQuant™ mass spectrometry method, which utilizes isotope-labeled peptides for accurate quantification of selected protein targets. To ensure analytical robustness, samples were randomized into 12 measurement batches and analyzed in triplicate, resulting in 1,080 MS runs. Among 20 proteins identified by the Human Protein Atlas as highly specific to brain tissue, only S100B is included in the Kardiovize target panel, highlighting its potential as a non-invasive biomarker detectable in tear fluid.

## Acknowledgment

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# Identification of Bordetella pertussis infection biomarkers in exhaled breath condensate using mass spectrometry-based proteomics

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## Abstract

Exhaled breath condensate (EBC) collection is a cost-effective and non-invasive approach to obtain samples from the human respiratory tract. EBC is a valuable source of biomarkers that can offer insights into both respiratory and systemic diseases. Utilizing proteomic analysis of EBC holds promise for detecting early changes in the respiratory system and potentially in other organs. This technique may eventually supplement or replace more invasive procedures and serve as a non-invasive screening tool for lung disorders.

In this work, which is part of the PERISCOPE study, we focused on identifying biomarkers associated with whooping cough, an infection caused by Bordetella pertussis. We analysed EBC samples obtained from healthy individuals who were intranasally inoculated with B. pertussis and later treated with antibiotics to eliminate colonization, either 14 days after inoculation or upon the emergence of symptoms, whichever occurred first.

To collect both nasal and oral fractions of exhaled breath from inoculated participants, we developed a specialized collection method involving a resuscitation mask connected to a collection system. Samples were gathered using the Turbo 14 Turbo DECCS System (Medivac, Italy). Proteomic analysis was performed using mass spectrometry, employing a gel-free sample preparation protocol, Orbitrap-based HPLC-MS, and advanced data processing tools to achieve a high number of protein identifications.

We analysed EBC samples from individuals participating in the Bordetella challenge trial, each contributing between 1 and 6 samples collected at different stages of the study. Two samples were taken prior to inoculation (7 days before and on the day of inoculation), and four were collected post-inoculation (on days 3, 7, 14, and 28). Post-inoculation samples were categorized as Bordetella-positive or negative based on culture results from nasal wash, nasopharyngeal swab, and throat swab taken on days 3, 7, and 14.

In total, we processed 257 EBC samples from the trial, corresponding to 45 participants. Each sample was analysed by mass spectrometry in triplicate, yielding 771 raw files. Across the cohort, we identified 4,443 unique proteins. Through statistical analysis, we proposed candidate biomarkers for B. pertussis infection and identified proteins that showed significant differences between culture-positive and culture-negative samples. We also compared these results with serology results and safety results (such as CRP, white cell count, neutrophils, lymphocytes, viral co-infections).

## Acknowledgment

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# Claire: A cloud-enabled software for detection of rare peptides with enhanced sensitivity and efficiency

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## Abstract

Detection of peptides and their diverse variants forms the critical initial stage in most bottom-up proteomics analyses. Although standard peptide detection is well-established, reliably identifying rare peptide variants remains challenging, particularly when dealing with spectra of low signal-to-noise ratio. Here, we report significant advancements made in Claire, our cloud-enabled software, aimed at addressing these challenges. We conducted extensive comparisons with state-of-the-art software using synthetic spectra, controlled proteomes, and complex proteomic samples, demonstrating Claire's substantial detection advantage, especially for rare peptides and low-quality spectra. Further internal enhancements include the automated construction of large-scale protein and peptide databases, allowing accurate detection from expansive resources such as six-frame translations, the entire TrEMBL protein dataset, or exhaustive alternative splicing events in human proteins. Core algorithm efficiency was dramatically improved through AI-driven reimplementation and optimization of functionality into C and C++, notably accelerating processes such as open search and protein inference. Additionally, we implemented novel scoring metrics, along with their implementation in complete score histogram calculations, further enhancing peptide discrimination capabilities. These developments position Claire closer to publication readiness, providing a robust, efficient, and comprehensive tool for detecting rare peptides grounded in rigorous theoretical foundations.

## Acknowledgment

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# X

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## Abstract

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## Acknowledgment

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# Amyloidosis, state-of-the-art

*Martina Navrátilová[1], Tomáš Pika[2], Dušan Holub[3], Dominik Hraboš[1], Jiří Minařík[2], Jaroslav Michálek[1], Petr Džubák[3], Patrik Flodr[1]*

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## Abstract

The number of human amyloid proteins increased on 42 in October 2022 (ISA 2022 and 2024). In general amyloidosis is a heterogeneous acquired (32 subtypes), hereditary (20) or both (6), systemic (22) or localised (32) disease that results from an abnormal deposition of β-pleated sheet fibrillar protein aggregates in various tissues with variable distribution in extracellular space. Hybrid amyloidosis is rarest in rare form with variable fibril protein combinations. We analysed more than 800 FFPE and native samples of which 379 tissues were positive in Congo red and/or Saturn red as a detection step with consequent immunohistochemical analysis (IHC) a proteomic analysis (laser captured microdissection-liquid chromatography/tandem mass spectrometry - LMD-LC/MS/MS) as a typing steps with completely analysed 263 specimens. Multiplex fluorescent IHC assay was applied in thriple-hybrid amyloidosis.

## Acknowledgment

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# Amyloidosis associated with localised B-cell neoplasia of undetermined significance

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## Abstract

The number of human amyloid proteins increased on 42 in October 2022 (ISA 2022, ISA 2024), 4 candidate proteins are under the investigation. In general amyloidosis is a heterogeneous acquired (32 subtypes), hereditary (20) or both (6), systemic (22) or localised (32) disease that results from an abnormal deposition of β-pleated sheet fibrillar protein aggregates in various tissues with variable distribution in extracellular space. Hybrid amyloidosis is rarest in rare form with variable fibril protein combinations. Different types of B-cell non-Hodgkin lymphomas (MM, plasmocytoma, MGUS, MZL, LPL, B-CLL/SLL, FL, MCL, DLBCL) with plasmacytic differentiation and may produce AL/AH amyloid locally or as a part of systemic AL/AH amyloidosis. Literary relatively new entities are "AL amyloidosis with a localized B-cell neoplasia of undetermined significance" and "AL amyloidosis with a an associated predominant kappa or lambda light chain expressing plasma cell population without evidence for clonality".

## Acknowledgment

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# Ferrioxamine derivates for PET/CT imaging of bacterial infections

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## Abstract

Introduction
In recent years, there has been a growing interest in using gallium-68-labeled siderophores, microbial iron-scavenging molecules, for positron emission tomography (PET) imaging of bacterial infections. Contemporary research is directed toward synthesizing artificial siderophores with enhanced pharmacokinetic characteristics and improved specificity for bacterial targets. In a prior study [1], we evaluated the potential of ferrioxamine E (FOX E) and its synthetic derivate, ferrioxamine 2-5 (FOX 2-5), to image infections caused by Aspergillus fumigatus and Staphylococcus aureus. The current study aims to expand on this research by evaluating these compounds for imaging of additional bacterial types and their potential specificity.

Methods
For this study, a panel of clinically significant bacterial pathogens was selected: Staphylococcus aureus (SA), Klebsiella pneumoniae (KP), Pseudomonas aeruginosa (PA), and Acinetobacter baumannii (AB). Escherichia coli (EC) served as the negative control. Both siderophores were radiolabelled with gallium-68 and their radiochemical purity measured using radio-HPLC. The uptake of [68Ga]Ga-FOX E and [68Ga]Ga-FOX 2-5 was assessed in each bacterial species and compared to uptake levels in heat-inactivated and iron-saturated cultures. Additionally, in vivo PET/CT imaging was conducted in immunosuppressed BALB/c mice in acute myositis infection model. Radiotracer accumulation was quantified in each infected limb separately based on the CT scan.

Results
In vitro assays revealed that both AB and SA showed substantial uptake of [68Ga]Ga-FOX E and [68Ga]Ga-FOX 2-5. In contrast, KP and PA demonstrated elevated uptake of [68Ga]Ga-FOX E, but very limited uptake of [68Ga]Ga-FOX 2-5. No significant uptake was detected in EC, consistent with its role as a negative control, nor in any heat-inactivated and iron-saturated bacterial cultures. The in vivo PET imaging findings were consistent with these in vitro results, showing high radiotracer accumulation for most pathogens with [68Ga]Ga-FOX E and reduced signal intensity for PA and KP when using [68Ga]Ga-FOX 2-5.

Conclusion
Our results demonstrate the potential of [68Ga]Ga-FOX E and its analogue as radiotracers for imaging bacterial infections, as showed by both in vitro uptake assays and in vivo PET/CT imaging. Notably, the reduced uptake of [68Ga]Ga-FOX 2-5 by KP and PA suggests a degree of pathogen-specific interaction. This observation encourages further investigation into [68Ga]Ga-FOX E modifications and bacteria specific imaging. Currently, experiments involving other imaging isotopes are being conducted.

# Radiopharmaceuticals for oncological theranostic applications: Comparative evaluation of $^{161}$Tb-labeled PSMA ligands and a $^{161}$Tb-Labeled *HER2*-targeting antibody

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## Abstract

The development of radiopharmaceuticals labeled with terbium-161 ($^{161}$Tb), a promising $\beta^-$-emitter with additional Auger and conversion electron emission, offers unique potential for targeted cancer therapy and diagnostics at the same time. In this study, we compared the ex vivo biodistribution and imaging characteristics of two PSMA-targeting ligands, $^{161}$Tb-PSMA-617 and $^{161}$Tb-PSMA-I&T, in prostate cancer models, and evaluated the in vivo behavior of $^{161}$Tb-labeled pertuzumab, a *HER2*-targeting monoclonal antibody as potential theranostic tracer for the breast cancer.

SPECT/CT imaging and ex vivo biodistribution studies were performed in LNCaP tumor-bearing mice for PSMA ligands, and in a dual tumor mouse model bearing HER2-positive SKOV3 and *HER2*-negative MDA-MB-231 xenografts for$^{161}$Tb-pertuzumab. The comparison of PSMA ligands revealed differing pharmacokinetics and tumor uptake: $^{161}$Tb-PSMA-I&T demonstrated slower renal clearance and slightly lower tumor retention compared to $^{161}$Tb-PSMA-617. The *HER2* targeting $^{161}$Tb-pertuzumab showed high and specific accumulation in *HER2*-positive SKOV3 tumors with no uptake in HER2-negative controls, reflecting high target specificity and favorable pharmacodynamics of the antibody-based radioconjugate.

These findings underscore the theranostic versatility of $^{161}$Tb across molecular platforms, from small molecules to antibodies. Tailored agent selection based on tumor type and target expression remains essential for optimizing the clinical potential of $^{161}$Tb-labeled radiopharmaceuticals. Preclinical therapy study with $^{161}$Tb-labeled pertuzumab is currently under preparation.

## Acknowledgment

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# 68Ga/89Zr-labelled desferrioxamine-B analogues: Evaluation for molecular imaging with PET/CT

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## Abstract

Introduction: Desferrioxamine B is a chelator that is used for the treatment of acute iron poisoning but is also being investigated in the field of nuclear chemistry, for its ability to label

biomolecules with radionuclides [1, 2]. Gallium-68-labelled desferrioxamine-B has been shown to be effective for PET imaging of microbial infections [3, 4]. Due to the lower stability of radiocomplexes, desferrioxamine-B derivatives - DFOstar, DFOstarSP, oxoDFOstar - have been developed [5]. These derivatives, labelled with gallium 68 and zirconium 89, are now being investigated for their in vitro and in vivo properties and for the possibility of imaging bacterial infections using photon emission tomography.

Results/discussion: All studied DFO-B derivatives were labelled with 68 Ga and 89 Zr with high (>=90%) radiochemical purity. All the tested 68 Ga/ 89 Zr-DFO-B derivatives showed hydrophilic properties, high stability in PBS, human serum, high in vivo stability (>90%) in blood after 5 min, and favorable in vivo behavior, which was manifested by rapid renal

excretion and low blood levels even in a short period of time after application (90 min for gallium-68, 3 h for zirconium-89). In vitro uptake showed that the labelled derivatives are taken up by bacteria such as S. aureus, S. agalactiae, P. aeruginosa. In the case of 89 Zr-DFO-B there is a possibility of imaging infections even 24 h after application of radiolabelled derivative.

Conclusions: All tested DFO-B analogues can be labelled with 68Ga and 89Zr and show promising in vitro and in vivo properties for PET imaging applications. Using these derivatives, it is possible to image bacterial infections caused by S. aureus, S. agalactiae, and P. aeruginosa.

## Acknowledgment

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# Surface-functionalised magnetic nanoparticles for multifunctional biomedical applications

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## Abstract

Magnetic nanomaterials are promising tools in bionanomedicine due to their intrinsic magnetic properties, biocompatibility, and variety of surface modifications. Among these, iron oxide-based magnetic nanoparticles (Fe3O4 MNPs) have been widely studied for a range of biomedical applications, including magnetic resonance imaging (MRI), targeted drug and gene delivery, cell tracking, and tissue engineering. Through advanced surface engineering and hierarchical functionalisation, these nanoparticles can be tailored as multifunctional agents for combined diagnostic and therapeutic use.

This study focuses on developing Fe3O4 MNPs for biomedical applications—specifically imaging, liquid biopsy, and integrated theranostic systems. A series of MNPs were synthesised and functionalised with various coatings, including polydopamine (PDA), polyvinylpyrrolidone (PVP), polyacrylic acid (PAA), tetraethyl orthosilicate (TEOS), (3-aminopropyl)triethoxysilane (APTES), and oleic acid (OA). These surface modifications were designed to enhance colloidal stability and biocompatibility, while preserving magnetic responsiveness and enabling further conjugation.

For imaging applications, PAA-coated MNPs were functionalised with deferoxamine (DFO) to facilitate gallium-68 radiolabelling, enabling dual PET/MRI. In the context of liquid biopsy, PAA-coated MNPs functionalised with streptavidin, along with submicron magnetic beads with silica shells based on OA-stabilised magnetic cores, were developed for nucleic acid capture.

Ultimately, this work aims to establish a nanoplatform that integrates magnetic properties with diagnostic and therapeutic capabilities, contributing to the development of magnetically guided, smart theranostic systems.

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# GenCReM: de novo design guided by explainable docking

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## Abstract

De novo generation methods represent a promising alternative to conventional virtual screening by enabling a more efficient exploration of the vast chemical space, which is too extensive for exhaustive enumeration and screening. Nonetheless, ensuring the synthetic feasibility of de novo-generated molecules remains a significant challenge for many current approaches. In our work, we employed the CReM[1] method, which inherently accounts for the synthetic accessibility of generated compounds. This was combined with a genetic algorithm and molecular docking to traverse chemical space efficiently. The primary goal of our tool is structure optimization, scaffold decoration, and exploration of local chemical space around a lead compound. The main feature is traversing chemical space guided by explainable docking preserving well-fitted 3D fragments while modifying suboptimal atoms, allowing the algorithm to converge faster than regular procedure. The optimized objective function can incorporate parameters such as docking scores, physicochemical and drug-likeness properties, and ligand-protein interaction fingerprints to preserve crucial interactions, etc. To some extent, the tool can be applied for unrestricted de novo generation and exploration of wider chemical space.

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# Searching for new CDK16 inhibitors in ultra-large libraries guided by de novo design

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## Abstract

The currently available combinatorial libraries of synthetically accessible compounds contain billions of structures, which can propose new chemotypes and scaffolds that greatly improve novelty of identified biologically active compounds. To efficiently explore these ul-tra-large chemical libraries, we suggested a protocol which includes a quick de novo gener-ation of structures able to fit to the binding site of a protein of interest followed by using these structures as queries for fast similarity search. As a primary aim of this study, we chose CDK16, which is involved in cell cycle regulation and is overexpressed in several can-cers (e.g., lung, prostate, breast, and malignant melanoma) [1].

De novo generation of compounds was carried out using the previously developed CReM-dock software, which integrates only open-source components, including MolGPka [2] for protonation state prediction. To enhance the synthetic accessibility of the generated ligands, fragment sources were derived from the Enamine and ChEMBL databases. Initial fragment docking was performed, followed by iterative fragment growth to optimize docking scores. The resulting structures were subsequently used as queries for similarity searches within the Enamine REAL database which contains 9.6 billion structures.

To identify an optimal setting of the suggested pipeline we investigated the impact of different fragment databases on the structural and physicochemical characteristics of the retrieved hits and their docking scores. We analyzed how the choice of molecular fingerprints influences the outcomes of similarity searches and properties of finally selected hits.

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# Translational modeling of tauopathy using differentiated SH-SY5Y cells: Validation with prion-like Tau aggregates and Oleocanthal modulation

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## Abstract

Tauopathies involve genetic factors such as MAPT mutations, which reduce tau's microtubule-binding ability and enhance its aggregation. These mutations contribute to differences in disease onset, progression, and pathology. To better model these mechanisms, we developed a rapid, two-step differentiation protocol for SH-SY5Y cells stably expressing inducible TauP301L-EGFP. Treating cells first with retinoic acid, then BDNF, generated cortical-like neurons with increased neurite complexity, cholinergic features, and strong expression of the 2N4R tau isoform. This provided a more physiologically relevant model for tauopathy studies.

To validate the model, differentiated cells were exposed to synthetic P301L tau fibrils to assess their susceptibility to prion-like seeding. Fibril exposure induced hallmark features of early tau pathology, including neuritic varicosity formation, reduced neurite diameter, and increased detergent-insoluble fractions of both endogenous and transgenic tau. These changes included increased pathological phosphorylation at Ser262 and higher T22 immunoreactivity. Co-treatment with oleocanthal, a small molecule known to disrupt tau fibrillization, mitigated these pathological changes, confirming the model's utility for pharmacological evaluation.

We are now using this differentiation protocol in 3D cultures to form structured neuronal assemblies that more closely resemble simplified brain-in-a-dish models. This extension increases the physiological relevance of the model and supports future development of organoid-based tauopathy systems. Together, these advances establish a practical and scalable human cell-based platform for studying tau pathology and testing therapeutic strategies.

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# Purpurin and Oleocanthal inhibit MAPT mutant Tau aggregation with strain selectivity

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## Abstract

Tau aggregation driven by pathogenic MAPT mutations is a hallmark of frontotemporal dementia (FTD), yet no approved therapies effectively target mutant tau species. Although rare in Alzheimer's disease (AD), these mutations provide critical insights into aggregation-prone domains of tau that are relevant across tauopathies. Here, we evaluated the in vitro anti-aggregation potential of two small molecules, purpurin (an anthraquinone derivative) and oleocanthal (a phenolic compound), against aggregation-prone tau variants using surface plasmon resonance (SPR) and thioflavin T (ThT) fluorescence assays. SPR analyses on the Bruker Sierra SPR-24 platform revealed preferential binding of both compounds to mutant tau peptides over wild-type (WT) tau. Purpurin displayed moderate affinity for WT tau ($K_D = 4.35 \times 10^{-6}$ M) and enhanced binding to N279K and V287I mutants, but weaker interaction with P301L ($K_D = 1.48 \times 10^{-5}$ M), likely due to faster dissociation kinetics ($K_{off} = 3.90 \times 10^{-4}$ $s^{-1}$). In contrast, Oleocanthal demonstrated broader binding across all tested mutants with significantly slower dissociation rates ($K_{off} = 8.26 \times 10^{-5}$ $s^{-1}$ for P301L, $8.69 \times 10^{-5}$ $s^{-1}$ for V287I, and $6.87 \times 10^{-5}$ $s^{-1}$ for N279K), indicating greater complex stability. Functionally, Purpurin selectively inhibited the aggregation of R2R3 domain mutants (V287I, N279K), while Oleocanthal showed broad-spectrum inhibition, including effective disruption of the P301L variant. These findings offer mechanistic insights into tau strain-selective inhibition and highlight oleocanthal as a promising therapeutic candidate for FTD.

Key Words: Tau aggregation, MAPT mutation, Surface Plasmon resonance, Oleocanthal, Purpurin

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# Focused siRNA screen for regulators involved in neurodegeneration

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## Abstract

RNA interference has been used for discovery and validation of new drug targets. Cell-based siRNA screen can be employed to identify genes that are involved in regulation of pathological processes. In the talk will be presented the cell-based model of a neurodegenerative disease and high-throughput assay for identification of responsible genes. The results will be confronted with effect of small-molecules as positive controls.

## Acknowledgment

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# A high-throughput binding assay for screening novel inhibitors of coronavirus infections

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## Abstract

The HTS (high-throughtput screening) technique accelerates the screening of a large numbers of potential biological modulators against selected and specific targets. This approach is routinely used in the pharmaceutical industry as well as in academic institutions as a primary tool for early-stage drug discovery. Presented screening campaign was focused on discovering novel antiviral compounds that inhibit CoV infection.

Our goal was to identify compounds that block the binding of the SARS-CoV-2 S-protein Receptor Binding Domain (RBD) to human angiotensin-converting enzyme 2 (hACE2), which is essential for infection. It is one of the key targets in the first phase of the SARS-Cov-2 replication cycle, where the binding of CoV to hACE2 or TMPRSS2 transmembrane protease serine 2 (TMPRSS2) allows entry into the host cell. This method, based on the enzymatic activity of hACE2, has been successfully miniaturized and implemented into HTS platform. In the primary screen, the inhibition activity of over 95.000 compounds from ECBL library were tested at one concentration (10 μM). To quantify the suitability of the assay in HTS, the Z-factor was determined for each plate. Data were analyzed by Dotmatics software.

Results obtained from the primary screening campaign will be presented and discussed.

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# Identifying the onset of cognitive changes in adulthood using four longitudinal studies

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## Abstract

Cognitive disorders represent a major health, social and economic burden for the world. Several studies have suggested that cognitive changes are observable as early as 40-50 years of age. However, the exact timing of these changes remains uncertain. The aim of this study is to identify the moment in adulthood when the first changes in cognitive performance can be identified. In this study, we use data from four longitudinal studies (ADNI, CHARLS, MIDUS, KV) and three continents, involving a total of 15,000 participants aged 26–94 years. Preliminary results suggest that the first cognitive decline occurs after the age of 40 and that significant symptoms appear with a 10-20 year delay. Further analyses using more refined analytical methods (using harmonization of cognitive data across datasets and mixed regression models) are being carried out to obtain definitive findings.

## Acknowledgment

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# Investigating the role of amyloid precursor protein in reactive astrogliosis during Alzheimer's disease

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## Abstract

Recent research is increasingly focusing on the influence of reactive astrogliosis during Alheimer´s disease (AD) progression. In our recently published work, the amyloid precursor protein (APP) overexpression led to reactive state of astrocytes and enhanced cytokine expression using in vitro model. However, the relationship between APP and astrocytic reactivity in AD is still subject of extensive research.

Here, we exlored morphology variability, as well as APP and interferon levels in AD reactive astrocytes in humans. Postmortem brain sections which included 3 healthy subjects and 3 AD patients were subjected to detailed morphological analysis and the optical density of APP and IFN was measured within the astrocytic cells.

GFAP stained sections show reactive astrocytes in AD which vary statistically in the majority of the observed morphological parameters in comparison to healthy subjects.

A significant difference was observed in the level of optical density for APP/GFAP and IFN/GFAP ratio between healthy and AD groups. There was also a distinctiveness in cell body for APP/GFAP and in both cell body and cell processes for IFN/GFAP ratio.

The outcome of this study confirms and corroborates our previous findings and highlights the impact of astrocytic APP as a valuable target for developing novel therapies to modulate reactive astrogliosis.

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# Revolutionizing aminoglycosides: Potent, safe, and orally bioavailable antibiotics against AMR

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## Abstract

Antimicrobial resistance (AMR) remains a pressing global health crisis, rendering many conventional antibiotics, including aminoglycosides, increasingly ineffective (1). Although aminoglycosides are celebrated for their broad-spectrum activity, their clinical use is limited by ototoxicity, nephrotoxicity, and poor oral bioavailability. Our research aims to develop a novel class of aminoglycosides that overcomes these challenges while maintaining and enhancing their antimicrobial efficacy.

Central to our approach is the integration of high-throughput (HT) synthesis and screening to accelerate the discovery and optimization of lead compounds (2). We have generated a structurally diverse library of aminoglycoside derivatives, systematically designed to improve their pharmacological properties. HT screening enables rapid evaluation of these derivatives, identifying candidates with the most favorable balance of antimicrobial activity, toxicity profile, and pharmacokinetics.

Structural modifications focus on mitigating ototoxic effects by reducing off-target interactions with cochlear cells, while simultaneously enhancing binding specificity for bacterial ribosomes to improve potency against resistant pathogens. Furthermore, we are addressing the challenge of oral bioavailability by introducing modifications that enhance membrane permeability and stability under gastrointestinal conditions.

Promising candidates identified through HT screening have demonstrated encouraging antimicrobial activity in preliminary assays, guiding the selection of compounds for further in-depth testing. Ongoing efforts include computational studies and the evaluation of key properties to refine lead candidates for future toxicity and pharmacokinetic studies.

This study underscores the transformative potential of combining HT synthesis and screening with modern computational methods to revolutionize antibiotic development. By delivering a novel class of aminoglycosides that are not only safer and more effective but also orally bioavailable, our work addresses critical unmet needs in the fight against AMR. These findings pave the way for more accessible and patient-friendly treatments for resistant bacterial infections, contributing to global efforts to combat this escalating threat.

## Acknowledgment

This research has been supported (AD) through the ERA Chair grant ACCELERATOR (101087318), the ERC Advanced grant AMADEUS (101098001), and the VIDEC grant (872195). This study was supported (AD) by the National Institute for Cancer Research—Programme EXCELES (ID Project No.LX22NPO5102), funded by the Cancer Research Czech Republic, and the Dutch Cancer Society (KWF Kankerbestrijding, KWF) grant (14712).

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# One-pot access to amenamevir: Process development and optimization in aqueous conditions

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## Abstract

Amenamevir, an antiviral drug used to treat herpes zoster, was synthesized via a one-pot Ugi four-component reaction (Ugi-4CR) in a simple, rapid, and sustainable manner. The focus of this study was on process development and optimization to enhance purity and yield. A thorough screening of solvents, reaction temperatures, times, and reagent equivalents was conducted, where water emerged as the optimal solvent, offering the best balance of efficiency, selectivity, and environmental safety. Reaction conditions were finely tuned to minimize byproducts and streamline purification. The final recrystallization protocol ensured high-quality material suitable for pharmaceutical application. This optimized process is scalable, cost-effective, and eco-friendly, outperforming existing synthetic methods.

# A rapid approach for identifying cell lines lacking functional cytidine deaminase

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## Abstract

Cytidine deaminase (CDA) is a key enzyme in the pyrimidine salvage pathway and plays a critical role in the metabolism of widely used cytidine analog chemotherapeutics, such as gemcitabine and cytarabine. Its activity level is a major determinant of both drug efficacy and toxicity, with high expression leading to chemoresistance. The ability to quickly and reliably identify cell lines lacking functional CDA is therefore crucial for cancer research, including studies on chemoresistance and the development of new CDA inhibitors. We have developed a novel, rapid, and cost-effective functional assay to identify CDA deficiency in intact cells. The method is based on the direct enzymatic transformation of 5-fluorocytidine (FC) to 5-fluorouridine (FU) in cells with functional CDA. The resulting FU is subsequently incorporated into cellular RNA, where it is detected in situ via immunofluorescence. The analysis is performed using standard fluorescence microscopy. The assay was validated on a panel of six human cell lines with known CDA protein expression levels. A strong and statistically significant fluorescent signal ($p < 0.05$) was observed exclusively in CDA-proficient cell lines (NCI-H2009, A549, HeLa, and HepG2). Conversely, CDA-deficient cell lines (hTERT RPE-1 and IMR-90) showed no detectable signal ($p > 0.05$). The results of our functional assay showed a perfect correlation with the presence or absence of CDA protein as determined by Western blot analysis. The developed approach is significantly faster and more cost-effective than existing techniques and provides a clear, qualitative 'yes/no' result for functional CDA activity. This tool has significant potential for basic research applications in chemoresistance modeling and the development of new anticancer therapies.

## Acknowledgment

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# Evaluation of biologic activity of organoselenium and organotellurium derivatives

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## Abstract

Organoselenium compounds such as ebselen display potent cytoprotective effects by mimicking glutathione peroxidase (GPx) activity. In our high-throughput screening we identified organoselenium compounds of several structural classes with the ability to protect cells against various stress inducers. Remarkably, several of these analogues demonstrated significantly stronger protective activity than ebselen. Unlike ebselen, however, they exhibited much weaker GPx-like activity while more effectively scavenging a broad spectrum of radical species.

## Acknowledgment

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# Response to diverse stress modalities in Tardigrades

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## Abstract

Tardigrades can survive extreme stresses ranging from complete desiccation to high doses of radiation and temperature extremes. Yet the molecular underpinnings of this resilience remain largely obscure. It is also unclear whether a single, universal defense strategy underpins their resistance to such a broad spectrum of stress.

We investigated survival of several Tardigrade species following exposure to X-rays, a mix of gamma and neutron radiation and heat stress, using automated microscopy and image analysis. While the resilience of tardigrades in their active stages to ionizing radiation is impressive (with LD50 values in the thousands of Gy), they are unable to withstand temperatures above 42°C. We also report results of exposure of Hypsibius exemplaris to oxidative stress, UV radiation and temperature and evaluation of cross-resistance studies.

Additionally, we report sensitivity of H. exemplaris to metals—our automated analysis optimized for robust tardigrade detection in complex images allowed for parallel testing of all relevant salts, a capability unprecedented in invertebrate toxicology. We propose that tardigrades could be used as a novel model organism in (eco) toxicology. Finally, we share insights from our experience with tardigrade DNA barcoding.

## Acknowledgment

The project was supported by infrastructural project CZ-OPENSCREEN (LM2023052) and the project National Institute for Neurological Research (Program EXCELES, ID Project No. LX22NPO5107) - Funded by the European Union - Next Generation EU from the Ministry of Education, Youth and Sports of the Czech Republic (MEYS).

# Antimycobacterial property of compounds from proprietary library

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## Abstract

Tuberculosis, a disease caused by M. tuberculosis remains a serious health problem due to high incidence and mortality globally. The emergency of drug-resistant strains of M. tuberculosis and the increase in disease incidence, stresses the need for development of novel drugs. The objective of this study was to identify the novel compounds with activity against three different mycobacteria including two Mycobacterium bovis substrain Russia, standard Mycobacterium tuberculosis standard H37Rv reference strain and multidrug resistant Mycobacterium tuberculosis clinical isolate. The activity of compounds from proprietary library were tested for activity of compounds of proprietary library against these bacteria in a high throughput growth inhibition assay. One primary high throughput screen of 4800 compounds for antimycobacterial activity against M. bovis BCG Russia showed that fifty compounds were identified as the primary hits, which at concentration of 50 μM inhibited the bacterial growth at the rate of ≥ 50%. The dose-response analysis showed that forty-two compounds had the activity against both strain with IC50 smaller than 10 μM. However, as the seventeen hits were ignored due to their previously published activity and eighteen hits were cytotoxic to human BJ fibroblast and/or mouse J774 cells, only seven secondary hits were tested for activity against intracellular M. bovis substrain Russia in the infected mouse J774 cells. Five secondary hits with the IC50 against intracellular bacteria smaller than 10 μM were selected as the final hits. Four of these final hits were also active against standard H37Rv reference strain and multidrug resistant isolate of M. tuberculosis in dose response assays. The other primary high throughput screen of 3500 compounds identified 221 compounds with activity against M. bovis substrain Russia. The focus of ongoing analyses is on these compounds.

## Acknowledgment

The study was supported by grants from the Czech Ministry of Education, Youth and Sports (CZ-OPENSCREEN-LM2018130, EATRIS-CZ-LM2018133), European Regional Development Fund-Project ENOCH (No. CZ.02.1.01/0.0/0.0/16_019/0000868), IGA_LF_2021_036 (Palacký University in Olomouc) and the European Union - Next Generation EU (The project National Institute for Cancer Research (Programme EXCELES, ID Project No. LX22NPO5102) and The project National Institute of Virology and Bacteriology (Programme EXCELES, I

# Multi-platform analytical validation and computational analysis of high-throughput covalent library quality control

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## Abstract

Quality control of large compound libraries remains a critical bottleneck in drug discovery. We present a comprehensive data-driven comparison of three complementary analytical platforms—$^1$H NMR, UPLC-UV-MS, and acoustic ejection mass spectrometry (AEMS)—applied to a 1,235-member acrylamide library synthesized via precipitation-driven Ugi chemistry. Our computational workflow employed machine learning-enhanced PeakSel® software pipeline, converting heterogeneous data formats (.raw, .mzML) into standardized matrices for cross-platform statistical analysis. AEMS utilized Echo® MS+ with ZenoTOF 7600, generating mass spectrometric datasets processed via ProteoWizard for extracted ion chromatogram (XIC) generation and direct quantitative comparison with UPLC-MS standards.

Confusion matrix analysis revealed AEMS's superior sensitivity (91.2%) but lower specificity (65.0%) versus UPLC-MS ground truth, with ionization mode significantly impacting performance: negative mode showed stronger correlation (Pearson $r = 0.74$, $p < 0.001$) than positive mode ($r = 0.54$, $p < 0.01$) due to reduced total ion current (TIC) background interference. Principal component analysis distinguished targeted covalent inhibitors from discovery compounds, revealing platform-specific detection biases. The integrated approach provided complementary insights: AEMS excelled at rapid compound verification and degradation kinetics, while UPLC-MS offered superior impurity profiling through chromatographic separation.

Two-year DMSO storage stability analysis revealed 89% compound detectability by AEMS, with dual-platform validation confirming 82% stability. Area-under-curve correlation analysis demonstrated platform-specific quantitative reliability, establishing analytical confidence intervals for library assessment. This work validates AEMS as a transformative high-throughput library QC tool and establishes a computational framework for systematic analytical platform optimization in drug discovery workflows.

**Keywords:** Acoustic ejection mass spectrometry, computational analysis, library quality control, data processing pipeline, multiplatform validation, drug discovery analytics

## Acknowledgment

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# CDK PROTACs: Advancing targeted protein degradation for pediatric neuroblastoma

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## Abstract

Triazolopiperazines represent a privileged molecular scaffold in pharmacology, present in both marketed and developmental drugs due to their unique structural features and inherently low cLogP values. Current synthetic routes to these compounds are often lengthy, costly, reliant on expensive transition metal catalysis, and offer limited scope for structural variation. We have designed an innovative, one-step synthetic access to the triazolopiperazine core, leveraging multicomponent reaction chemistry. This approach aims to circumvent metal catalysis entirely, thereby enabling access to an unprecedented variety of products with three distinct points for substitution. The proposed mechanism involves a Ugi-type reaction featuring an in-situ Wittig ring closure, followed by a condensation and a second ring closure to afford the target triazolopiperazine, either through isolation of an intermediate oxadiazole or via a streamlined one-pot procedure. Optimization of the reaction conditions is planned using High-Throughput Experimentation (HTE) with parallel synthesis equipment. The scalability of the reaction is projected to be established from the nano to the tens of millimole scale, including multi-gram syntheses of the marketed diabetes drug Sitagliptin and otherwise unobtainable derivatives via existing routes. Big data generation through nanoscale synthesis on 1536-well plates and high-throughput analytics will provide an unprecedented wealth of structure-reactivity relationship insights. Ultimately, we intend to demonstrate that these libraries of triazolopiperazines serve as interesting intermediates for additional transformations, leading to further products of unparalleled complexity in just a few synthetic steps.

# Triazolopiperazines enabled by high throughput experimentation

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## Abstract

Triazolopiperazines represent a privileged molecular scaffold in pharmacology, present in both marketed and developmental drugs due to their unique structural features and inherently low cLogP values. Current synthetic routes to these compounds are often lengthy, costly, reliant on expensive transition metal catalysis, and offer limited scope for structural variation. We have designed an innovative, one-step synthetic access to the triazolopiperazine core, leveraging multicomponent reaction chemistry. This approach aims to circumvent metal catalysis entirely, thereby enabling access to an unprecedented variety of products with three distinct points for substitution. The proposed mechanism involves a Ugi-type reaction featuring an in-situ Wittig ring closure, followed by a condensation and a second ring closure to afford the target triazolopiperazine, either through isolation of an intermediate oxadiazole or via a streamlined one-pot procedure. Optimization of the reaction conditions is planned using High-Throughput Experimentation (HTE) with parallel synthesis equipment. The scalability of the reaction is projected to be established from the nano to the tens of millimole scale, including multi-gram syntheses of the marketed diabetes drug Sitagliptin and otherwise unobtainable derivatives via existing routes. Big data generation through nanoscale synthesis on 1536-well plates and high-throughput analytics will provide an unprecedented wealth of structure-reactivity relationship insights. Ultimately, we intend to demonstrate that these libraries of triazolopiperazines serve as interesting intermediates for additional transformations, leading to further products of unparalleled complexity in just a few synthetic steps.

## Acknowledgment

This research has been supported through the ERA Chair grant ACCELERATOR (101087318), the ERC Advanced grant AMADEUS (101098001), and the VIDEC grant (872195). This study was supported by the National Institute for Cancer Research—Programme EXCELES (ID Project No. LX22NPO5102), funded by the Cancer Research Czech Republic.

# Innovative design of K-Ras G13C covalent inhibitors

*Mayur Mukim, Alexander Dömling, George Gouridis, Atilio Reyes Romero, Zeinab Saedi, Foteini Efetzi, Marian Hajduch, Martin Onda, Lukas Lenart, Marzieh Mosazadeh, Jan Kroenke*

## Abstract

KRAS G13C is the second most common KRAS mutation in cancer, but unlike the well-studied G12C mutation, it has remained undrugged due to the challenge of targeting its highly conserved and GTP-occupied binding pocket. This research focuses on developing the first covalent inhibitors specifically targeting KRAS G13C by designing small molecules that mimic GTP and selectively bind to the cysteine at position 13. The approach aims to overcome the high binding affinity and intracellular concentration of GTP by covalent modification. Key challenges include developing stable, cell-permeable GTP mimics and achieving selective C13 reactivity. Using structure-based design and protein mass spectrometry screening, this work seeks to deliver the first drug-like G13C inhibitors, with broader potential for targeting other GTPases in disease.

## Acknowledgment

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# Exploring Kras inhibition: A biophysical perspective

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## Abstract

Ras proteins are membrane-bound GTPases that act as molecular switches, cycling between inactive GDP-bound and active GTP-bound states to regulate cell proliferation, survival, and differentiation. Among Ras isoforms, KRas is often called the "Holy Grail" of cancer research, as activating mutations—particularly at residues G12 and G13—occur in approximately 30% of human cancers, including pancreatic, lung, and colorectal tumors, some of the deadliest cancer types in the USA [2].

Although G12 mutations are more prevalent, G13 mutations account for around 14% of KRas-driven tumors, with the G13C mutation representing 6%. This mutation affects nearly 7,000 lung cancer patients annually in the U.S., highlighting its clinical importance [1]. Our research aims to develop G13C-targeted nucleotide analogues that bind to the nucleotide-binding pocket and covalently modify the mutant cysteine. Thermal shift assays show that these compounds react under optimized conditions that differ from those previously described.

In parallel, we have designed a new class of guanine isosteres, synthesized using innovative chemical strategies that preserve essential hydrogen bonding interactions. Using biophysical techniques, we confirmed their binding to the GTP/GDP pocket and compared their affinities to those of natural nucleotides.

Additionally, we investigate pan-Ras allosteric inhibitors inspired by molecules such as BI-2852, which stabilize Ras dimers in an isolated in vitro model [3]. By locking KRas into an inactive conformation, these inhibitors disrupt interactions with guanine nucleotide exchange factors (GEFs), GAPs, and downstream effectors like RAF, effectively blocking oncogenic signaling. This broad-spectrum mechanism offers a promising strategy to overcome resistance commonly seen with mutation-specific therapies. To validate the activity of our compounds, we employ nucleotide exchange assays (NEA) to assess their efficacy in vitro, including testing SOS1 inhibitors as complementary agents.

Together, these strategies mark a significant advance in targeting Ras-driven malignancies and offer renewed hope for more effective cancer therapies.

## Acknowledgment

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# Accelerated access to rna-aided small molecule discovery

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## Abstract

For many years, RNA has been largely overlooked as a pharmaceutical target in favor of proteins. Since the first protein structure was crystallized and deposited in the Protein Data Bank (PDB) [1], more than 92% of the deposited structures have been proteins, whereas only 3% are RNA structures—and even fewer are RNA–small molecule complexes—highlighting a significant underrepresentation. Despite the completion of the Human Genome Project, only a small fraction of the genome has been successfully drugged. Approximately 1.5% of the genome encodes proteins (roughly 20,000 in total), and it is estimated that only 10–15% of these (~2,000–3,000, representing 0.2% of the genome) are disease-related. Currently, fewer than 700 of these proteins (~0.05% of the genome) are targeted by therapeutic drugs [2–4]. Targeting RNA could substantially expand the druggable portion of the genome, as potential RNA targets include messenger RNAs encoding disease-related proteins—particularly those considered “undruggable”—as well as various non-coding RNAs (e.g., microRNAs, riboswitches, ribosomal RNAs, and long non-coding RNAs) implicated in disease processes.

In this work, we present the purification of several highly structured RNAs for high-throughput crystallography. We also show preliminary biophysical screening data for the discovery of small molecules acting as molecular glues, with potential applications in antibiotic development or rare neurological diseases. In collaboration with industrial and academic partners and supported by the recent ERC-funded AMADEUS platform—which focuses on the “miniaturization + automation” mantra—this project aims to establish a high-throughput RNA crystallography pipeline. This will help overcome current limitations in RNA-targeted drug discovery, expedite structural determination, and ultimately contribute to the advancement of RNA-focused therapeutics.

## Acknowledgment

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# Structure standardization and curation pipeline

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## Abstract

Within the project a chemical structure curation workflow was developed based on the open-sourceRDKit library to replace proprietary Chemaxon tools used before. The workflow has twoapplications: I) checking structures before uploading to the database to identify possible issues andreport them, ii) standardize and prepare structures for modeling. The workflow is consisted ofmultiple individual checks which may be combined in a specific order to get a desired result. Thereare steps such as removing salts and inorganic fragments, neutralizing formal charges, whilepreserving molecular metadata. The pipeline also checks and corrects stereochemical features,including invalid wedge/dash bonds and undefined stereocenters or double bonds. Additionally, aseries of SMARTS-based transformations is applied to normalize reactive or ambiguous functionalgroups like nitro, azide, and diazo groups. The RDKit-based implementation offers a flexible, open,and maintainable solution for routine structure curation tasks.

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