XXIX Gliwice Scientific Meetings

Gliwice, November 20-21, 2025
http://gsn.io.gliwice.pl/

ORGANIZERS OF GLIWICE SCIENTIFIC MEETINGS 2025:

Association for the Support of Cancer Research

Maria Skłodowska-Curie National Research Institute of Oncology

Silesian University of Technology

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Maria Sklodowska-Curie
National Research
Institute of Oncology

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GLIWICE SCIENTIFIC MEETINGS 2025

SCIENTIFIC PROGRAM

THURSDAY, 20TH NOVEMBER 2025

9:00 – 9:15 Opening of the Conference
Welcome addresses
Remembering Prof. Mieczysław Chorąży (1925-2021)

9:15 – 10:00 ***Prof. Mieczysław Chorąży Memory Lecture 2025:***
Marc Baumann (*University of Helsinki, Helsinki*): All you need to know about Alzheimer's disease.

10:00 – 13:30 Session ***„Brains and Genomes"***
Chairpersons: Roman Jaksik & Wiesława Widłak
Claude R. Cloninger (*Washington University, St. Louis*): How and why has human personality evolved?
Giordano Lippi (*The Scripps Research Institute, La Jolla*):
MicroRNA mechanisms of neuronal diversification.

Coffee Break
Joanna Rzeszowska (*Silesian University of Technology, Gliwice*):
Amplifications and deletions in the human genome.
Gunter Meister (*University of Regensburg, Regensburg*):
Contribution of non-coding RNAs to social fear adaptation in mice.
Aleksandra Pękowska (N*encki Institute of Experimental Biology, Warszawa*):
Evolution of astrocytes in primates: from genes to phenotypes.
Andrew Yoo (*Washington University, St. Louis*): MicroRNAs as potent cell-fate effectors and modeling late-onset neurodegeneration via direct somatic reprogramming.
Łukasz Chrobok (*Jagiellonian University, Krakow*): Deciphering circadian rhythms in gene expression in the brainstem satiety centre - implications for obesity management.

13:30 – 14:30 Lunch

14:30 – 16:30 **Poster Session** (Part I – odd numbers and Part II – even numbers) and Coffee

16:30 – 18:00 Session „Brains and Artificial Intelligence":
Chairpersons: Krzysztof Fujarewicz & Andrzej Polański
Krzysztof Fujarewicz (*Silesian University of Technology, Gliwice*):
Biological inspirations in machine learning.
Igor Zwir (*Washington University, St. Louis*): Brain AI for digital mental health: multi-
-layer machine learning across omics, imaging, and neuromodulation.
Jacek Rumiński (*Gdańsk University of Technology, Gdańsk*): Deep learning in brain imaging studies.
Andrzej Polański (*Silesian University of Technology, Gliwice*): Modeling slow degradation processes with the use of Fisher–Kolmogorov wave.

19:30 - Social Event

FRIDAY, NOVEMBER 21, 2025

8:30 – 11:00 Session: ***„Extracellular Vesicles in neuronal communication”***:
Chairpersons: Monika Pietrowska & Piotr Widłak
Eva-Maria Krämer-Albers (*Johannes Gutenberg University Mainz, Mainz*): Extracellular vesicles in neuron-glia communication and CNS homeostasis.
Rossella Di Giaimo (*University of Naples Federico II, Naples*): Extracellular vesicle-mediated trafficking of molecular cues during human brain development and disease alteration.
Katalin Lumniczky (*National Centre for Public Health and Pharmacy, Budapest*): The role of microenvironment in radiation-induced brain tumorigenesis – the DISCOVER project.
Eulalia Marti (*University of Barcelona, Barcelona*): Plasma Extracellular Vesicle-associated small RNAs as Early Biomarkers in Huntington's Disease.
Urszula Wojda (*Nencki Institute of Experimental Biology, Warszawa*): Prospects of exosomal miRNAs and artificial intelligence in the diagnostics and prognostics of neurodegenerative diseases.

11:00 – 11:15 Coffee Break

11:15 – 13:20 Session: ***„Education and Implementation of Research”***
Chairpersons: Katarzyna Lisowska & Dorota Ścieglińska
Barbara Kraj (*Old Dominion University, Norfolk*): Introducing molecular diagnostics into the clinical laboratory education.
Olena Danylyuk (*Henry Ford Innovation Institute, Detroit*): Transforming Ideas into Impact: IP, Commercialization, and Partnerships in Academic Medical Centers.
Anna Habryka-Pawlowska (*Preclinical Services, Revvity, Cambridge*): From Screening to Success: Supporting Early Discovery Programmes from Inception to Clinic.
Dorota Iwaszkiewicz-Grześ (*Medical University of Gdańsk, Gdańsk*): T regulatory cells in the treatment of autoimmune diseases – from bench to bedside.
Iwona Ługowska (*MSCI National Research Institute of Oncology, Warszawa*): Polish Cancer Mission Hub – the pentahelix model of stakeholder cooperation.

13:20 – 14:00 Lunch

14:00 – 17:45 Session: ***„New Trends in Medical Biotechnologies”***
Chairpersons: Magdalena Skonieczna & Petr Humpolíček
Kevin Dean (*UT Southwestern Medical Center, Dallas*): Decoding Biology with Autonomous Multiscale Imaging.
Seweryn Gałecki (*UT Southwestern Medical Center, Dallas; Silesian University of Technologies, Gliwice*): Highly multiplexed imaging of subcellular architectures with nanoscale precision.
Petr Humpolíček (*Tomas Bata University, Zlin*): Novel methods of preparation of conductive composites based on polypyrrole.
Jan Vícha (T*omas Bata University, Zlin*): Anticancer drug carriers: a relic of the past or the future of cancer therapy?

Coffee Break
Marcin Kamiński (St. *Jude Children's Research Hospital, Memphis*): How mitochondrial metabolism controls T cell activation, i.e., 'How to fix an old engine using metabolic tracing analysis'?
Domenico Vittorio Delfino (U*niversity of Perugia, Perugia*): When nature meets precision medicine: natural compounds against NPM1-mutant AML.
Jan Hansmann (U*niversity of Applied Sciences Würzburg-Schweinfurt, Schweinfurt*): How engineering technology supports life science.

17:45 – 18:15 **Poster Awards** and Closing of the Conference

THIS YEAR MARKS THE 100TH ANNIVERSARY OF THE BIRTH OF PROFESSOR MIECZYSŁAW CHORĄŻY (1925-2021)

The Professor worked at the Institute of Oncology in Gliwice for 70 years (1951-2021) and headed the Department of Tumor Biology for 37 years (1958-1995).

During his scientific career, he was passionate about research on cancer cell metabolism, the mechanisms of DNA entry into eukaryotic cells, genome structure and function, environmental mutagenesis, molecular epidemiology, chaos theory, the self-organization of matter, and the origin of life on Earth.

He was a Member of the Polish Academy of Sciences, the Polish Academy of Arts and Sciences (PAU), the Polish Commission for UNESCO, the European Association for Cancer Research, NSZZ Solidarność (Solidarity Trade Union), and many others.

In the 1960s, Prof. Chorąży started the tradition of scientific meetings in Gliwice (since 1997, they have become the annual Gliwice Scientific Meetings).

He was the initiator of the Popular Science University of the Polish Academy of Arts and Sciences (Wszechnica PAU) in Gliwice, for which he was awarded the title of a "Man of the Gliwice Land" (foto). He initiated the Polish edition of the textbook "Teaching about Malignant Cancers in Schools. A Guide for Teachers." He also organized the scholarship program for scientists from Eastern Europe, financed by NCI-NIH and UNESCO, implemented at the Institute of Oncology in Gliwice (1996-2011).

Prof. Chorąży was an insurgent fighting in the Warsaw Uprising of 1944, a Knight of the Order of the White Eagle (the most prestigious Polish state decoration), and many other state decorations, a mentor and educator of many generations of scientists at the Institute of Oncology, a good man, and our friend.

PROF. MIECZYSŁAW CHORĄŻY MEMORY LECTURE

To honor and commemorate Professor Chorąży, since 2021 Gliwice Scientific Meetings conference has started with an *in-memoriam* keynote lecture.

Previous speakers:

2021 **Kari Hemminki** (*Charles University, Pilsen & DKFZ, Heidelberg*): Science and society.

2022 **Philip Maini** (W*olfson Centre for Mathematical Biology, Oxford*): Can mathematical modelling help us understand cancer growth and progression?

2023 **Victor Ambros** (*University of Massachusetts, Worcester*) (Nobel Prize winner 2024): Genetic regulatory networks involving noncoding RNA.

2024 **Theresa L. Whiteside**, (*University of Pittsburgh Medical Center, PA, USA*): Exosomes: the ubiquitous intracellular communication system in human health and disease.

PROF. MIECZYSŁAW CHORĄŻY SCHOLARSHIP FOR YOUNG SCIENTISTS

Professor Mieczysław Chorąży was an eminent Man, an outstanding scientist, soldier of the Warsaw Uprising, moral authority for generations of fellow researchers, and an exceptionally warm person.

To commemorate Him, the Association for the Support of Cancer Research has funded a scholarship for young researchers who seem to follow his path.

Previous Awardees:

2021 **Alexander Cortez**, PhD (M*aria Skłodowska-Curie National Research Institute of Oncology, Gliwic*e)

2022 **Paulina Marona**, PhD (*Jagiellonian University, Kraków*)

2023 **Anna Sobiepanek**, PhD Eng. (*Warsaw University of Technology, Warsaw*)

2024 **Marcelina Jureczko**, PhD Eng. (*Silesian University of Technology, Gliwice*)

PROF. MIECZYSŁAW CHORĄŻY MEMORY LECTURE 2025

Prof. Marc Henrik Baumann, Ph.D
Research Director,
Meilahti Clinical Proteomics Core Unit,
Biomedicum Helsinki, Finland

Born in Geneva/Switzerland (April 1, 1954)

UNIVERSITY DEGREES:
1984 – MB; 1991 – PhD; 1995 – Adj.Prof., 1996 – Ass.Prof., 2001 – MBA

ACADEMIC POSITIONS:
1982-1984 Faculty of Medicine, University of Zurich; 1985-1987 – Institute of Biotechnology, University of Helsinki; 1987-2001 – Institute of Biomedicine, Department of Medical Chemistry, Faculty of Medicine, University of Helsinki; 1989-1991 – Bio-Rad Laboratories; 1993-1995 – Department of Pathology, New York University Medical Center; 1999 – Medical Faculty, Second University of Napoli; 2013-2014 – Medical Faculty, University of Salerno, 2014-2016 – Faculty of Medicine, Milano University; 2001-ongoing – Meilahti Clinical Proteomics Core Unit, Biomedicum Helsinki, University of Helsinki.

RESEARCH INTERESTS:
The main focus of Dr. Baumann is neurology, neuroscience, and neuromedicine with a particular interest in misfolding diseases (incl. Alzheimer's Disease, Prion Diseases, Amyloidoses, etc.). His main scientific achievement is the discovery of the clinicopathological cascade leading to Alzheimer's disease. This knowledge, today largely accepted, was a pioneering work in the late 90-ties, and led to the discovery and registration of several patents for drugs designed for Alzheimer's disease treatment. A drug designed by Dr. Baumann to treat AD was licensed to Serono (later acquired by Merck). Today, humanized monoclonal antibodies, based on similar thoughts of AD treatment, have been introduced to the market by Esai/Biogen and Eisai/BioArctic.

Currently, the research interests of Dr. Baumann include studies on the development of new microchip-based tools for clinical diagnostics. His work has led to patents and products in collaboration with SONY/Shimadzu/Tethis/SpA/Stratek. In 2001, Dr. Baumann founded a company intending to develop research instrumentation based on microfluidics and SMART surfaces.

Dr. Baumann has over 140 publications in peer-reviewed scientific journals (his papers were quoted almost 7,000 times), several patents, book chapters, and has made appearances on TV and radio. Additionally, he is actively involved in teaching medical students and participating in educational activities.

LECTURE ABSTRACTS

Prof. Mieczysław Chorąży Memory Lecture

ALL YOU NEED TO KNOW ABOUT ALZHEIMER'S DISEASE

Marc Baumann

Medicum, Faculty of Medicine, University of Helsinki, Helsinki, Finland.

Alzheimer's disease is one of the most common forms of dementia, a brain disorder that slowly destroys a person's memory and behaviour. It is characterized by the loss of cognitive functioning – thinking, remembering, and reasoning. Eventually, people with Alzheimer's disease lose the ability to perform simple daily tasks, such as eating or walking. Current estimates suggest that more than 55 million people worldwide suffer from AD. Since Age is the biggest known risk factor for Alzheimer's disease, and people just live longer and longer, the disease is expected to rapidly rise in number as the population ages. For many years and with a lot of effort (over 250,000 scientific publications in PubMed (https://pubmed.ncbi.nlm.nih.gov)), scientists have tried to reveal the cause of this devastating disease, and yet, only very recently have we seen some light at the end of the road. Why has the path for the treatment been so difficult, and what have we missed all these years? In my lecture, I will describe step-by-step how the disease is initiated and how it progresses to the final stage of no return. I will also give the audience a summary of other similar diseases and their link to Alzheimer's disease. In the end, I will envision the effects of current treatments and how they will, if they will, change the field of Alzheimer's disease patient care.

Session

„Brains and Genomes”

HOW AND WHY HAS HUMAN PERSONALITY EVOLVED?

C. Robert Cloninger

Washington University, St. Louis, MO, USA

Human personality reflects the dynamic organization of brain functions that underlie our ability to learn, adapt, and flourish across changing environments. In this lecture, I will present recent interdisciplinary advances that clarify the evolutionary development of personality as a system of three integrated learning networks – temperament, intentionality, and self-awareness – that shape the psychobiological core of the human experience. Drawing from comparative genomics, we trace how *Homo sapiens* evolved distinct neurogenetic systems supporting creativity, prosocial behavior, and healthy longevity, features that distinguish our species from Neanderthals and other hominids.

I will examine the emergence of self-awareness in human evolution, its neural and genetic foundations, and its measurable impact on well-being, resilience, and social connectedness. Three nearly disjoint networks of genes – mapped through over 900 genetic loci – correspond with specific profiles of personality and learning. Notably, 95% of the genes unique to modern humans are non-protein coding RNAs that regulate gene co-expression in brain regions responsible for self-awareness and emotional integration.

These findings illuminate how self-awareness has enhanced human adaptability by fostering complex symbolic thinking, artistic expression, and intergenerational caregiving – a view supported by the grandparenting hypothesis of human creativity. I will also discuss implications for medicine, artificial intelligence, and public health in the genomic era, highlighting the necessity of integrating pharmacological, emotional, cognitive, social, and spiritual approaches to promote human health and well-being.

This presentation offers a novel synthesis of genetics, neurobiology, and psychology that reshapes our understanding of human personality as a core mechanism for survival, health, and cultural evolution.

MICRORNA MECHANISMS OF NEURONAL DIVERSIFICATION

Giordano Lippi

Scripps Research Institute, La Jolla, CA, USA

MicroRNAs are critical for brain development; however, if, when, and how microRNAs drive neuronal subtype specification remains poorly understood. To address this, we engineered technologies with vastly improved spatiotemporal resolution that allow the dissection of cell-type-specific miRNA-target networks. Fast and reversible miRNA loss of function showed that miRNAs are necessary for Purkinje cell (PC) differentiation, which previously appeared to be miRNA independent, and identified distinct critical miRNA windows for dendritogenesis and climbing fiber synaptogenesis, structural features defining PC identity. Using new mouse models that enable miRNA-target network mapping in rare cell types, we uncovered PC-specific post-transcriptional programs. Manipulation of these programs revealed that the PC-enriched miR-206 and targets Shank3, Prag1, En2, and Vash1, which are uniquely repressed in PCs, are critical regulators of PC-specific dendritogenesis and synaptogenesis, with miR-206 knockdown and target overexpression partially phenocopying miRNA loss of function. Our results suggest that gene expression regulation by miRNAs, beyond transcription, is critical for neuronal subtype specification.

AMPLIFICATIONS AND DELETIONS IN THE HUMAN GENOME

Roman Jaksik, Joanna Rzeszowska

Silesian University of Technology, Gliwice, Poland

Human genomes exhibit considerable variability, particularly within non-coding regions. This genetic diversity manifests through single-nucleotide polymorphisms (SNPs), as well as structural variations such as duplications, deletions, inversions, and translocations of DNA fragments of varying sizes. Understanding the mechanisms and implications of such variation—especially those affecting development and brain function-has become a key focus in both biological and artificial intelligence research.

In this study, we analyzed the genomes of 32 donors, including several family trios (parents and children). Genomic DNA was isolated from blood cells using standard procedures and sequenced by a professional service provider. We examined the distribution of SNPs, amplifications, and deletions across entire genomes and individual chromosomes. Family-based analyses revealed de novo amplifications and deletions in all offspring, with deletions consistently more frequent than amplifications across all individuals.

Copy number variation (CNV) analysis identified three distinct groups of alleles present in atypical copy numbers in some or all donors. The first group comprised sequences that were exclusively amplified. The second group included sequences amplified in some individuals but deleted in others. The third group consisted of sequences that were exclusively deleted. Among the most frequently affected sequences, we identified protein-coding genes, pseudogenes, and long non-coding RNAs (lncRNAs).

Of the 760 alleles analyzed, 220 exhibited either amplification or a standard two-copy configuration, while 250 alleles showed deletions exclusively, with no amplifications observed in any donor. These findings suggest the existence of coordinated amplification and deletion programs active during embryogenesis and within specialized cellular populations of the organism.

CONTRIBUTION OF NON-CODING RNAS TO SOCIAL FEAR ADAPTATION IN MICE

Gunter Meister

University of Regensburg, Regensburg, Germany

Gene expression is regulated at many post-transcriptional steps and is, among others, mediated by non-coding RNAs, including microRNAs (miRNAs) or long non-coding RNAs (lncRNAs). MiRNAs bind to 3' untranslated regions of target mRNAs and repress their expression. The functions of lncRNAs are more diverse, and many of them function in the nucleus of cells, associate with chromatin, and affect transcriptional output. However, various other functions have also been discovered. Both non-coding RNA classes play critical roles in diverse cellular and physiological processes and have been associated with the manifestation and progression of many diverse diseases.

Social interactions are critical for mammalian survival and evolution. Dysregulation of social behavior often leads to psychopathologies such as social anxiety disorder, denoted by intense fear and avoidance of social situations. Such dysfunctions can be monitored in a mouse model for social fear conditioning (SFC). Using this mouse model, we profiled miRNAs and lncRNAs in the septum, a brain region important for social behavior. We particularly compared animals with successful and unsuccessful social fear extinction after acquisition and functionally characterized miR-132 as well as the lncRNA Meg3 in this process. Our work highlights the relevance of non--coding RNAs for social behavior in mice, which could potentially also be important for human social disorders.

EVOLUTION OF ASTROCYTES IN PRIMATES: FROM GENES TO PHENOTYPES

Aleksandra Pękowska

Dioscuri Centre for Chromatin Biology and Epigenomics, Nencki Institute of Experimental Biology, Polish Academy of Sciences, Warsaw, Poland

Astrocytes contribute to the establishment and regulation of the brain's higher-level functions. Evolutionary changes in astrocyte activity during development and adulthood likely help establish the unique cognitive capacities of the human brain. However, while the transcriptional differences between human and non-human primate (NHP) adult astrocytes are increasingly better defined, the molecular signature of fetal astrocyte evolution is unknown.

We used human, chimpanzee, and macaque induced pluripotent stem cell-derived astrocytes (iAstrocytes) as a robust source of fetal astrocytes. Human iAstrocytes are bigger and more complex than NHP iAstrocytes. We found new loci and cellular pathways related to the interspecies differences in astrocyte size and complexity. Strikingly, genes that feature lower expression in humans than in NHP iAstrocytes frequently relate to neurological disorders, including intellectual disability, opening new questions on the relationship between evolution and the higher-level mental capacities of our brain.

Evolution is largely fuelled by changes in gene activity, which in turn arise as a corollary to genetic modification of distal DNA regulatory elements, including enhancers. Enhancers evolve fast. Yet, whether there are general and broadly applicable sequence changes that lead to functional activation of enhancers in evolution remains enigmatic. Our multilevel regulome analysis and machine learning revealed that functional activation of astrocytic enhancers coincides with a previously unappreciated, pervasive gain of binding sites of 'stripe' transcription factors, which are general transcriptional regulators. Altogether, we uncover genes and pathways linked to fetal astrocyte evolution and shed new light on a mechanism driving the acquisition of the regulatory potential of enhancers

MICRORNAS AS POTENT CELL-FATE EFFECTORS AND MODELING LATE-ONSET NEURODEGENERATION VIA DIRECT SOMATIC REPROGRAMMING

Andrew Yoo

Department of Developmental Biology, Washington University School of Medicine, St. Louis, MO, USA

Brain-enriched microRNAs (miRNAs), miR-9/9* and miR-124 (miR-9/9*-124), show cell fate-reprogramming activities when ectopically expressed in non-neural somatic cells such as dermal fibroblasts. These miRNAs effectively recognize gene networks that define the non-neuronal identity of fibroblasts, target multiple components of chromatin modifiers, and trigger extensive reconfiguration of the chromatin landscape. Through this chromatin switching, miR-9/9*-124 induces a highly efficient cell-fate conversion from fibroblasts to neurons. The miRNA--induced neuronal state can then be combined with additional transcription factors enriched in distinct brain regions to guide neuronal conversion to specific disease-relevant neuronal subtypes. Through this cell fate transition, directly reprogrammed neurons retain the age information stored in the starting fibroblasts and enable the modeling of late-onset neurodegenerative disorders in age-relevant, patient-specific neurons. Importantly, dissecting pathways associated with the ages of fibroblast donors in directly reprogrammed neurons implicates a consistent decline in protein clearance pathways, autophagy, and mitochondrial function. We have been investigating how age-related alterations in miRNA expression may contribute to this decline in cellular function in aged human neurons and have identified a number of miRNAs whose alterations with aging may underlie the loss of cellular function. Our results so far indicate that reversing these age-associated miRNA expressions towards the younger counterpart confers neuroprotection, which altogether suggests the benefit of reversing aging-associated pathways as an intervention strategy for late-onset neurodegeneration.

DECIPHERING CIRCADIAN RHYTHMS IN GENE EXPRESSION IN THE BRAINSTEM SATIETY CENTRE – IMPLICATIONS FOR OBESITY MANAGEMENT

Lukasz Chrobok

University of Bristol, School of Physiology, Pharmacology, and Neuroscience

Life on Earth is subordinate to periodic alterations in the environment, with most notable changes seen from day to night. To adapt to these cyclic changes, living organisms evolved endogenous 24h timekeeping mechanisms named circadian clocks. However, the regulation of these rhythms by environmental cues and lifestyle has not been fully understood.

Amongst of many behaviours regulated in a circadian fashion, daily partitioning of feeding is crucial for well--being and survival. The dysregulation of circadian rhythmicity seen in the modern 24/7 society leads to obesity, cardiovascular problems, metabolic syndrome and some kinds of cancer, constituting a major public health burden. Although the primary circadian clock is localised in the suprachiasmatic nuclei of the hypothalamus (SCN), local extra-SCN circadian timekeeping mechanisms are important for circadian physiology, including the daily patterning of feeding. Recently, we found that a brainstem satiety centre – the dorsal vagal complex (DVC) displays exceptionally robust timekeeping properties, which are additionally sensitive to diet.

In this talk, I will summarise our novel findings, which position the DVC as an autonomous circadian timing centre relevant to daily rhythms in feeding behaviour. We recently showed that genes for the molecular clock, as well as neurochemical receptors, are rhythmically expressed in the DVC *in vivo*. Moreover, in brain slices that do not contain the SCN, rhythms in clock gene expression can be monitored in the DVC for several days *ex vivo*, proving DVC's autonomy from the primary clock. Similarly, when recorded in brain slices, DVC neurons show day-to--night changes in the responsiveness to gut as well as other feeding-related neuronal signals, while the 24h profile of their electrophysiological activity exhibits pronounced day-night variation, which is grossly altered in animals fed a high-fat diet. From these and other findings, we hypothesized that time-restricted feeding shapes the 24h profile of gene expression, including that of the molecular clock in the DVC.

To test this, mice were held under light-dark conditions and acclimatised to feeding regimens whereby food pellets were available at one of three 6h-long windows (early day, early night, or late night) or *ad libitum*. Mice were killed at 4-6h intervals over the 24h cycle and gene expression in the DVC assessed by qPCR, NanoString nCounter, or RNAscope *in situ* hybridisation. Phase of the daily oscillations in the expression of clock and neurochemical signalling genes were systematically altered by the different schedules of feeding such that many were aligned to time of day at which food was available.

This research indicates that the daily expression patterns of clock and non-clock genes in the DVC is malleable, and that daily timing of food availability is a key factor in sculpting this 24h profile. This raises the possibility that oscillatory activity in the DVC is either part of or recruited by the SCN-autonomous food-entrainable oscillator. Further behavioural research is on-going to evaluate their importance to food anticipatory activity.

Session

„Brains and Artificial Intelligence”

BIOLOGICAL INSPIRATIONS IN MACHINE LEARNING

Krzysztof Fujarewicz

Silesian University of Technology, Gliwice

Machine learning has been inspired by biology from the very beginning, particularly by the functioning of the nervous system. Classical artificial neural networks were designed as simplified models of biological neurons, which integrate input signals and generate output depending on their activation. One of the key breakthroughs was the development of the backpropagation algorithm, which enables efficient weight adjustment in large networks by calculating error gradients – comparable to biological mechanisms of learning and synaptic plasticity, though realized in a fundamentally different way.

The talk will also address specific architectures inspired by neurobiology. The Hopfield network, as a model of associative memory, reflects the brain's ability to store and recall patterns from incomplete data. Kohonen networks (SOM – Self-Organizing Maps) imitate processes of self-organization and the formation of topographic maps in the cerebral cortex, allowing for unsupervised analysis and visualization of data.

In the final part, attention will turn to deep neural networks, which combine biological inspiration with immense computational power and big data, leading to breakthroughs in image, speech, and natural language recognition. The presentation will demonstrate that although today's AI models are largely mathematical and engineering constructs, their roots lie in the observation of nature, and further progress often results from attempts to understand and replicate the principles governing the brain.

BRAIN AI FOR DIGITAL MENTAL HEFALTH: MULTI-LAYER MACHINE LEARNING ACROSS OMICS, IMAGING, AND NEUROMODULATION

Igor Zwir

Washington University, St. Louis, MO, USA

This work aims to transform our understanding of mental health disorders and related dementias by integrating advanced machine learning and AI with multi-domain biomedical data. By combining multi-omics, genomics, multi-modal neuroimaging, brain neuromodulation measurements (e.g., stimulation parameters and evoked responses), and clinical datasets, we will develop an innovative, heterogeneity-aware, multi-layer integration framework that avoids traditional assumptions of data homogeneity. This approach is designed to reveal novel disease subtypes and identify causal, druggable biomarkers that underlie the complex trajectories of these conditions. Our strategy leverages multi-layer Graph Neural Networks (GNNs) to integrate longitudinal multi--omics data with neuroimaging and neuromodulation time series, addressing the limitations of imaging modalities in capturing subtle neuropathological changes over time. We will also develop a temporal, multi-domain alignment framework that systematically maps molecular alterations and neuromodulation responses to corresponding imaging and cognitive features, ensuring a holistic view of disease progression. Complementing these efforts, rigorous multi-omics analyses will uncover functional genes and proteins linked to mental-health risk, particularly in underrepresented populations. Further enhancing translational impact, we will create a Digital Twin AI Assistant (AIDA) that integrates causal networks with reinforcement learning to simulate individual disease trajectories and guide personalized interventions, including neuromodulatory strategies, ultimately improving diagnostic precision and therapeutic outcomes. Extending beyond existing platforms, our work both builds on established methodologies and pioneers new avenues for understanding and managing complex disorders. This comprehensive, precision--medicine approach has the potential to address critical gaps in current research and improve health outcomes across diverse populations.

DEEP LEARNING IN BRAIN IMAGING STUDIES

Jacek Ruminski

Gdansk University of Technology, Gdańsk, Poland

Introduction: Deep learning enables the effective training of multi-layer deep neural networks to address various clinical problems. An example of a relatively simple problem is to train the classification task using supervised representation learning. In this approach, labelled data (e.g., disease vs. control) are used to train networks that both perform the classification task and learn intermediate representations. The simplified interpretation of such representations is usually understood as a set of features. Such deep learning-based representations are usually as non-interpretable as traditional features, e.g., textures, shape, or image intensity descriptors. This work addresses different approaches to data representation learning in brain imaging studies. First, we describe self-supervised representation learning and related downstream tasks; next, we present a contrastive learning approach in brain imaging studies. Finally, we show a case study of representation learning using a generative AI approach, when GAN-based models learn representations from examples of healthy MRI images by randomly masking them (the inpainting problem).

Methods: Self-supervised representation learning (SSRL) does not require full labelling. Instead, it exploits data structure or proxy tasks (e.g., inpainting) to learn latent features. Studies such as Zhou et al., Nature Machine Intelligence (2024) demonstrate that SSRL can capture fine-grained anatomical structures, enabling more generalizable and data-efficient neuroimaging analysis. Contrastive learning (CL) is another representation-learning approach in which the model is trained to bring embeddings of "similar" pairs (positive pairs) closer together in latent space, and push embeddings of "dissimilar" pairs (negative pairs) farther apart. In this work, we adapted the CoMod GAN generative model to a set of 49280 T1w MRI images of healthy subjects (AOMIC Dataset, available online: https://nilab-uva.github.io/AOMIC.github.io/). The random generator was used to prepare masks that erase parts of the input images. The learning task of the MRI-CoMod GAN is to effectively reconstruct the masked images. We used several metrics, including PSNR, SSIM and FID, for the quantitative evaluation comparing true images and generated images.

Results and Conclusion: The self-supervised MRI-CoMod GAN model efficiently learned to reconstruct masked images for healthy individuals. The example metric values obtained for the validation set are PSNR=42.007 17.888; SSIM=0.973 0.026 and FID=0.0027. Such a model can be applied in various scenarios, including downstream classification, where MRI-CoMod GAN can serve as a backbone for further fine-tuning or to perform quality control of AI models. This is achieved by replacing the tumor area with "health-like" structures and observing the model's response, which is then inspected for quality control.

MODELING SLOW DEGRADATION PROCESSES WITH THE USE OF FISHER–KOLMOGOROV WAVE

Andrzej Polanski

Silesian University of Technology, Gliwice, Poland

The talk has three parts. The first is the overview of some fundamental genetic and evolutionary principles and effects, Hill-Robertson effect, Muller's ratchet, and Fisher–Kolmogorov mutation wave. They are discussed in a qualitative and descriptive way as they importantly support understanding of genetic and evolutionary mechanisms in populations dynamics. The interest concerns asexual evolution where mildly (weakly) deleterious mutations accumulate despite their genetic disadvantage. The progression of the mutation wave caused by Muller's ratchet force is a slow degeneration process, which can lead to population degradation or population extinction (mutational meltdown).

The second part is the list of some experimental studies where models/scenarios of the asexual evolution of populations of species, organisms, and cells with Muller's ratchet effect seen or hypothesized, including the Y chromosome, mitochondria, aging, dementia, viral infections, and cancers. Some available recently posted genomic sequencing datasets are also highlighted.

The third part is devoted to computational and mathematical modelling of slow degeneration evolutionary processes driven by the Muller's ratchet force. Existing approaches are listed and discussed, including stochastic simulations, stochastic differential equations, Markovian models, and deterministic models comprising systems of differential equations and partial differential equations. Finally, methods of confronting models with existing experimental data are discussed, and some conclusions regarding the importance of modeling and the potential profits of modeling are highlighted.

Session

„Extracellular Vesicles in neuronal communication”

EXTRACELLULAR VESICLES IN OLIGODENDROCYTE-NEURON COMMUNICATION AND AXONAL MAINTENANCE

Eva-Maria Krämer-Albers

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Oligodendrocytes form the myelin sheath and are essential for maintaining axonal integrity. How axons are maintained and the mechanisms of extrinsic support by oligodendroglia are poorly understood. Our research revealed that EVs, exhibiting morphological and biochemical characteristics of exosomes, promote axonal homeostasis by enhancing stress resistance and facilitating axonal transport. Mice with impaired oligodendroglial exosome release, cargo and function exhibit progressive axonal degeneration demonstrating that EV transfer from oligodendrocytes to neurons is required for long-term axonal maintenance. Here, I will focus on our recent work employing CreERT2-reporter mouse models to track the oligodendrocyte-to-neuron delivery of EV-cargo in vivo. The CreERT2 protein is included in oligodendroglial EVs and upon endosomal escape in target cells mediates reporter gene recombination, which allows their identification in the mouse brain. EV-mediated cargo transfer was verified by conditional Alix deletion. Using this reporter system, we provide evidence that oligodendroglial EVs deliver functional cargo to neurons and astrocytes. While astrocyte targeting remains to be elucidated, our findings highlight the importance of glial EV transfer to neurons in maintaining axonal integrity and sustaining neural networks.

EXTRACELLULAR VESICLE-MEDIATED TRAFFICKING OF MOLECULAR CUES DURING HUMAN BRAIN DEVELOPMENT AND DISEASE ALTERATION

Rossella Di Giaimo

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The extracellular environment, including extracellular vesicles (EVs), plays a pivotal role in brain development by regulating neural processes such as proliferation, differentiation, and migration. In this study, we sought to elucidate the pathogenesis of progressive myoclonus epilepsy type 1 (EPM1), a rare neurodegenerative disorder primarily caused by mutations in the *CSTB* gene. Using cerebral organoids derived from somatic cells of EPM1 patients (*EPM1-COs*), we have previously demonstrated that CSTB contributes to human cortical development and regulates extracellular signaling, cell proliferation, interneuron recruitment, and synaptic physiology.

Here, we show that EPM1-COs display altered electrophysiological activity compared to control COs, suggesting a disrupted excitatory/inhibitory balance. In order to dissect interneuron trajectory and maturation, we focused on ventrally patterned COs (EPM1-vCO), which give rise mainly to interneurons. EPM1-vCOs reveal abnormal neural cell fate specification, characterized by a shift toward dorsal neuron identities that can affect the excitatory/inhibitory balance. CSTB was previously found to be secreted via EVs during neurodevelopment, exerting cell-non-autonomous effects on neighboring cells. Therefore, we investigated EV biogenesis and cargo composition, finding pathological alteration in EPM1-vCOs. Interestingly, we found disrupted Sonic Hedgehog (SHH) signaling. By exposing control neural progenitor cells to EPM1 EVs, we were able to show that pathological EVs lead to inactivation of the intracellular SHH pathway in the receiving cells, pointing out the role of SHH signaling via EVs as a molecular hint toward impaired neuronal differentiation and cell fate determination in EPM1 brain development.

Together, our findings indicate that both intrinsic and extrinsic mechanisms drive EPM1 pathology, and highlight extracellular vesicle–mediated pathways as potential targets for therapeutic intervention.

THE ROLE OF MICROENVIRONMENT IN RADIATION-INDUCED BRAIN TUMORIGENESIS – THE DISCOVER PROJECT

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While accumulating DNA damage in target cells is a major driver of radiation carcinogenesis by now it is increasingly recognized that the role of the tumor microenvironment as well. Microenvironmental changes such as inflammation, immune response, and impaired intercellular communication can contribute to cancer development.

The DISCOVER project (Dissecting Radiation Effects into the Cerebellum Microenvironment Driving Tumour Promotion), as part of the European Partnership for Radiation Protection Research (PIANOFORTE) is supported by Horizon-EURATOM and brings together four European research institutions. The main objective of the project is to investigate how radiation-induced changes in the cerebellum and altered interactions among cerebellar cell populations contribute to medulloblastoma formation. Using Ptch1+/- mice – a radiosensitive model genetically predisposed to MB – the project uses complex experimental approaches, such as in vivo irradiation, ex vivo cerebellar slices, and in vitro co-cultures of medulloblastoma cells and different cell types within their microenvironment.

The current presentation will give a short overview of the main objectives of the project, major experimental approaches, and present some first preliminary results. In particular, we will present our first results on the impact of irradiation and extracellular vesicles on the activation of DNA-sensing pathways within the microenvironmental cells and how microenvironmental signals impact the physiological migratory capacity of granular progenitor cells, considered as the main target cells in medulloblastoma development.

Funding: *The DISCOVER project has received funding from the Euratom research and training programme 2022-2027 in the framework of PIANOFORTE under grant agreement No 101061037.*

PLASMA EXTRACELLULAR VESICLE-ASSOCIATED SMALL RNAS AS EARLY BIOMARKERS IN HUNTINGTON'S DISEASE

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Introduction. Small regulatory RNAs (sRNAs) are dynamic modulators of gene expression that act through both cell-autonomous and non-autonomous mechanisms, particularly when secreted into the extracellular space. Their tightly regulated networks are essential for brain homeostasis, and accumulating evidence implicates sRNA dysregulation in the pathogenesis of neurodegenerative diseases. Due to their sensitivity to early molecular changes, sRNAs in biofluids have emerged as promising biomarkers for preclinical disease detection, addressing critical needs in diagnosis, prognosis, and patient stratification. In biofluids, sRNAs can be found freely circulating, bound to proteins, or enclosed within extracellular vesicles (EVs).Huntington's disease (HD) is a dominant genetic disorder caused by a CAG repeat expansion in the coding region of the *HTT* gene and is characterized by progressive motor, cognitive, and psychiatric symptoms. Beyond the toxic effects of mutant HTT protein, recent findings highlight a pivotal role for RNA-based mechanisms in disease progression. We have shown that sRNAs are strongly dysregulated in HD brains and are sufficient to induce neuropathogenic changes, including motor alterations, when injected into mice. In this study, we analyzed sRNA profiles in different plasma subfractions, both vesicular and extravesicular, and evaluated their biomarker potential in HD.

Methods. EVs were isolated from plasma by size-exclusion chromatography (SEC) and ultrafiltration. To characterize sRNAs across distinct plasma compartments, we used SEC to collect both EV-enriched and non-EV protein-enriched fractions. sRNAs were profiled using high-throughput sequencing, and differential expression was assessed through a rigorous bioinformatic pipeline combining ExceRpt and SeqCluster tools. Candidate sRNAs were validated by quantitative RT-PCR in an independent cohort. Longitudinal samples were analyzed to assess temporal changes in sRNA levels, and correlations with cognitive performance were evaluated to determine biomarker potential.

Results. Using an optimized EV purification protocol and a comprehensive sRNA sequencing pipeline, we identified EVs as the plasma subfraction with the most stable sRNA content. We showed that EV-associated sRNAs are downregulated early in *HTT* mutation carriers at premanifest stages, and this dysregulation correlates with premanifest cognitive performance. We established a plasma EV-sRNA biosignature that was validated in additional subjects, demonstrating significant diagnostic accuracy at premanifest stages. Specific sRNA species also exhibited longitudinal changes in premanifest patients.

Conclusions. These findings suggest that within the complex landscape of plasma sRNAs, those enclosed in EVs exhibit early and specific deregulation in HD. This alteration appears to coincide with molecular changes occurring before the onset of motor symptoms. Taken together, these results support the potential of selected sRNA species as valuable biomarkers for early detection and monitoring of HD progression.

PROSPECTS OF EXOSOMAL MIRNAS AND ARTIFICIAL INTELLIGENCE IN THE DIAGNOSTICS AND PROGNOSTICS OF NEURODEGENERATIVE DISEASES.

Urszula Wojda

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Early and accessible biomarkers of Alzheimer's disease (AD) – the most common neurodegenerative disorder – are urgently needed to enable reliable diagnosis before neurodegeneration becomes irreversible. Current fluid biomarkers, such as amyloid-β and tau proteins, capture only selected aspects of the complex, multifactorial nature of AD. This drives the search for molecular indicators that better reflect the broad network of dysregulated processes underlying disease onset and progression. Among such candidates, microRNAs (miRNAs) have gained considerable attention due to their stability in blood, their essential roles in intra- and inter-neuronal communication, and extensive evidence linking numerous miRNAs to AD pathogenesis and progression in both brain tissue and peripheral circulation.

Particular interest has been directed toward miRNAs packaged in extracellular vesicles, especially exosomes, which cross the blood–brain barrier, protect their cargo, and mediate neuron-to-neuron and neuron-glia signalling. Recent multicentre studies demonstrate that panels of neuron-derived exosomal miRNAs can identify individuals in preclinical AD years before symptom onset, underscoring their strong diagnostic and prognostic potential.

Our own approach to identifying a blood-based molecular signature of AD assumes that early miRNA alterations in blood may originate not only from the brain but also from peripheral tissues, reflecting metabolic dysregulation and low-grade inflammation – both increasingly recognised as contributors to AD risk. Therefore, we analyse miRNA signatures present in the entire circulating pool, including those transported in exosomes, AGO2-protein complexes, HDL particles, and microvesicles.

Using this strategy, we developed a diagnostic panel of plasma miRNAs together with a dedicated machine-learning pipeline. This integrative model improved the classification of AD, presymptomatic AD, and non-AD groups across several independent cohorts. Importantly, the resulting composite miRNA signature provides a level of diagnostic specificity and sensitivity not previously achievable with single-biomarker approaches. Together, circulating miRNAs and AI-assisted analytics open new perspectives for minimally invasive, biologically meaningful biomarkers that better capture the complexity of AD and enable earlier and more accurate risk stratification.

Session

„Education and Implementation of Research”

INTRODUCING MOLECULAR DIAGNOSTICS INTO THE CLINICAL LABORATORY EDUCATION

Barbara Kraj

Macon & Joan Brock Virginia Health Sciences at Old Dominion University, Norfolk, VA, USA

Molecular diagnostics has been fundamental to clinical laboratory operations for pathogen detection, genetic testing, oncology diagnostics, transplantation monitoring, and personalized medicine. Since the late 20th century, the introduction of molecular techniques has revolutionized diagnostic practice by providing sensitive and specific tools that often surpass traditional methods. However, in the early 2000s, most medical technology or clinical laboratory science educational programs in the United States-now formally known as Medical Laboratory Science (MLS) programs-lagged behind. Curricula offered limited theoretical exposure and scarce hands-on training, leaving graduates underprepared to confidently perform clinical molecular assays, creating a mismatch with laboratory workforce expectations.

Several external developments accelerated the adoption of molecular methods in U.S. laboratories, indirectly pressuring MLS education. One key driver was the European Union requirement for nucleic acid testing of plasma-derived products, prompting manufacturers and blood banks to adopt equivalent practices. At the same time, the U.S. Food and Drug Administration approved multiple molecular assays, embedding them into transfusion medicine and infectious disease diagnostics. Recognizing these shifts, in 2001, the National Accrediting Agency for Clinical Laboratory Science mandated the inclusion of molecular diagnostics in the curricula of educational programs, yet implementation across programs has remained inconsistent. While most programs taught some molecular content, few offered dedicated courses or laboratory exercises developing essential psychomotor competencies.

Persistent barriers to integration included inadequate expertise of faculty; financial constraints associated with purchasing reagents and specialized instrumentation, and content overload with little room to add new subjects within the baccalaureate curriculum. Access to molecular laboratories for clinical rotations has been inequitable. Additionally, the competencies expected of new graduates have been described ambiguously. The certification content guidelines, published by the American Society for Clinical Pathology, remained limited, prompting other laboratory advocacy organizations to develop specific cognitive and psychomotor objectives for the medical laboratory professionals at various levels of practice. Today, integration of molecular diagnostics into MLS curricula is imperative but continues to be challenged by factual and perceived barriers.

This presentation highlights historical drivers, obstacles, and strategies to embed molecular diagnostics into clinical laboratory education, emphasizing the need for verbalizing specific competencies, faculty development, resource investment, teaching creativity, and collaboration with molecular facilities, providing opportunities for hands-on training focusing on skills expected upon entry into the workforce.

TRANSFORMING IDEAS INTO IMPACT: IP, COMMERCIALIZATION, AND PARTNERSHIPS IN ACADEMIC MEDICAL CENTERS

Olena Danylyuk

Henry Ford Innovation Institute, Henry Ford Health, Detroit, MI, USA

Hospitals and academic medical centers are uniquely positioned at the intersection of discovery and care, where clinical insight drives innovation and patient needs shape translational research. This lecture explores how intellectual property (IP) management and technology transfer functions within U.S. hospital systems and academic institutions serve as catalysts for transforming medical breakthroughs into real-world applications that improve lives and deliver innovative healthcare solutions.

Key components of a successful technology transfer program in a clinical setting will be outlined, including invention disclosure, patenting, licensing, startup development, and collaboration with industry. Special attention will be given to the challenges and opportunities of managing clinician-led innovations and aligning commercialization efforts with the mission and values of healthcare institutions. Real-world case studies will illustrate diverse commercialization pathways and the impact of these efforts on patient care, inventor engagement, institutional growth, and broader societal benefit.

Whether you are a clinician, researcher, innovation leader, or external partner, this session will provide current tools and practical strategies to help transform healthcare ideas into impactful, patient-centered solutions – advancing not only medical progress but also the core mission of institutions like Henry Ford Health to improve people's lives through excellence in science, care, and innovation.

FROM SCREENING TO SUCCESS: SUPPORTING EARLY DISCOVERY PROGRAMMES FROM INCEPTION TO CLINIC

Anna Habryka-Pawlowska

Preclinical Services, Revvity, Cambridge, UK

Revvity, a leading biotech company, is dedicated to advancing biomedical research through its provision of comprehensive life science solutions, technologies, and services. Our mission is to support academia, pharmaceutical companies, clinical laboratories, healthcare professionals, and contract research organizations (CROs) in their journey from bench to bedside.

At Revvity, we revolutionize early discovery programmes with a particular focus on translational multi-omics, biomarker identification, imaging, prediction, screening, detection, diagnosis, informatics, and more. This lecture will provide an overview on how our preclinical CRO services are designed to accelerate the drug discovery journey for biotechnology and pharmaceutical clients worldwide. With industry-leading cell-based screening solutions, we leverage an immense wealth of experience and knowledge to support cell model design and preclinical screening requirements across various modalities, from target identification to therapeutic validation studies.

By partnering with Revvity, clients can focus on their core priorities while outsourcing to our experienced scientific team and advanced technologies. We provide reliable, robust data to support decision-making, helping to understand therapeutic mechanisms and identify *in vitro* predictive biomarkers and oncology indications associated with optimal outcomes.

With expertise in small molecules, biologics, oligo-based therapeutics, and more, we cover pre-clinical applications such as target identification, validation, mechanism of action studies, and drug-gene interaction studies. For nearly two decades, we have specialized in oncology assays and high-throughput screening, and are trusted by many top pharmaceutical companies.

In this session, you will gain insights into Revvity's innovative approaches and how advanced technologies can transform early discovery programmes from inception to clinic, driving success in the pursuit of new therapeutic breakthroughs.

T REGULATORY CELLS IN THE TREATMENT OF AUTOIMMUNE DISEASES – FROM BENCH TO BEDSIDE

Dorota Iwaszkiewicz-Grześ

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Regulatory T cells (Tregs) have long represented a promising approach to immune modulation, offering the potential to suppress pathological autoreactivity in autoimmune diseases. Our research into Tregs began at the basic science level, focusing on their differentiation, phenotypic stability, and suppressive mechanisms. These studies laid the foundation for the development of ex vivo expansion and modification protocols, including the generation of antigen-specific Tregs and CAR-Tregs, which now form a core component of PolTREG's therapeutic pipeline.

Within PolTREG's clinical programs, autologous polyclonal Tregs (PTG-007) have been administered to patients with type 1 diabetes (T1D) both shortly after diagnosis and at pre-symptomatic stages. The company is also exploring Treg-based therapies for multiple sclerosis (MS), including intrathecal delivery in neuroinflammatory models. Long-term clinical data from treated T1D patients demonstrate durable partial remission for up to 12 years, and in some individuals, insulin independence lasting 18–24 months, without serious adverse events. These outcomes support the concept of Tregs as a disease-modifying therapy rather than a merely symptomatic intervention.

In parallel, PolTREG is advancing next-generation strategies such as TCR-Tregs, in vivo induction of Tregs (e.g., via mRNA-based vaccines), and CAR-Tregs designed to achieve precise, antigen-specific immune modulation. In the MS field, PolTREG has obtained patents for intrathecal administration and is preparing trials in both relapsing-remitting and primary-progressive MS. Supported by GMP-certified facilities and more than 17 years of experience in Treg biology and manufacturing, the company is well-positioned to scale production and expand clinical applications.

POLISH CANCER MISSION HUB – THE PENTAHELIX MODEL OF STAKEHOLDER COOPERATION

Iwona Ługowska

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National Cancer Mission Hubs (NCMHs) constitute a structure of governance of the EU Mission on Cancer operating across national, regional, and local levels in each Member State. By fostering collaborations, dialogues, and partnerships between diverse stakeholders, including representatives of policymakers, healthcare professionals, researchers, business, patients, and citizens, NCMHs play a pivotal role in uniting efforts and accelerating research and innovation on cancer prevention, cancer care, and the quality of life of cancer survivors and carers. Convening national stakeholders on the fight against cancer and ensuring the collaboration between Member States is central to the successful implementation of both Cancer Mission and EBCP's objective of improving the lives of 3 million people living with cancer by 2030. The mission of NCMHs is to empower and promote a collaborative ecosystem serving the implementation of both the EU Mission on Cancer and Europe's Beating Cancer Plan. By facilitating co-design, co-creation, co-implementation, and co-evaluation activities on cancer between multiple stakeholders, NCMHs must work to improve cancer understanding, prevention, early detection, treatment, care, and quality of life in a people-centric way. When applicable, NCMHs may also contribute to the implementation of National Cancer Control Strategies (NCCS) or National Cancer Control Plans (NCCP).

Polish Cancer Mission Hub (Polish CMH) is coordinated by the Maria Skłodowska-Curie National Research Institute of Oncology (MSCI), which acts to ensure alignment of the EU Mission on Cancer and Europe's Beating Cancer Plan with Poland's National Cancer Strategy and National Cancer Network.

The Polish CMH will:

- Foster collaboration with key stakeholders, including the Ministry of Health, Ministry of Science, academia, the Medical Research Agency (ABM) with its Warsaw Innovation Hub, and Infarma (Association of Employers of Innovative Pharmaceutical Companies), to ensure strategic alignment between European and national cancer-related initiatives.

- Strengthen partnerships with patient advocacy groups and civil society organizations to ensure a patient-centered approach in research, care, and policymaking.

- Act as the key communication and coordination hub between EU and national institutions, facilitating the exchange of knowledge, best practices, and policies.

- Build capacity and increase engagement in co-programmed calls, improving the positioning of Polish stakeholders for participation in Cancer Mission calls.

By establishing strong collaboration between research, policy, industry, and civil society, Poland's NCMH will contribute to enhancing cancer prevention, diagnosis, treatment, and survivorship, in full alignment with EU goals and national priorities.

Session

„New Trends in Medical Biotechnologies”

DECODING BIOLOGY WITH AUTONOMOUS MULTISCALE IMAGING

Kevin Dean

UT Southwestern Medical Center, Dallas, TX, USA

Rare and spatially complex biological phenomena often elude conventional imaging techniques, which struggle to capture cellular events in their full tissue context. We present an integrated software-hardware framework that combines an intelligent imaging platform, navigate, with a multiscale cleared-tissue light-sheet microscope (MCT-ASLM) to enable event-driven, multiscale acquisition workflows. The open-source navigation software provides a Python-based graphical user interface for building reconfigurable, decision-based imaging pipelines across diverse light-sheet microscopy modalities, empowering investigators without programming expertise to implement real-time event adaptive microscopy. Paired with MCT-ASLM's ability to map centimeter-scale volumes at cellular (micron-scale) resolution and seamlessly transition to ~300 nm isotropic resolution for targeted subcellular imaging, this system can rapidly pinpoint and interrogate rare biological events in situ. By focusing high-resolution acquisition only on features of interest, these tools dramatically reduce data volume and imaging time while preserving the surrounding tissue context. We demonstrate applications of this autonomous multiscale approach across developmental biology, neuroscience, and cancer research – from mapping intricate neuronal circuits to visualizing organ innervation and profiling metastatic tumor microenvironments – underscoring its broad utility in unveiling how localized events drive tissue- and organ-level phenomena.

HIGHLY MULTIPLEXED IMAGING OF SUBCELLULAR ARCHITECTURES WITH NANOSCALE PRECISION

Seweryn Gałecki

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The spatial organization of proteins and organelles within eukaryotic cells orchestrates fundamental biological processes. Super-resolution imaging of multiple biological structures is critical for understanding both normal physiology and the molecular mechanisms that drive disease. Yet, despite significant advances in optics, spectral overlap typically restricts biological imaging to a handful of fluorophores, and often lacks the resolution needed to visualize events at sub-diffraction scales. To overcome these challenges, we introduce Cyclically Multiplexed Expansion Microscopy (Cy-ExM) – a workflow that integrates cryo-fixation of the specimen, expansion microscopy, and cyclic immunofluorescence labeling. Cy-ExM enables highly multiplexed imaging of proteins, cytoskeletal architectures, and cellular organelles with preservation of their native spatial organization. We validate this approach across multiple mammalian cell types using spinning disk, oblique plane, and axially swept light-sheet microscopy. Unlike DNA-barcoded or photoswitchable fluorophore strategies, Cy-ExM relies on standard reagents and instrumentation, making it readily adaptable to most research laboratories. Together, these advances provide an accessible platform for volumetric, high-plex imaging at nanoscale resolution-offering new opportunities to dissect the molecular organization of cells in health and disease, and to illuminate the nanoscale origins of oncogenic transformation.

NOVEL METHODS OF PREPARATION OF CONDUCTIVE COMPOSITES BASED ON POLYPYRROLE

Petr Humpolíček

Tomas Bata University in Zlín, Zlín, Czech Republic

Conducting polymers, particularly polypyrrole (PPy) and polyaniline (PANI), represent a unique class of materials combining electronic and ionic conductivity that makes them attractive for biomedical applications. However, their practical utilization has been traditionally hampered by poor processability, limited biocompatibility knowledge, lack of mechanical properties, and their problematic incorporation into functional composite systems, for example, due to delamination. Our research journey began with establishing the fundamental biological properties of conducting polymers. We conducted the first systematic biocompatibility comparison between PPy and PANI, demonstrating that both polymers in their base forms exhibit comparable and acceptable cytotoxicity profiles when properly prepared. This foundational work revealed that biocompatibility depends more significantly on polymer modification (salt vs. base forms) than on the specific polymer type, overturning previous assumptions about inherent toxicity differences. Building on this knowledge, we pioneered the development of colloidal PPy dispersions as a solution to the processability limitations of conventional PPy powders. These homogeneously stabilized particles demonstrated remarkable biological properties: absence of cytotoxicity, potent free-radical scavenging activity, significant antibacterial activity, and, most importantly, pronounced immunomodulatory capabilities. The next critical advancement involved incorporating these colloidal PPy particles into composite matrices. We developed cytocompatible 3D scaffolds based on sodium hyaluronate that effectively distributed PPy particles throughout hydrophilic networks, achieving conductivities comparable to human tissues while maintaining excellent mechanical properties and biocompatibility. Our methodology culminated in the development of conductive films and surface coatings. We created an in situ polypyrrole coating technique for nano- and microfibrous substrates, enabling precise control over layer morphology, thickness, and biological performance. The pinnacle of our research is a patented method for covalent anchoring of PPy. Composites exhibit extraordinary multifunctional properties: conductivity, antibacterial activity, antioxidant capacity, and remarkable immunomodulatory effects.

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ANTICANCER DRUG CARRIERS: A RELIC OF THE PAST OR THE FUTURE OF CANCER THERAPY?

Jan Vícha

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Despite billion-dollar annual investments and exponential growth in research publications—ranging from 23 manuscripts in 1995 to over 25,000 in the 2020s—less than 20 nanoparticle-based anticancer drug carriers have achieved global regulatory approval and entered clinical practice. Most of these failures can be attributed to insufficient efficacy rather than toxicity issues, which can be traced to several factors.

The primary issue is the overreliance on the enhanced permeability and retention effect (EPR). Unlike simplified xenograft models that exhibit exaggerated EPR effects, human tumors feature a variable density of extracellular matrix, nonhomogeneous endothelial fenestration, and elevated interstitial fluid pressure, which severely limit nanoparticle penetration. Moreover, to achieve a meaningful EPR effect in humans, long circulation times are required.

PEGylation, initially hailed as the solution to nanoparticle clearance, introduced unexpected immunological complications. Anti-PEG antibodies, now detected in over 40% of healthy humans, can trigger accelerated blood clearance and complement activation-related pseudoallergy. Further development led to carriers targeting specific markers or pathways. Although highly potent in vitro, these approaches have underestimated the clinical heterogeneity of tumors, which possess hundreds of non-synonymous mutations. These complex, multi-component systems also fail to translate from the laboratory to GMP manufacturing due to highly challenging synthesis.

Future carriers thus require fundamental paradigm shifts: abandoning the universal EPR assumption, embracing tumor heterogeneity and the microenvironment as design parameters rather than obstacles, accepting manufacturing realities, and developing patient-specific rather than universal solutions. Several new approaches are currently emerging. These include biological rather than chemical solutions, such as phage-mediated drug delivery, patient-specific antibody/drug conjugates, targeting of cancer-associated glycans, and the use of biologically active carriers, which not only carry and target the drug but also provide complementary therapeutic effects. Drug delivery thus still holds considerable potential, provided that we learn from past mistakes.

Acknowledgment: *This work was supported by the Ministry of Health of the Czech Republic, Grant NW24-03-00331 and TBU Internal Development Project RP/CPS/2024-28/001 financed by the Ministry of Education, Youth and Sports of the Czech Republic.*

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HOW MITOCHONDRIAL METABOLISM CONTROLS T CELL ACTIVATION, I.E., 'HOW TO FIX AN OLD ENGINE USING METABOLIC TRACING ANALYSIS'?

Marcin Kamiński

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T cell activation leads to a spectrum of signaling, transcriptomic, and metabolic changes. In that respect, TCR triggering induces a rapid generation of low, non-toxic levels of reactive oxygen species (ROS). This "oxidative signal" regulates T cell activation-evoked transcription, and thus, differentiation. Interestingly, in the elderly, T cell--dependent immune response is often impaired due to aberrant T cell activation.

Mass spectrometry (MS)-based metabolic tracing (monitoring of ^{13}C-labelled isotopomers) is a powerful analytical technique that could be implemented to address a large variety of cell biological questions. This presentation showcases how metabolic tracing analysis, by providing unique insights into the regulation of T cell activation, enables putting forward new anti-immune aging therapy approaches.

According to the mitochondrial theory of aging, age-acquired mutations of mitochondrial DNA (mtDNA) result in a vicious cycle of ROS-dependent mitochondrial damage. To investigate changes in mitochondrial function of aging T cells, we applied a mtDNA polymerase γ (PolG) 'Mutator' progeroid mouse model where gradual accumulation of mtDNA mutations results in premature aging. Aiming to analyze the underlying cause of T cell dysfunction present in Mutator mice, we applied bone-marrow (BM) chimeric mice and analysis of metabolic tracing. Our results demonstrate unexpected consequences of mitochondrial impairment for T cell function. Importantly, we could also demonstrate how mitochondrial metabolism controls T cell activation and propose how to alleviate accelerated aging-induced T cell dysfunction.

WHEN NATURE MEETS PRECISION MEDICINE: NATURAL COMPOUNDS AGAINST NPM1-MUTANT AML

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Nucleophosmin (NPM1) mutations represent one of the most frequent genetic alterations in acute myeloid leukemia (AML), defining a distinct disease entity with unique molecular features and clinical implications. Despite therapeutic advances, treatment of NPM1-mutant AML remains challenging due to disease heterogeneity, relapse risk, and limited targeted options. In this context, natural compounds are gaining attention as promising modulators of leukemogenic pathways, bridging traditional pharmacognosy with precision medicine.

Recent investigations have highlighted the modulatory activity of Cucurbitacin D, a triterpenoid isolated from Elaeocarpus hainanensis, on leukemic cell signaling. Specifically, Cucurbitacin D was shown to downregulate the oncogene ZNF217, a transcriptional regulator implicated in proliferation, survival, and therapy resistance in NPM1-mutated AML cells. This selective effect suggests that bioactive natural products may exert fine-tuned regulatory activity on key molecular drivers, complementing existing therapeutic strategies.

The integration of natural compound discovery with genomic and transcriptomic profiling offers a powerful route toward individualized therapies. By targeting aberrant transcriptional programs such as NPM1-driven leukemogenesis and ZNF217 upregulation, Cucurbitacin D exemplifies how natural molecules can be repositioned within a precision medicine framework.

Altogether, these findings support the concept that nature-inspired agents hold the potential to overcome the therapeutic limitations of conventional regimens, paving the way for innovative combinatorial strategies against NPM1-mutant AML.

HOW ENGINEERING TECHNOLOGY SUPPORTS LIFE SCIENCE.

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Tissue engineering is transforming drug candidate testing by enabling the development of human tissue models. In life science laboratories, automation plays a crucial role in this field, offering significant enhancements in throughput, precision, and efficiency. The Robotic-enabled Biological Automation platform (ReBia) exemplifies this by automating the complete tissue engineering workflow, which enables the production of three-dimensional human tissue models, such as epidermal skin, airway epithelia, and tumor spheroids, for drug testing. This comprehensive approach aligns with the 3R principles-replacement, reduction, and refinement of animal testing—by enhancing model availability and standardization. AI integration within ReBia ensures close monitoring of critical steps and key events in cell culture processes, boosting system reliability. AI serves as a powerful tool for analyzing data and supporting experimental processes, with tumor models as a prime example. By employing machine learning, the system can identify morphological changes in tumor spheroids in response to drug treatments, thereby advancing the screening and evaluation of drug efficacy. Comparative drug screening studies will demonstrate the transformative impact of automation and AI in the life sciences.

Sections of the Poster Sessions

Section I

„Bioinformatics and Mathematical Modeling”

[I-1] PREDICTING CIRCRNA – EXOSOME RELATED PROTEIN INTERACTIONS IN GLIOMA WITH UTILIZATION OF PUBLICLY AVAILABLE BIOINFORMATICS DATA

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Introduction: High-grade gliomas are lethal tumors of the central nervous system. Among them, glioblastoma is the most common primary CNS malignancy in adults, characterized by poor prognosis with a median overall survival reaching approximately 15 months. Thus, a thorough exploration of the molecular pathology of diffuse gliomas is still immensely needed. Recent studies in the field have been focused on circRNA– a class of non-coding RNA with a covalently closed loop-like structure. CircRNAs can exert various molecular functions, including interacting with miRNAs and various proteins (RNA-binding proteins- RBP). Similarly to other classes of RNA, they can also be packed into extracellular vesicles such as exosomes and subsequently secreted into the tumor microenvironment. The aim of this work was to seek potential circRNA–exosomal protein interactions in glioma with the utilization of publicly available bioinformatics data.

Methods: To construct a glioma-specific circRNA–exosome related protein interaction network, we integrated data from several publicly available repositories, including Gene Ontology (GO), Cancer-Specific CircRNA Database 2.0 (CSCD2) and The Human Protein Atlas. We filtered through all cancer–specific CLIP-seq–derived circRNA-RBP interactions deposited in CSCD2 with RNA-seq evidence for circRNA detection in glioma. Then, by using org.Hs.eg.db and AnnotationDbi packages, we intersected the obtained list with genes annotated in exosome--related ontologies and narrowed it to protein products with glioma IHC detection evidence from The Human Protein Atlas. This integration was utilized mainly with dplyr package. For Gene Ontology Enrichment Analysis, we utilized clusterProfiler package. All packages were utilized in RStudio 2025.05.01.

Results: The constructed glioma-specific network revealed 29 protein-coding genes (including AGO2, HNRNPC and PTBP1) related to GO cellular component term GO:0070062; extracellular exosome, and their 1 536 interactions associated with 493 various circRNAs. The Gene Ontology Enrichment analysis of biological processes for the protein-coding component of the network showed significant enrichment in terms related mainly to RNA metabolic processes and regulation of various splicing events. Interestingly, 27 circRNAs from the obtained network were transcribed from SPAG9 gene, which possesses a well-documented role in the pathogenesis of various human malignancies.

Conclusions: Our bioinformatics approach highlighted the relevance of mRNA processing and alternative splicing events in regard to exosome metabolism. Those findings pose potential questions regarding the role of circRNA in exosome biogenesis and exosomal cargo composition and its impact on the development of tumors of glial origin. Further experimental investigation with the utilization of appropriate laboratory models is warranted to deepen the understanding of this subject.

[I-2] DEVELOPMENT OF A POLISH MEDICAL LANGUAGE CORPUS

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Introduction: A corpus refers to a digitally stored collection of written or spoken texts used for studying language in context. Although numerous corpora have been developed for other languages, currently available Polish corpora are mostly general-purpose (such as the National Polish Language Corpus) and do not include medical language. This project focuses on building a Polish medical language corpus with applications in infertility and gynaecology, utilising existing corpus-creation tools. Specifically, the corpus is needed to create a domain-specific natural language processing (NLP) model for supporting infertility treatment.

Methods: The corpora were built from several Polish-language sources, including: (i) a clinical text database from mostwiedzy.pl; (ii) selected medical publications and e-books from Medycyna Praktyczna; (iii) a dictionary of abbreviations related to the diagnosis and infertility treatment in Poland. Raw data were carefully examined and pre-processed to remove non-linguistic elements, such as headers, footers and advertisements. Next, files were cleaned, converted to CSV format, and underwent post-processing while maintaining UTF-8 encoding. Then, three corpus-creation approaches were compared: (i) Sketch Engine; (ii) Korpusomat; (iii) a self-developed algorithm. Each approach was evaluated for supported formats, output quality and suitability for linguistic analysis and NLP tasks.

Results: Three TXT files containing cleaned and properly formatted corpus material were generated at the final stage. The output from Sketch Engine was mostly properly formatted, while Korpusomat produced separate files that required merging into a single TXT file. Moreover, two specialised linguistic files were obtained in VERT (Sketch Engine) and CoNLL-U (Korpusomat) formats, each containing linguistic annotations. The most time-consuming step was data pre-processing, which required careful manual investigation of the input data and the definition of data filtering steps. While both Sketch Engine and Korpusomat are powerful tools, for our subsequent task, manual corpus creation proved to be sufficient for producing a single, clean TXT corpus file.

Conclusions: Regardless of existing tools, the self-developed pipeline gave the cleanest Polish medical language corpus. The resulting corpora should provide a valuable resource for future NLP model development in medical diagnosis and treatment.

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[I-3] POST-GWAS GENE AND PATHWAY INTEGRATION REVEALS IMMUNE-RELATED MECHANISMS UNDERLYING TYPE 1 DIABETES

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Type 1 diabetes (T1D) is an autoimmune disorder characterised by the destruction of pancreatic β-cells. The exact causes and mechanisms are not fully understood.

Genome-wide association studies (GWAS) have identified numerous single-nucleotide polymorphisms (SNPs) associated with the risk of T1D. However, translation of single-variant findings into gene- and pathway-level insights remains a crucial post-GWAS step to facilitate better understanding of the molecular mechanisms and biological context.

GWAS was conducted among a cohort of Ukrainian individuals, encompassing 2598 individuals diagnosed with T1D and 4240 healthy controls. In order to integrate SNP-level p-values into gene-level association statistics, two complementary approaches were applied: the first method is MAGMA, which accounts for linkage disequilibrium and gene boundaries. The second method is Fisher's combined probability test, a classical method for aggregating multiple p-values. The concordance between the two gene-based approaches was evaluated. Gene rankings derived from both integration strategies were further analysed using gene set enrichment analysis (GSEA) based on MSigDB collections.

The MAGMA and Fisher gene-level statistics demonstrated strong agreement, thereby supporting consistent detection of T1D-related genetic signals. Independent GSEA analyses yielded highly overlapping gene sets for both methods, thereby confirming the robustness of the functional interpretation pipeline. Amongst the 116 significant pathways that were identified as being shared between the two approaches, the majority of these belonged to the C5 GO collection, with a particular emphasis on immune-related biological processes and cellular components. These included MHC protein complexes, antigen processing, and T cell activation. The results of the study have highlighted additional enriched pathways from the C2 curated collection, which have provided further evidence to support the immunogenetic basis of T1D. The pathways in question have been shown to be relevant to autoimmune mechanisms, such as systemic lupus erythematosus signaling.

This study shows that combining post-GWAS gene-based aggregation with functional enrichment analysis improves interpretation of GWAS results. Two independent integration methods show similar results, highlighting immune-related pathways. This provides insights into the causes of T1D. The findings show the importance of antigen presentation and adaptive immune activation in driving T1D risk, offering targets for future research.

[I-4] COMPARATIVE ANALYSIS OF 5'-UTRS CAPTURE EFFICIENCY ACROSS COMMON COMMERCIAL WES TARGET ENRICHMENT KITS

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Introduction: Whole-exome sequencing (WES) is a next-generation sequencing approach that enables profiling of protein-coding regions of almost all human genes. It has become widely adopted due to its high capacity, rapid and scalable workflows, and substantially lower costs compared with whole-genome sequencing. Over the years, multiple commercial WES enrichment protocols have been introduced, offering various target designs and competing in terms of capture efficiency. Although overall performance across major protocols is considered comparable, efficiency may substantially vary within 5′ untranslated regions (5′-UTRs). Therefore, the aim of this project was to conduct a comparative analysis of 5′-UTR capture efficiency across commonly used WES target enrichment kits.

Methods: 623 samples obtained from both publicly available and proprietary datasets were analyzed. The following kits were tested i) Agilent SureSelect (CRE, CRE V4, Human All Exon V4+UTRs, V5, V6, V7), ii) IDT xGen Exome Research Panel, iii) Nimblegen (Roche) SeqCap EZ Human Exome Library v3.0 and iv) Twist Bioscience Core Exome kits. Quality control was performed using FastQC, and reads were trimmed with Trimmomatic when necessary. Sequencing reads were aligned to the GRCh38 (hg38) reference genome or lifted over to hg38 if required. Annotated 5′-UTR regions were extracted from reference genome BED files with kit-specific target regions. Coverage calculations were performed using bedtools. Kit performance was evaluated by: (i) mean coverage of target regions; (ii) percentage of covered 5′-UTR positions; and (iii) a normalized 5′-UTR coverage metric, calculated as the ratio of covered to targeted 5′-UTR positions, adjusted for the proportion of 5′-UTR length included in each kit's design.

Results: The results showed that in terms of coverage of kit-targeted 5'-UTRs, the latest WES designs achieved high and comparable performance. Older generations of enrichment kits, however, demonstrated noticeably lower accuracy, yet still maintained moderately good coverage, usually exceeding 90%. However, when using the normalized metric accounting for differences in 5'-UTRs targeting, performance varied markedly across compared kits. The highest normalized 5'-UTRs coverage was achieved in SureSelect V4+UTRs kit, and then followed by the IDT xGen Exome Research Panel and Nimblegen SeqCap EZ Human Exome Library v3.0.

Conclusions: Choice of accurate WES target enrichment kit is crucial for reliable exome studies. Our results show that despite the high nominal coverage across modern kits, their true efficiency in 5'-UTRs landscapes varies considerably once differences in target design are taken into account. This highlights the need for a careful kit selection, especially when summarization of genetic information into gene-level is performed.

[I-5] IMPROVING VARIANT AND CLONALITY DETECTION IN FFPE SAMPLES: COMPARATIVE ASSESSMENT OF COMPUTATIONAL AND ENZYMATIC CORRECTION STRATEGIES

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Formalin-fixed, paraffin-embedded (FFPE) tissues are a crucial resource for molecular oncology, enabling retrospective genomic studies from archived clinical material. Despite their utility, formaldehyde fixation introduces characteristics DNA alterations – most notably cytosine deamination leading to C>T or G>A transitions. It can complicate accurate variant detection in sequencing data. To address these artifacts, several computational tools such as ideafix, microSEC, DeepOmics, FFPolish, and SOBdetector have been developed to filter out sequencing artifacts.

In our study, we assessed the performance of multiple approaches for artifact filtering in FFPE-derived sequencing data. Among the computational methods tested, DeepOmics demonstrated the most balanced performance, achieving a favorable trade-off between sensitivity and precision in variant calling when compared to fresh-frozen references. Moreover, enzymatic repair with NEBNext® FFPE DNA Repair v2 Module showed the most reliable recovery of native variant profiles, outperforming all computational approaches.

Based on these findings, we extended our analysis to evaluate clonal architecture across four data types: fresh-frozen, FFPE, FFPE processed with DeepOmics and FFPE repaired with NEBNext. Clonal inference was performed using SciClone, based on variant allele frequency (VAF) distributions of somatic single-nucleotide variants. While DeepOmics initially showed promising accuracy in artifact removal, it proved suboptimal for downstream clonality analysis. NEBNext-treated samples, by contrast, best reflected the clonal landscape of the original fresh-frozen material.

Together, these results highlight that although computational tools such as DeepOmics are effective in reducing artifacts and improving variant calling, enzymatic repair remains indispensable for preserving biologically meaningful clonal information in FFPE-based genomic studies.

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[I-6] THE EFFECT OF NON-CLASSICAL FACTORS ON THE INCIDENCE OF ACUTE MYOCARDIAL INFARCTION

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Background: Acute Myocardial Infarction (AMI) occurs when a coronary artery is narrowed or closed by an intravascular thrombus, thus impairing or cutting off blood flow to the heart muscle. In the majority of cases, it is a life-threatening condition that may lead to hemodynamic and electrical instability and sudden cardiac death. The multifactorial nature of AMI makes it challenging to understand the specific role of each contributing risk factor fully. Therefore, analyzing a variety of datasets that incorporate both clinical and environmental data is essential for effectively studying the relationships between potential risk factors and the prevalence of AMI.

Objective: The aim of the study was to evaluate the effect of meteorological factors, including temperature and changes in atmospheric pressure, as well as the time of day, on the incidence of AMI within the population of Gliwice County, and to characterize the clinical profile of patients in the selected groups.

Methods: We utilized data collected from 3778 consecutive patients with AMI, sourced from the hospital in Gliwice between 2007 and 2025. AMI cases were classified according to the International Classification of Diseases, which provides a standardized system for categorizing health conditions. Meteorological data were obtained from the meteostat Python library. To investigate the potential influence of air pollution on the prevalence of AMI, environmental data, specific to the dates on which each AMI occurred, were obtained from the Chief Inspectorate for Environmental Protection. We further extended the analyses by incorporating additional parameters: morphological, echocardiographic, and coronarographic data reflecting the patients'clinical status between 2017 and 2025.

Results: Within the study population, the incidence of AMI was higher in men and typically occurred at younger ages than in women. Interestingly, the time of day was found to influence patients'clinical profiles, with statistically significant associations observed for troponin, potassium, lymphocytes, and eGFR. At the current stage, significant associations with meteorological factors were noted only in certain cases, although the incidence of non-ST--segment elevation myocardial infarction (NSTEMI) tended to increase following drops in atmospheric pressure greater than 5hPa.

Conclusions: The analysis confirmed the typical presentation of patients with AMI. Although elevated air pollution levels and rapid meteorological changes were significantly associated with AMI incidence only in certain cases, sudden drops in atmospheric pressure may represent non-classical risk factors, particularly in NSTEMI. Patients with reduced left ventricular ejection fraction also appeared more susceptible to such pressure changes. These observations underscore the potential value of considering environmental triggers in forecasting NSTEMI incidence and in developing tools to support healthcare resource management or new risk scores.

[I-7] GENOMIC DETERMINANTS AND CONSEQUENCES OF MMEJ IN HRD CANCERS

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Homologous Recombination Deficiency (HRD), frequently caused by mutations in genes such as BRCA1/2, leads to genomic instability in various cancers, with ovarian cancer (OV) being one of the most affected. Under HRD conditions, cancer cells often rely on error-prone, DNA repair mechanisms, such as microhomology-mediated end-joining (MMEJ), which uses short homologous sequences to rejoin DNA ends. The aim of this study was to characterize the genomic consequences and determinants of MMEJ activity in HRD tumors, with ovarian cancer (OV) as a key model.

We performed a comprehensive analysis of whole-genome sequencing (WGS, n = 121 patients) and whole-exome sequencing (WES, n = 506 patients) data from OV samples and 30 additional cancer types (n = 7591 patients). We have also developed a bioinformatic tool, MHDetect, for classification of indels generated through MMEJ activity.

We observed significant variation in the frequency of MMEJ-mediated deletions across cancer types, with the highest proportions detected in TGCT (Testicular Germ Cell Tumor), PCPG (Pheochromocytoma and Paraganglioma), and THCA (Thyroid Carcinoma). In OV, increased MMEJ activity, reflected by a higher number of MMEJ-dependent deletions, was associated primarily with RB1 loss of function. Survival analysis revealed that a higher number of MMEJ-dependent deletions correlated with improved patient survival, indicating the prognostic potential of this pathway.

These results provide new insights into the contexts in which MMEJ becomes overactivated and reveal genomic features associated with its activity in HRD tumors.

[I-8] PREDICTION OF METASTATIC ONSET BASED ON MODELING OF TGF-B–INDUCED EPITHELIAL–MESENCHYMAL TRANSITION

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Transforming Growth Factor Beta (TGF-β) is a cytokine that plays a critical role in regulating cell growth, differentiation, apoptosis, and immune responses. In the context of cancer, TGF-β exhibits a dual function: it acts as a tumor suppressor in early stages by inhibiting epithelial cell proliferation, but promotes tumor progression and metastasis in later stages by inducing epithelial–mesenchymal transition (EMT). Elevated levels of TGF-β are correlated with increased metastatic potential in a wide range of cancers, including breast, lung, pancreatic, and colorectal malignancies [1].

In this study, we developed a model that integrates tumor growth and cell proliferation with the simultaneous modeling of intracellular processes associated with EMT and the dynamics of TGF-β levels within the tissue. We proposed a simplified TGF-β dynamics dependent on tumor size, taking into account the fact that tumor cells synthesize and secrete substantial amounts of TGF-β [1]. The level of TGF-β was fitted to reproduce the increase in tissue levels observed in non-small cell lung cancer (NSCLC) tumors between stages T1–T2 and T3–T4 [2]. For modeling EMT, we employed the framework proposed in [3], incorporating a heterogeneous cell population resulting, for example, from asymmetric cell divisions. The heterogeneity was introduced into the model by randomizing kinetic parameters of the model.

The implemented model allows tracking the levels of EMT-associated proteins, such as E-cadherin and N-cadherin, in individual cells, enabling their classification as epithelial or mesenchymal. We estimated the cumulative density function (CDF) for the probability of metastasis occurrence based on the number of mesenchymal cells at a given time, which was then used to model metastasis with a compartmental ODE model (based on [4]), where the metastatic compartment is switched on when a metastatic event occurs.

The results indicate that incorporating TGF-β dynamics and the EMT model allows prediction of metastasis timing, showing good agreement with clinical Overall Survival (OS) data in a cohort of patients with NSCLC. It should be emphasized that TGF-β dynamics was fitted to population-averaged data. If fitted to individual patient measurements, the model could be used in personalized medicine.

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[I-9] INTEGRATING POPULATION-SPECIFIC ANALYSIS WITH AUTOMATED ACMG-BASED CLASSIFICATION FOR GERMLINE VARIANT INTERPRETATION

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Accurate classification of germline variants remains one of the central challenges in human genetics. Traditional filtering strategies, often based solely on functional annotation or allele frequency thresholds, tend to be overly broad and insufficiently specific. As a result, hundreds of potentially pathogenic variants are typically retained for each individual, even though the vast majority have no real connection to disease mechanisms. The ACMG guidelines have provided essential standardization for clinical variant interpretation. However, numerous modifications and refinements have been proposed, many of which are not integrated into existing implementations. Moreover, no unified, scalable based on ACMG framework currently exists that could be easily applied to large genomic datasets consisting of hundreds of thousands of variants. This gap limits both reproducibility and efficiency in variant interpretation. The aim of this project is to develop an automated classification approach that enables accurate prioritization of truly pathogenic germline variants.

The analysis consisted of two complementary components, both based on Whole Genome Sequencing (WGS) data from 32 Polish individuals. The first focused on germline variants potentially involved in carcinogenesis, while the second addressed the classification of all potentially pathogenic variants, irrespective of disease context. In the first stage, after functional annotation, multi-step filtering retained exonic variants predicted to affect protein structure or function within oncogenes and tumor suppressor genes. Allele frequency differences between the 1000 Polish Genomes project and gnomAD were assessed using the χ^2 test with Bonferroni correction to identify variants specific to the Polish population. The second stage involved developing an automated classification based on ACMG rules integrating functional, population, and gene level evidence, along with recent rule refinements, to enable reproducible interpretation of germline pathogenicity.

The analysis identified 111 variants in 63 genes present in a group of 32 patients, characterized by significant differences in allele frequency between the Polish population and the global population. These include variants that are very low in gnomAD but increase significantly in the Polish population (e.g. BRCA1, JMJD1C, COL7A1). Applying only the most stringent ACMG criterion (PVS1) to all potentially pathogenic variants reduced the dataset from an average of approximately 4 million variants per patient to about 125 variants per patient (38 homozygous and 87 heterozygous).

These findings reveal population-specific germline variants, likely influenced by founder effects or environmental factors. Integrating specific for population data with standardized pathogenicity rules enables efficient, reproducible prioritization of truly pathogenic variants, supporting scalable interpretation and informing personalized genomics and risk assessment.

[I-10] WHEN AI READS GENES: LARGE LANGUAGE MODELS IN GENE SET ANALYSIS

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In bioinformatics, there are a broad range of algorithms dedicated to analyzing gene sets/ signaling pathways based on results from statistical inference. One of them is Overrepresentation Analysis (ORA), which uses as input only the list of statistically significant genes. On the other hand, Large Language Models (LLMs) have gained considerable popularity and are now being applied in various fields. The LLMs also as input needs a list of genes and a short prompt on what users want to do with it. Thus, the aim of this study was to examine the potential and capabilities of free or paid, untrained LLMs in analyzing gene overrepresentation compared to the ORA algorithm.

Six different models (GROK 3, Gemini 2.5 Pro, GPT-5o, Llama 4, DeepSeek-V3, and Phind-70B Model) were tested by assigning four different queries, ranging from more general to specific one. Next, outputs were examined to determine how many gene sets were covered in relation to the ORA. In both cases, the Reactome database was used to identify enriched pathways. Next semantic similarity was compared using MedCPT. Finally, the hierarchy of Reactome pathways returned by the LLMs was analyzed to investigate how detailed the results provided by the models were.

None of the LLMs returns the same number of pathways as the ORA. The best coverage was achieved by GPT-5o and Gemini 2.5 Pro with levels 9.5% and 7.8% respectively. Interestingly, part of the LLMs gave Reactome's ID, but after verification, certain of those IDs do not exist, or the names were inconsistent with the originals, which suggests hallucination of models. Next, the identified signal paths from LLMs appear to be more general compared to ORA, as indicated by their hierarchical level. Yet, each of the outputs presents similar semantics to results from ORA with cosine metric over 0.95.

This study shows that the untrained LLMs are able to assign genes to specific pathways and provide correct labels. Nonetheless, their capability in overrepresentation analysis remains rather limited. The results also suggest that, in future studies, the models like GPT-5o and Gemini 2.5 Pro show the greatest potential for training to for practical tools in gene set analysis.

[I-11] TOOL FOR MIRNA ENRICHMENT ANALYSIS – CATNAP

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The CATNAP application is a bioinformatics tool developed for the analysis of genes regulated by microRNAs (miRNAs) and their associated biological pathways. The tool was built in the R programming language using the Shiny framework, which enables an intuitive and user-friendly operation directly through a web browser.

The application accepts user input for a selected miRNA name and parameters such as the minimal prediction score and the minimal pathway size. Data are automatically retrieved from a locally stored miRDB database and subsequently analyzed using the clusterProfiler and KEGGREST packages. The results are presented in the form of dynamic tables and plots, with an option to export them for further use.

Functionally, the application is conceptually related to existing tools such as ShinyGO 0.82, STRING, or miRNet, but it focuses specifically on the miRNA–gene–pathway relationships and does not emphasize oncological pathways, unlike miRNet. CATNAP serves as a simple and efficient alternative, allowing for rapid biological interpretation and visualization of miRNA-related analyses.

[I-12] OPTIMIZATION OF PLASMID SEGMENTATION IN ATOMIC FORCE MICROSCOPY IMAGES USING CLASSICAL IMAGE PROCESSING METHODS

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Introduction: Microscopic image analysis is an important step in biological research. The automation of microscopic image processing and analysis methods provides significant support for researchers, enabling much faster analysis of large amounts of data and offering greater consistency compared to the human eye. Among imaging techniques, atomic force microscopy (AFM) has gained particular interest because it enables visualization of even very small biomolecular structures, such as plasmid DNA. The observations and their analysis enable the evaluation of plasmid characteristics and properties, which is valuable for studies examining the effects of specific factors on these molecules. The main objective of this work was to find the best methods for image denoising and plasmid segmentation in order to automate the plasmid analysis process.

Materials and Methods: AFM pUC19 plasmid images were used to test various classical image processing methods. The focus was on background denoising, maximizing the distinction between the regions of interest (plasmid regions) and the background, and obtaining a binary mask containing the ROIs. Individual steps were analyzed to obtain the most effective approach. The noise reduction process involved testing a combination of different filtering steps, such as: total variation filter, non-local means, wavelet denoising or bilateral filter, histogram equalization (traditional or CLAHE), as well as the influence of Gaussian background subtraction.

Results: As a result of the conducted tests, the performance of various approaches to microscopic image denoising was evaluated. The final stage of image preparation was binarization, which was also tested using various thresholds and algorithms. The image processing methods were assessed visually and using the BRISQUE method. Binary segmentation results were compared against manually annotated plasmids on a subset of images.

Conclusions: The tested methods enabled the identification of the optimal pipeline for plasmid segmentation. They successfully reduced strong noise and delineated plasmid regions in the test images. The obtained methods will allow for the automation of AFM plasmid image analysis and the use of the results in further, more advanced stages of research.

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[I-13] IMPLANT SEGMENTATION FROM COMPUTED MICROTOMOGRAPHY IMAGES USING SUPERVISED AND UNSUPERVISED MACHINE LEARNING METHODS

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Development of metallurgy, chemical engineering and materials engineering enables to synthesis of alloys with different properties, used in e.g. biomaterials engineering. An example of this may be magnesium alloy's implants, developed to improve the comfort of patients demanding the use of an implant screw. The most important feature of the implant is its biodegradability. The aim of the project was to determine the rate of decrease in implant volume and the process of bone formation around it over time.

Analysis and image processing was made in Python programming language. The images obtained were Z-stack from micro-computed tomography examinations at various times during which the implant was present in the bone. The script was written for the purpose of segmentation bone, newborn bone, tissue, background, gas inside the bone and implant from every Z-stacks' image. The implant segmentation was performed using the proprietary Region Growing algorithm, which searched for similar pixels in three dimensions.

The next step was to check the quality of the segmentation script. For this purpose, the unsupervised machine learning method k-means was used. This algorithm, after getting the number of clusters, was supposed to classify the image pixels itself.

The last step was to test a supervised machine learning method. For this purpose, the U-net neural network architecture was written. Training data were images obtained by manual segmentation with applied labels.

Initial manual segmentation, as well as the use of supervised and unsupervised machine learning methods, revealed a gradual decrease in implant volume over time. Image analysis also indicated changes in the amount of gases produced during the degradation process. Simultaneously, an increase in newly formed bone tissue was observed within the area of implant-induced damage.

The applied supervised and unsupervised neural learning methods allowed us to observe that the k-means algorithm did not select centroids appropriately, which resulted in distorted segmentation due to noise resulting from clustering of inappropriate pixels from the image. However, despite the inaccuracy of some algorithms, we can observe that the design of the magnesium alloy implants meets its assumptions, and the implants degrade over time.

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[I-14] RNA-SEQ-BASED ANALYSIS OF DIFFERENTIAL GENE EXPRESSION IN THYROID CANCER

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Introduction: Thyroid cancer is a type of malignancy that originates in the thyroid gland, the organ located at the base of the neck. It is one of the most common endocrine cancers and includes several subtypes. This research aimed to investigate gene expression differences between healthy and tumor thyroid tissues based on RNA sequencing – a powerful high-throughput technique for the transcriptome analysis.

Methods: The thyroid cancer data came from The Cancer Genome Atlas (TCGA) project and were downloaded from the Genomic Data Commons (GDC) portal in the STAR counts form. The pairs of tumor and healthy samples from 59 patients were analysed. The data were normalized using a variance stabilizing transformation from DESeq2 R package. Principal component analysis (PCA) served for the initial assessment of the general expression pattern. The paired Mann-Whitney U-test followed by the Benjamini-Hochberg correction method were used for the comparative analysis. Gene set enrichment analysis was conducted with the CERNO test.

Results: PCA showed a distinct separation between healthy and cancer samples, suggesting strong biological divergence in gene expression profiles. A large number of statistically significant genes (over 21 thousand) were identified using the comparative analysis, which confirmed the widespread expression changes in thyroid cancer tissues. The CERNO test revealed 11 gene sets showing significant enrichment. Among them, the most consistently enriched modules, supported by high AUC and low q-values, were associated with “cell cycle and transcription”, “immune activation – generic cluster”, and “inflammation”.

Conclusion: These findings indicate distinct differences in gene expression profiles of cancer and healthy tissues. Moreover, they provide insight into the biological pathways involved in thyroid cancer. The study also lays the foundation for further research.

Keywords: thyroid cancer, gene expression profiling, RNA-seq, comparative analysis

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[I-15] PARAMETER ESTIMATION OF A HYBRID MODEL OF CANCER RADIOCHEMOTHERAPY USING ADJOINT SENSITIVITY ANALYSIS

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In this work, we used a hybrid approach [1] and adjoint sensitivity analysis [2] to parameter estimation of a dynamical model of combined radiochemotherapy for a group of patients. In the estimation process, we utilized clinical data for lung cancer patients obtained from the National Institute of Oncology, Gliwice branch. The hybrid approach assumes that parameters of the dynamical model aren't directly estimated for the whole group of patients, but rather those parameters are a function of additional clinical data for each patient. The results show that using a hybrid approach and adjoint sensitivity analysis, we can predict more precisely (in comparison to the classical approach) the overall survival times of patients.

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[I-16] SCALING UP THE MORAN MODEL FOR ASSESSING THE CLONAL STRUCTURE OF SIMULATED CANCER CELL POPULATIONS

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The Moran model proposed by P.A.P. Moran in 1957 [1] allows stochastic simulation of the dynamics of finite populations. Modifications and extensions to the algorithm enable it to be used in specific studies on the evolution of variability in the chosen population. Kurpas, M. K., & Kimmel, M. (2022) [2] used an extended Moran model to simulate changes in fitness of cancer cells as a result of 'driver' and 'passenger' mutations. The model they created was implemented in Matlab and simulated a population of a hundred cells. A significant increase in population size was impractical for this implementation, due to memory usage. In this work the algorithm implemented by Kurpas, M. K., & Kimmel, M. has been modified using sparse matrices to achieve lessened memory usage, enabling simulations using larger populations of cells. This new implementation allowed for a simulation of even a million cells, while preserving key characteristics of evolutionary dynamics such as selective ousting of clones. The model has been fitted to sequencing data of triple-negative breast cancer (TNBC) to showcase its veracity to experimental datasets. The created solution enables the simulation of carcinogenic processes and the adaptation of cancers on a scale more similar to actual in vitro experiments. With a more similar population size the risk of unnatural conclusions based on effects of small populations, such as disproportionately larger impact of random effects, is minimized, which is an important step in obtaining a biologically precise model of carcinogenic processes.

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[I-17] OPTIMIZING MORAN-MODEL PARAMETERS TO REPRODUCE TUMOR VARIANT-FREQUENCY SPECTRA

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Mathematical modeling of tumor growth provides a framework for studying mechanisms underlying clonal expansion, accumulation of mutations, and treatment resistance. The stochastic Moran process[1] describes evolutionary dynamics in finite tumor cell populations. In this study, we apply the Model A formulation[2] to simulate tumor growth and explore how evolutionary parameters shape intratumoral diversity.

Our approach focuses on three key parameters: the selective advantage of driver mutation (s), the fitness cost of passenger mutation (d), and the driver mutation probability (p). These jointly determine whether tumor evolution favors clonal coexistence or rapid dominance of aggressive subpopulations.

Variant-allele-frequency spectra from TCGA and Silesian University of Technology cohorts reveal substantial heterogeneity between and within tumor types. To capture this diversity, we generate site-frequency spectra using stochastic Moran-model simulations and optimize parameters by fitting simulated to empirical data. The fitting process combines deterministic, heuristic, and machine-learning–based optimization to ensure robust and automated parameter estimation.

Preliminary results show that optimized selection coefficients vary across tumor types, reflecting differences in evolutionary pressure and clonal architecture. The proposed framework provides a quantitative method for characterizing tumor evolution and holds diagnostic potential for assessing heterogeneity, predicting resistance, and improving individualized therapies.

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[I-18] MODELING CLONAL EVOLUTION IN CANCER: A COMPUTATIONAL OPTIMIZATION FRAMEWORK

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Understanding tumor evolution requires integrating biological data analysis with efficient mathematical modeling. This study develops a computational framework to simulate cancer cell population dynamics using genomic data. The core of the model is a modernized Moran process algorithm implemented in C++. To enable large-scale simulations, key data structures use dynamic memory allocation, significantly reducing RAM usage. The model is calibrated to experimental data by fitting the simulated Variant Allele Frequency (VAF) distribution. We applied Bayesian optimization to identify key parameters, such as selection coefficients, that produce VAFs aligning with empirical observations. This combined approach of a memory-optimized model and Bayesian inference provides a powerful tool for reconstructing evolutionary histories, revealing specific pathways of progression.

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[I-19] SEGMENTATION AND CO-REGISTRATION OF HIP X-RAY IMAGES TO ASSESS HIP ENDOPROSTHESIS DISPLACEMENT

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Introduction: The hip joint is one of the most critical joints in the human body, and arthroplasty, the surgical procedure for hip joint replacement, is one of the two most frequently carried out orthopaedic surgeries worldwide. Conventional radiography is regularly used to evaluate joint prostheses after implantation. X-rays help detect potential abnormalities affecting both the implant and the surrounding bone. These include, for example, periprosthetic fracture, dislocation, osteolysis, shaft collapse, or even minor displacement of the endoprosthesis. The aim of this study is to develop a program for assessing the displacement of a hip prosthesis using image analysis, particularly segmentation and image co-registration, and to test various algorithms for these tasks.

Materials and methods: X-ray images of the hip joint from three patients, taken in two different planes and at two time points, were provided by the Trauma and Orthopedic Department of the District Hospital of Orthopaedics and Trauma Surgery in Piekary Śląskie. First, the X-ray images of the implant was segmented using two methods (thresholding and k-means clustering) to determine the superior approach, and the results were assessed using the Dice coefficient. Then, various colours and transparency levels for the registered images were tested. In the final stage, different image co-registration transformations were evaluated to select the best one using the same metric and to assess whether displacement occurred.

Results: Both segmentation methods yielded nearly identical, highly accurate results. The Dice coefficient did not fall below 0.97 compared to the manually segmented reference implant for each image. Default colour schemes were used for overlaying: grayscale for the fixed image and the "turbo" colormap for the moving image, with a transparency level of 0.45. Among the transformations, the worst results were obtained with the "Rigid" transformation, while no significant differences were observed among the other three transformations, and the metric did not indicate values below 0.95. Visual evaluation demonstrated no displacement of the implant over time.

Conclusions: Due to its greater automation potential, the k-means clustering segmentation method appears to be the better choice. Considering the computational resources required and minimal differences in results, the "Similarity" transformation was selected for further analysis. The chosen colour schemes and transparency levels enable efficient visual assessment of the registered images, which confirmed that no migration of the hip prosthesis occurred in the analysed patients.

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[I-20] COMPARATIVE ANALYSIS OF 2D U-NET AND 3D U-NET FOR GLIOMA SEGMENTATION IN MULTI-SEQUENCE MRI BRAIN IMAGES

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Introduction: Accurate segmentation of gliomas in magnetic resonance imaging (MRI) brain images is essential for diagnosis, treatment planning, and monitoring of disease progression. This study presents a comparative analysis of two widely used deep learning architectures: 2D U-Net and 3D U-Net for glioma segmentation with the focus on assessing the influence of multi-sequence MRI data on segmentation performance and identifying the most effective approach to enhance accuracy and robustness in automated brain tumor analysis.

Materials and methods: The study used the BRATS 2021 dataset, which contains multi-sequence MRI images (T1, T1ce, T2, FLAIR) with expert-annotated glioma segmentations. This dataset offers a set of brain tumor images, covering various tumor grades and types. The performance of 2D U-Net models, processing images slice by slice, was compared to 3D U-Net models that leverage volumetric context by analysing the entire MRI volume and capturing spatial relationships between slices. Experiments evaluated segmentation accuracy using different combinations of MRI sequences to determine how sequences influence the model's accuracy. Evaluation metrics included the Dice similarity coefficient, measuring the overlap between predicted and ground truth segmentations, as well as sensitivity and specificity, which reflect the model's capability to correctly identify tumor tissue and minimize false positives. Models were trained following standardised procedures to ensure fair comparison and reliable performance assessment across architectures.

Results: Preliminary results indicate differences in segmentation performance between 2D and 3D U-Net models depending on the number and type of MRI sequences used. 3D U-Net models benefit from volumetric context but require more computational resources, whereas 2D U-Net models show robust performance on smaller datasets and with fewer sequences. The choice of sequences affected model accuracy, underscoring the importance of integrating multiple sequences for optimal glioma segmentation.

Conclusions: The study demonstrates that both network architecture and the selection of MRI sequences notably impact glioma segmentation performance. While 3D U-Net shows potential for improved volumetric segmentation, 2D U-Net provides a computationally efficient alternative for data-limited scenarios. Future work will explore hybrid approaches and optimised multi-sequence integration to enhance segmentation accuracy further.

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[I-21] CAN DIET PREDICT INSULIN RESISTANCE? A DATA-DRIVEN EXPLORATION OF NUTRITIONAL HABITS

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Introduction: Insulin resistance is a metabolic condition in which the body's cells exhibit reduced sensitivity to insulin, leading to increased levels of both insulin and glucose in the blood. The aim of this study was to evaluate the contribution of these dietary factors to the risk of developing insulin resistance. This project is conducted to better understand how nutrition influences metabolic health and to identify potential strategies for preventing insulin resistance through dietary modifications.

Methods: In this project, epidemiological and laboratory data from the NHANES 2021-2023 database were used, comprising 30 features measured in 3,158 patients. For this study, seven diet-related features were selected, including nutrient intake, caloric value, and the amount of HDL ("good") cholesterol. The dataset was first preprocessed by cleaning and imputing missing values using the k-nearest neighbors (kNN) algorithm. The normality of variable distributions was assessed using the Shapiro–Wilk test, and pairwise correlations between features were examined. Three classification models were tested and compared: logistic regression, random forest, and Support Vector Machine (SVM). The dataset was split into training and testing subsets in an 80:20 ratio. Model performance was evaluated using balanced accuracy, sensitivity, and specificity.

Results: The Shapiro-Wilk test revealed that several variables deviated from a normal distribution. Among the tested classification methods, the logistic regression model achieved the highest overall performance, with a balanced accuracy of 0.66, sensitivity of 0.72, and specificity of 0.60. The most influential feature identified by the model was the HDL cholesterol level, while the other diet-related features showed comparable importance among the analyzed parameters.

Conclusions: The results indicate that the developed classification models can predict the likelihood of developing insulin resistance with moderate accuracy. Although the achieved performance metrics suggest a promising direction, further research should include a broader range of dietary and clinical features to improve model sensitivity and generalizability.

[I-22] ANALYSIS OF THROMBIN ACTIVITY RELEASED DURING STEPWISE DISSOLUTION OF BLOOD CLOTS: DEPENDENCE ON SURFACE DENSITY

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Thrombin plays a key role in blood coagulation and clot degradation. The aim of this study was to investigate the dynamics of thrombin release during controlled dissolution of blood clots and to assess the influence of surface density on enzymatic activity.

Clots were subjected to dissolution for 6 hours, and fractions were collected at regular intervals. Thrombin activity was determined using a fluorescence-based assay, and concentrations were calculated from calibration curves fitted with a polynomial model in a logarithmic scale. Surface density was calculated from clot length and mass measurements.

An increasing trend in mean thrombin activity was observed during the final hours of the experiment in clots with higher surface density. In contrast, clots with lower density showed earlier and more uniform enzyme release.

Clot structural density affects the kinetics of thrombin release. Denser clots exhibit delayed but more pronounced enzyme release in later stages, which may result from limited diffusion and retention of thrombin within the compact fibrin network.

[I-23] PRELIMINARY COMPARISON OF NEURAL NETWORK EFFECTIVENESS FOR BREAST LESION SEGMENTATION IN DCE-MRI

Michał Rajzer[1], Bartłomiej Szkodny[1], Jakub Wieczorek[1], Jakub Sanecki[1], Michał Czyż[1], Piotr Bartosz[1,2], Michał Lasak[3], Tomasz Michalik[4,5], Alicja Staśczak[4,5], Sebastian Student[4,5], Damian Borys[4,5]

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Magnetic Resonance Imaging (MRI) is a highly sensitive modality for detecting breast cancer but the accurate segmentation of lesions remains a challenging and time-consuming task for radiologists. Automated segmentation methods, particularly those based on deep learning, offer significant potential to improve diagnostic workflow and reproducibility. This study presents a preliminary comparison of the effectiveness of various neural network architectures for breast lesion segmentation in MRI data.

In our study, we utilised a Mamma Mia dataset, which is a set of breast DCE-MRI scans with corresponding ground-truth segmentations delineated by expert radiologists. The dataset consisted of DCE-MRI images of 1271 patients with images in axial view. We focused on comparing three distinct neural network architectures for semantic segmentation: the standard U-Net architecture, Segresnet, and a NN-Unet model. Additionally, the YOLO model was explored for initial lesion localisation (bounding box detection) to create a region-of-interest-based segmentation pipeline. For baseline comparison, traditional clustering algorithms, namely Fuzzy C-Means (FCM) and X-means, were also applied. The results were verified using Dice Sørensen Score, IoU, HD95 and MSD evaluation metrics. Approaches such as SegResNet, 3D U-Net, and NN-UNet vary in their suitability for 3D MRI segmentation. SegResNet is tailored for volumetric imaging with deep residual blocks, achieving high accuracy and robustness at a high computational cost. 3D U-Net remains a widely used and efficient baseline, balancing accuracy, interpretability, and training complexity. NN-UNet builds on the U-Net framework by automatically adapting its architecture and preprocessing to the dataset, consistently delivering superior performance with minimal manual tuning.

In conclusion, deep learning-based methods clearly outperform traditional clustering approaches for breast lesion segmentation in DCE-MRI. Among the evaluated models, NN-UNet achieved the most accurate and reliable results, while SegResNet and 3D U-Net provided strong and complementary performance. These findings confirm NN-UNet as a robust and generalizable solution for automated MRI segmentation in clinical applications.

Calculations were carried out using the infrastructure of the Ziemowit computer cluster POIG.02.01.00-00--166/08 POIG.02.03.01-00-040/13

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[I-24] COMPARATIVE ANALYSIS OF CANCER DATA FROM TCGA AND SILESIAN UNIVERSITY OF TECHNOLOGY PROJECT DATASET

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Genomic analysis of cancer provides insights into tumor progression, genetic changes and potential therapies. Breast (BRCA), lung adenocarcinoma (LUAD) and head and neck (HNSC) cancers show diverse mutational profiles and copy number variations (CNV), which are linked to disease development and reactions to therapy. Comparative analysis of large public datasets, such as TCGA, together with sequencing data from the Silesian University of Technology project (POLSL) enables assessment of data reproducibility and detection of genomic patterns unique to specific groups. Such data can serve as a basis for mathematical modeling of tumor evolution and guiding experimental confirmation.

Sequencing data from BRCA, LUAD and HNSC tumors were obtained from TCGA and the POLSL dataset. POLSL samples for BRCA and HNSC were derived from formalin-fixed, paraffin-embedded (FFPE) tissue, while LUAD samples were frozen. TCGA datasets were based mostly on frozen tissue. Initial analyses of the POLSL dataset revealed formalin-fixation artifacts in BRCA and HNSC samples. False-positive mutation calls were filtered out using DEEPOMICS software [1]. Analyses included descriptive statistics, identification of recurrent driver mutations and copy number variation assessment. Mutation frequency plots and CNV heatmaps were among the visualization methods used for comparisons between datasets.

Across both datasets, key driver mutations were consistent. TP53 was the main driver in HNSC and LUAD, while BRCA showed mutations in both TP53 and PIK3CA. CNV analysis revealed characteristic amplifications and deletions for each cancer type. Despite differences in data origin, general trends in mutation frequencies and CNV were comparable between TCGA and POLSL datasets. Variant class distributions differed slightly. POLSL data showed fewer insertions and deletions, but filtration improved substitution proportions, especially in BRCA. Across cancer types, BRCA showed higher copy number levels, as typically observed for this cancer type. HNSC data showed lower consistency before and after filtration, likely due to higher sample heterogeneity, whereas LUAD results closely matched TCGA.

This comparative genomic analysis of BRCA, LUAD, and HNSC tumors adds to the understanding of key genomic alterations across public and institutional datasets. Identification of overlapping as well as unique features between datasets can guide future analyses combining genomic, clinical, and computational data and support the development of predictive models of tumor evolution.

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[I-25] EXTENSION OF THE CHEMICAL INVENTORY AND CATALOGUING APPLICATION TO INCORPORATE LOCATION TRACKING FUNCTIONALITY AND THE CAPABILITY TO GENERATE GHS-COMPLIANT LABELS

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In chemical laboratories, efficient management and labelling of chemical reagents are essential for ensuring safety, organization, and effective resource use. Researchers at the Faculty of Chemistry of the Silesian University of Technology often face difficulties in locating, identifying, and cataloguing reagents. To address this issue, we focused on expanding the existing computer system for inventorying chemicals and introducing new functionalities that would streamline daily laboratory work. The updated version of the system integrates a PostgreSQL database containing detailed information on reagents, including safety data such as H and P precautionary codes. It enables users to generate standardized GHS (Globally Harmonized System) labels, manage reagent records, and quickly identify substances using QR and barcode technology. The project also includes the development of a responsive web interface and a dedicated mobile application, allowing laboratory staff to access and update reagent information directly in the laboratory space.

The realization of this project will result in a comprehensive and user-friendly system for managing laboratory reagents, improving safety and efficiency, reducing redundant purchases, and significantly streamlining laboratory work at the Silesian University of Technology. Additionally, it will provide students with valuable experience in database design, web and mobile application development, and team collaboration.

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[I-26] COMPUTATIONAL ANALYSIS OF REACTIVE OXYGEN SPECIES NEUTRALIZATION IN DIFFERENT CANCER CELL TYPES

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Reactive Oxygen Species (ROS) are known mainly as factors that negatively influence cells, causing aging and death. Although considered toxic, they are fundamental byproducts of existing in an aerobic environment, and often, they can have a beneficial impact on cells. To counteract the detrimental effects of free radicals, each cell possesses various tools and mechanisms that enable rapid neutralization of ROS, thereby protecting the cell from their detrimental effects. The objective of this research was to enhance understanding of how these systems operate and how their activity varies across different cell types and tissues.

Based on the collected data, a mathematical model was developed and applied to compare the effectiveness of different pathways for ROS neutralization in cancer cells sourced from various tissues. Particular attention was paid to hydrogen peroxide (H_2O_2), a key molecule regulating cellular redox status, which can function as a signaling molecule in intracellular and extracellular signaling. Computer simulations revealed that the process of neutralizing ROS (H_2O_2) varied not only among cancer cell lines from different tissues but also among those derived from the same tissue. Notably, these simulations facilitated a comparison of ROS neutralization in both radiosensitive and radioresistant cancer cell lines, indicating that the effectiveness of specific ROS neutralization pathways could be linked to the cells' capacity to survive radiation exposure. The identified differences in radiosensitivity among various types of cancer cells may be linked to their capacity to neutralize H_2O_2, indicating the potential use of this feature as a biomarker of radiosensitivity.

Additionally, the research gathered and organized knowledge about reactive oxygen species and the mechanisms of their regulation in various cell types. Both universal and tissue-specific expression patterns of genes involved in redox processes were identified. It has been shown that ionizing radiation triggers a coordinated transcriptional response in cells by modulating the expression of genes that are involved in the generation and neutralization of reactive oxygen species (ROS). Furthermore, the role of miRNAs as a feedback element in the regulation of ROS levels was shown, suggesting interaction with hydrogen peroxide and neutralizing enzymes.

The developed mathematical model, along with other research outcomes, has significantly advanced our understanding of the mechanisms that govern ROS neutralization and provide a valuable starting point for further research, particularly in the context of a better understanding of the mechanisms that differentiate cellular responses to oxidative stress and radiation.

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[I-27] DETECTION AND CLASSIFICATION OF PULMONARY DISEASES IN CHEST X-RAY IMAGES USING NEURAL NETWORKS

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Pneumonia remains one of the leading causes of hospitalization and mortality worldwide, particularly among children and the elderly. Chest radiography is the primary diagnostic tool due to accessibility and low cost, yet image interpretation is sensitive to subjectivity and variability among observers. Recent advances in artificial intelligence, especially convolutional neural networks, have enabled rapid and objective diagnosis of pulmonary diseases. A hierarchical deep learning framework was presented for automated detection and classification of lung abnormalities on X-ray images, aiming to improve diagnostic precision and interpretability. The proposed model was developed on EfficientNet architecture. The classification process proceeds hierarchically — first distinguishing healthy from pathological lungs, then differentiating among tuberculosis, COVID-19, and pneumonia, finally subdividing pneumonia into bacterial and viral forms. Publicly available datasets were integrated into a five-class dataset and exceeded 7,000 X-ray images. Data preprocessing included augmentation, normalization, and expert verification. Transfer learning with pretrained EfficientNet (B1 and B3 versions) models was applied in TensorFlow, and model performance was evaluated using standard classification metrics. Model interpretability was evaluated using Grad-CAM visualizations to highlight image areas critical to decision-making. The system achieved consistently high classification performance across all stages, effectively distinguishing between healthy and pathological lungs as well as among individual disease categories. Slightly lower accuracy results for distinguishing between subtypes of pneumonia. Grad-CAM heatmaps confirmed that the model generally focused on clinically relevant lung regions, supporting the reliability of its predictions. This study demonstrates that EfficientNet provides an accurate and interpretable approach for automated pulmonary disease classification. The hierarchical framework extends beyond traditional binary detection by addressing etiological differentiation of pneumonia, showing strong potential for clinical integration, particularly in resource-limited settings. Future work will focus on expanding dataset diversity and exploring innovative deep learning and explainable AI techniques to further enhance differentiation of pulmonary diseases.

[I-28] A PATHWAY FREQUENCY-BASED APPROACH FOR IDENTIFYING THE MOST FUNCTIONALLY RELEVANT MIRNA TARGETS – IN SILICO REVALIDATION STUDY

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Pathway enrichment databases help identify which proteins are involved in specific biological pathways. Our approach focuses on functionally revalidating the significance of specific miRNAs by identifying their target genes that are most frequently represented across enriched pathways. This strategy allows us to highlight the targets most likely responsible for the observed regulatory effects of the miRNAs. This study presents a pathway frequency-based analytical approach designed to identify the most functionally relevant target genes of selected microRNAs (miRNAs): miR-9-3p, miR-9-5p, and miR-129-5p. The goal of this work was to determine how frequently these miRNA target genes appear across enriched biological pathways and to rank them based on their frequency of occurrence.

Predicted miRNA targets were retrieved from the miRDB database and filtered for confidence scores ≥ 80. Enrichment analysis was conducted using the shiny0.82 platform, integrating data from Gene Ontology (BP, CC, MF), PANTHER, KEGG, Reactome, WikiPathways, and Hallmark databases. Pathways with FDR ≥ 0.05 were excluded. A custom R script was used to count gene occurrences and compile cumulative frequency lists across all databases. In total, more than 1300 enriched pathways were processed before filtering.

The analysis revealed the five genes that appeared most frequently across signaling pathways. WNT5A was present in 396 pathways and is a target of miR-129-5p, followed by ITGB1 (331 pathways, target of miR-9-3p), TGFB2 (318 pathways, target of miR-9-3p), SMAD4 (304 pathways, target of miR-129-5p), SIRT1 (299 pathways, target of miR-9-5p).

Those consistent targeting events by the miR-9 and miR-129 families – miRNAs down-regulated after reperfusion therapy in acute stroke patients – suggest that they represent robust candidates for revalidation as functional targets in the context of neurovascular injury and recovery.

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[I-29] DEVELOPMENT OF AN INTEGRATED COMPUTATIONAL PIPELINE FOR IDENTIFYING KEY REGULATORY VARIABLES IN OVARIAN CANCER NETWORKS THROUGH MULTI-ALGORITHMIC NETWORK ANALYSIS

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This research presents a comprehensive computational framework for systematic identification of key regulatory variables in complex biological networks, with specific application to ovarian cancer signaling pathways. The study addresses fundamental challenges in biological network analysis by developing an integrated pipeline that combines automated data extraction from multiple omics databases (Pathway Commons, AnimalTFDB, CellTalkDB, TCGA-OV) with complementary computational methodologies.

Our approach implements four distinct analytical techniques: Boolean network modeling for discrete-state dynamic simulation, PageRank algorithm for network centrality quantification, random walk simulations for stochastic exploration of information flow, and Recurrent Convolutional Neural Networks (RCNNs) for spatiotemporal pattern recognition in network dynamics. The constructed molecular interaction network comprised 1,872 nodes and 3,914 edges, representing a comprehensive map of ovarian cancer signaling. Multi-algorithmic convergence analysis consistently identified established master regulators, including NF-κB, p53, and ATM, across all methodological approaches, while also revealing novel candidates such as IKKα and TNFR1. Method-specific findings included Boolean modeling identification of crucial mRNA components (A20 mRNA, Wip1 mRNA, PTEN mRNA), PageRank detection of phosphorylated signaling intermediates (ATM-p, Chk2-p), random walk highlighting of DNA damage response elements, and RCNN emphasis on apoptosis-associated proteins (Bax, p21).

Framework validation across Cell Cycle and MAPK signaling pathways demonstrated robust identification of pathway-specific regulators, including Cyclin D, CDK4/6, EGFR, RAS, MEK, and ERK, confirming methodological generalizability. The research contributes an empirically validated computational framework that advances systems biology approaches to therapeutic target discovery, providing both methodological innovations in network analysis and substantive biological insights into ovarian cancer regulatory mechanisms.

Session II

„Bioactive Compounds and Medicinal Chemistry„

[II-1] NANOSTRUCTURED LIPID CARRIERS AS PLATFORMS FOR REPURPOSING NONSTEROIDAL ANTI-INFLAMMATORY DRUGS TOWARD ANTICANCER APPLICATIONS

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Cancer remains one of the leading causes of death worldwide, and its rising incidence calls for the development of effective and safe therapeutic strategies. One promising approach involves repurposing well-known nonsteroidal anti-inflammatory drugs (NSAIDs) as anticancer agents. Compounds such as dexibuprofen (DXI) and indomethacin (IND) exhibit documented proapoptotic and antiproliferative activity; however, their clinical application is limited by adverse effects and low bioavailability due to poor water solubility.

The aim of this study was to design and characterize nanostructured lipid carriers (NLCs) as controlled-release systems for the delivery of NSAIDs in cancer therapy. Both DXI-NLC and IND-NLC were produced using the hot high-pressure homogenization technique with a combination of beeswax and Miglyol® 812 as the lipid matrix and Tween® 80 as a surfactant. The obtained nanocarriers showed excellent physicochemical properties and very high encapsulation efficiency (>99%).

Both systems demonstrated high storage stability and prolonged drug release compared to free drugs. In vitro studies performed on prostate cancer (PC-3) and triple-negative breast cancer (MDA-MB-468) cell lines revealed significantly higher antiproliferative activity for the drug-loaded NLCs compared to non-encapsulated NSAIDs. DXI-NLC exhibited an IC_{50} value of 3.4 μM against MDA-MB-468 cells, while IND-NLC showed an IC_{50} of 15.7 μM, whereas free drugs displayed no significant cytotoxic effect. Additionally, rapid cellular internalization of the nanoparticles was observed, confirming their efficient uptake by cancer cells.

The developed DXI-NLC and IND-NLC formulations represent promising nanotechnology-based platforms for the targeted delivery of NSAIDs with anticancer properties. Combining high physicochemical stability, biocompatibility, and enhanced biological efficacy, these systems may contribute to the advancement of low-cost and effective cancer therapies based on drug repurposing.

[II-2] CELLULAR EFFECTS OF PHOTODYNAMIC THERAPY (PDT) IN SPATIALLY DISTRIBUTED 3D CO-INCUBATION SYSTEMS *IN VITRO*

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Nonsteroidal anti-inflammatory drugs (NSAIDs) such as ibuprofen inhibit cyclooxygenase and exhibit analgesic, antipyretic, and anticancer effects [1,2]. The project investigated metal complexes (Fe, Ag) with NSAIDs for their cytotoxicity against human normal and cancer cells. FePcOC compounds, which react with diclofenac and generate reactive oxygen species, were used. The products were analyzed for their cytotoxicity and anticancer activity, also in potential photodynamic therapy (PDT) protocols, using 3D cell culture models (spheroids and organoids) in vitro [3]. In vitro 2D and 3D cell cultures were subjected to PDT using a red light lamp (λ=685 nm). After 24 hours, intravital observations were performed using fluorescence and confocal microscopy to determine culture growth and cellular morphological changes, as well as the spatial distribution of specific molecular signals. Cell death was determined using viability assays and flow cytometry. Additionally, mitochondrial redox potential under the influence of induced oxidative stress was assessed. As a result, it turned out that the administration of new diclofenac derivatives yielded innovative results in inducing cell death, such as apoptosis, which, when regulated, could become a new therapeutic target. The mechanism of action of photosensitizers in in vitro cellular systems has been tentatively identified.

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[II-3] BIOTRANSFORMATION OF LAVENDER INFUSION BY HUMAN GUT MICROBIOTA

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Lavender (Lavandula officinalis) infusion is traditionally used to relieve mild anxiety and depression [1]. We characterized a commercial flower infusion by UHPLC-DAD-IT-MS and isolated major constituents by preparative HPLC with NMR confirmation. Thirty-one phenolics were identified, mainly flavone glycosides and methoxylated hydroxycinnamate derivatives (e.g., Z/E-melilotoside and 4-methoxymelilotosides). Ex vivo anaerobic incubation with human fecal slurries (n=6) showed extensive biotransformation: glycosides were hydrolyzed and reduced to smaller metabolites, including coumarin and herniarin from melilotoside isomers, o-coumaric and 4-methoxy-o-coumaric acids, and the flavone aglycones luteolin and apigenin. Importantly, the infusion did not induce dysbiosis; α-diversity remained stable and relative abundances of beneficial genera such as Faecalibacterium and Prevotella increased versus controls, indicating a prebiotic-like shift [3]. Because coumarin is a known human hepatotoxicant, its microbial formation highlights a safety consideration for chronic intake [2]. Collectively, these data provide a mechanistic link between lavender's polar phytochemicals and gut microbiota, suggesting that microbial production of postbiotic metabolites may contribute to reported anxiolytic effects, while underscoring the need to monitor coumarin exposure. Further in vivo studies should quantify these metabolites in humans and relate them to clinical endpoints.

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[II-4] DESIGN AND EVALUATION OF NEW 1,8-NAPHTHALIMIDE–MUCOCHLORIC ACID HYBRIDS AS POTENTIAL ANTI-CANCER AGENTS

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1,8-Naphthalimide derivatives have attracted considerable attention from researchers due to their unique properties. These compounds are potent inhibitors of enzymes involved in DNA replication, disrupting the helical structure through intercalation, and serve as key intermediates in the synthesis of fluorophores and dyes for cell staining. They exhibit high quantum yields and excellent photostability. In this study, we present a novel type of hybrids containing the 1,8-naphthalimide intercalating scaffold linked by an alkyl chain of different lengths to the highly bioactive 3,4-dichloro-2(5H)-furanone (mucochloric acid, MCA) molecule. N-(ω-Hydroxyalkyl)-1,8-naphthalimides (NI) substituted on carbon C4 of naphthalimide core were obtained by condensation of corresponding anhydrides with ω-amino alcohols possessing different lengths of alkyl chain. The condensation of the latter with 5-acetoxy-3,4-dichloro-2(5H)-furanone in DMF solution at elevated temperature led to the desired NI/MCA hybrids in good yield. The synthesized hybrids exhibit cytotoxic effect in an extent of 3.3 μM to 50 μM against model cancer cell lines, namely colorectal cancer CT26, breast cancer 4T1, and murine melanoma B16F10. Efficient penetration of the cell's membrane by hybrids was confirmed by the observed high fluorescence of excited cells. The synthesized hybrids initiated apoptosis and necrosis on different levels and reduced the cloning ability of the investigated cancer cells.

[II-5] ANTIBIOFILM ACTIVITY OF LYETX I MNΔK, A PEPTIDE DERIVED FROM THE VENOM OF THE SPIDER LYCOSA ERYTHROGNATHA, ON URINARY CATHETERS AND ITS TOXICITY EVALUATION IN VITRO AND IN VIVO

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Introduction: Globally, approximately 150 million people are affected by urinary tract infections (UTIs) each year, with 75–80% of cases caused by uropathogenic Escherichia coli (UPEC). In this study, we aimed to evaluate the antibiofilm activity of the LyeTx I mnΔK peptide against UPEC biofilms formed on urinary catheters, as well as its toxicity profile.

Methods: To evaluate the antibiofilm effects, LyeTx I mnΔK (1 μg/mL) was added to UPEC-inoculated medium also containing catheter segments and incubated at 37 °C for 24 h. After incubation, the catheters were washed with saline, fixed with 2.5% glutaraldehyde, and analyzed by scanning electron microscopy. The number of bacteria adhered to the catheter surface was determined by calculating the log reduction relative to the control. The peptide was incubated with human embryonic kidney cells (HEK-293) in 96-well plates for 24 h to assess its in vitro cytotoxicity. The in vivo toxicity was evaluated in six-week-old female BALB/c mice using different peptide concentrations (0, 1, 5, and 10 mg/kg). The animals were visually monitored daily for 14 days.

Results: The peptide reduced the number of bacteria adhering to urinary catheters by approximately 1 log. Scanning electron microscopy revealed that catheters incubated in cultures containing the peptide had markedly fewer adherent cells compared with the control samples. The in vitro cytotoxic concentration of the peptide for 50% of the cells (CC_{50}) was 32.72 μM. In vivo toxicity tests showed no deaths and normal weight gain in the treated animals. The peptide did not alter renal mass or cause edema; however, serum creatinine elevation and mild tubular degeneration were observed in animals treated with the highest dose (10 mg/kg).

Conclusion: LyeTx I mnΔK demonstrates promising antibiofilm activity against UPEC on urinary catheters with minimal cytotoxicity in vitro and in vivo, highlighting its potential as an effective prototype against quinolone-resistant UPEC strains

[II-6] BIODEGRADABLE BRANCHED POLYANHYDRIDES BASED ON SEBACIC ACID AND OLIGOSUCCINATES

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Polyanhydrides are a promising class of polymers for controlled drug delivery due to their favorable properties, such as biodegradability, surface erosion and low to no toxicity. They have a high reactivity with water, which results in a rapid hydrolytic degradation. Due to their tendency to degrade by surface erosion, they are an excellent material for controlled release carriers, because the rate of drug release can be controlled by the rate of polymer degradation. Polyanhydrides are approved by the Food and Drug Administration (FDA), for human use, as the degradation products are easily removed from the body by human metabolism. Branched polymers offer significantly different physical properties from linear polymers and can provide several advantages for drug delivery applications. Compared to linear polymers, branched polyanhydrides exhibit improved mechanical stability, controlled degradation kinetics, and increased drug encapsulation capacity, making them more promising for advanced pharmaceutical delivery systems. They can be used both as therapeutic molecule carriers and as active compounds.

The aim of this work was the synthesis and characterization of branched polyanhydrides based on sebacic acid and oligosuccinates. These copolymers can be used as carriers in drug delivery systems, in the form of microspheres.

Branched polymers were obtained by two-step melt polycondensation of sebacic acid and a derivative of succinic acid oligomers used as branching agents. Succinic acid is a non-toxic compound, while sebacic acid is approved by the FDA for use in drug delivery systems. The content of oligomer in the obtained copolymers was from 2 to 20 wt %. To fully characterize the obtained polyanhydrides, nuclear magnetic resonance (NMR) spectroscopy, infrared (IR) spectroscopy, gel chromatography (GPC), and differential scanning calorimetry (DSC) were used.

[II-7] APPLICATION OF BETULIN-BASED POLYANHYDRIDES IN THE PREPARATION OF ETOPOSIDE-LOADED MICROSPHERES

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In today's world, cancer is one of the most common causes of premature death of humans. The mortality caused by this disease increases year by year. After its detection, it is often too late for the treatment. Finding the suitable way of treatment increases the chances of recovery. Current knowledge about the types and methods of cancer treatment is very vide, though it does not guarantee the possibility of saving all the patients. Drugs with anticancer activity, apart from many advantages, also have a numerous of disadvantages, including harmful effects on healthy cells which leads to the weakening of the body. That is why it is so important to search for new, selectively acting drugs and methods of their direct delivery to diseased tissues in order to limit the harmful effects on healthy cells. One of the methods of delivering drugs to the body is to use polymer-drug systems.

The aim of this work was preparation and characterization of polymer-drug systems in the form of polymer microspheres obtained from linear polyanhydrides of betulin derivatives. The active compound which was coupled to the betulin-based carriers was etoposide, an anticancer drug, used to treat small cell lung cancer. The microspheres were obtained by emulsion (O/W) solvent evaporation method.

For obtained polymer-drug systems, parameters such as the actual etoposide content in the microspheres, encapsulation efficiency and drug loading were determined. The encapsulation efficiency and drug loading were dependent on the particle size and the starting amount of the drug.

Betulin and its derivatives have a broad spectrum of biological relevance, thus these compounds are promising as new, potential therapeutic agents. Microspheres prepared from betulin-based polyanhydrides may have significant application in drug delivery systems. These particles combined with chemotherapeutic agents will lead to a synergistic therapeutic effect.

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[II-8] EVALUATION OF CYTOTOXICITY OF COMBINATIONS OF 1-AMINOMETHYLENE-1,1-BISPHOSPHONIC ACID WITH SELECTED CYTOSTATIC AGENTS

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Bisphosphonates (BPs) are synthetic analogs of naturally occurring inorganic pyrophosphates characterized by a P–C–P backbone. These compounds exhibit a broad spectrum of biological activities, including antiresorptive and antitumor properties, and demonstrate a high affinity for bone tissue due to their ability to interact with hydroxyapatite (HAP). The therapeutic potential of BPs in oncology may be enhanced through combination therapies with conventional cytostatics, offering the possibility of synergistic or additive effects. This study aimed to preliminarily assess the cytotoxic interactions between 1-aminomethylene-1,1-bisphosphonic acid and selected chemotherapeutic agents.

Cytotoxicity assays were conducted using the MTS test on two human cell lines: the osteosarcoma line U-2 OS and the non-cancerous skin fibroblast cell line BJ1-hTERT. Cells were treated with the cytostatic agent alone, the BP derivative alone, or both compounds in combination. Cell metabolic activity was assessed after 48 hours of exposure. Additional comparative experiments were performed using zoledronic acid (BP used in clinical practice) instead of 1-aminomethylene-1,1-bisphosphonic acid to evaluate differences in cytotoxic effects between the reference compound and the tested derivative.

The combination of 1-aminomethylene-1,1-bisphosphonic acid with cytostatic agents enhanced the cytotoxicity against U-2 OS osteosarcoma cells. The most pronounced effects were observed in combinations with camptothecin, cisplatin, and etoposide. In contrast, analogous treatments involving zoledronic acid produced notably weaker cytotoxic responses. Importantly, the tested BP derivative did not increase the cytotoxicity of chemotherapeutic agents toward non-cancerous BJ1-hTERT fibroblasts compared to the cytostatics applied alone.

The results demonstrate that 1-aminomethylene-1,1-bisphosphonic acid can potentiate the cytotoxic effects of selected anticancer agents in U-2 OS cells, without increasing toxicity to BJ1-hTERT cells. Future studies should explore the efficacy of such combinations in other bone cancer models and investigate the molecular mechanisms underlying the enhanced cytotoxic effects observed.

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[II-9] SPILANTHOL AS REGULATOR OF COLORECTAL AND BREAST CANCER CELL PROLIFERATION

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Spilanthol is a fatty acid amide found in Acmella oleracea, widely used in cosmetics and traditional medicine due to its anti-inflammatory activity. Recent studies have shown that spilanthol may also act as an inhibitor of JAK1 and JAK2 proteins [DOI.10.1080/14786419.2023.2222220]. JAKs can both stimulate and inhibit cell proliferation depending on the cellular context and cell type [DOI.10.1186/s12964-022-00990-5; DOI.10.1111/jcmm.16289].

The aim of the study was to investigate the effect of spilanthol on the proliferation of HCT116 (colon cancer) and MCF7 (breast cancer) cancer cells. Cell proliferation was evaluated by cell counting and CCK-8 assay. Additionally, based on the KEGG database, we selected molecular targets of the JAK/STAT pathway associated with the cell cycle (p21) and apoptosis (BCL-2). Using RT-qPCR, we checked whether the expression levels of selected genes changed in cells treated with spilanthol.

The obtained results showed that spilanthol has therapeutic potential and acts on cancer cells in a concentration-dependent manner.

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[II-10] BETULIN GLYCOCONJUGATES – SYNTHESIS OF SELECTED MODELS USING THE CUAAC REACTION AND PRELIMINARY ASSESSMENT OF THEIR CYTOTOXICITY

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Numerous literature reports show wide interest in 3-lup-20(29)-ene-3β,28-diol (BN, betulin) among scientists. BN is perceived as a natural bioactive compound with significant synthetic and pharmacological potential with a broad biological activity (anticancer, antibacterial, anti-inflammatory, antiretroviral). An additional advantage seems to be its easy availability, low price and attractive in terms of modifications of the parent structure of betulin provide an opportunity to design new BN derivatives with favorable pharmacokinetic properties [1]. However, poor bioavailability and low intracellular accumulation limit its pharmaceutical application. A promising strategy to enhance BN's therapeutic potential seems to be glycoconjugation because the attachment of a sugar unit to skeleton of bioactive compound can improve the solubility and the possibility of targeting a specific molecular target, especially in case of tumors (leveraging cancer cells' heightened glucose demand and overexpression of glucose transporters) [2]. Furthermore, incorporating an N-heterocyclic linker, such as a 1,2,3-triazole ring, further enhances biological activity.

Studies on the synthesis of new BN derivatives, as well as results of assays of their cytotoxicity against selected cell lines, both cancerous (colon cancer cells, HCT-116 and breast cancer cells, MCF-7), and healthy (human fibroblasts, NHDF), will be presented [3].

Based on the previous results of cytotoxicity and selectivity studies of BN glycoconjugates, we have designed new, slightly modified structures that will be synthesized and studied soon.

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[II-11] ANTIPROLIFERATIVE ACTIVITY OF NOVEL FULLERENE NANOMATERIALS WITH SUPERPARAMAGNETIC IRON OXIDE AGAINST PANCREATIC CANCER

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Pancreatic cancer, particularly pancreatic ductal adenocarcinoma (PDAC), is classified as one of the most aggressive malignancies. It is characterized by high mortality and a 5-year survival rate of only 8%. Standard treatment for advanced pancreatic cancer involves gemcitabine-based chemotherapy, and more recently, combination therapy with erlotinib. Despite these approaches, there remains a critical and ongoing need to identify and implement more effective therapeutic strategies.

One promising direction in the development of nanomedicine is the use of fullerenes as theranostic agents or drug carriers that enable precise delivery of active substances. Thanks to their unique physicochemical properties and the possibility of functionalization, fullerenes open new perspectives in the treatment of diseases, including cancer [1].

Our recent studies demonstrate the high potential of a C60 fullerene–gemcitabine nanoconjugate to generate reactive oxygen species (ROS) upon exposure to blue light, which significantly enhances the effectiveness of pancreatic cancer therapy [2].

The latest research focuses on new hybrid fullerene-based nanomaterials with attached superparamagnetic iron oxide nanoparticles (SPIONs) and chemotherapeutic agents: gemcitabine and erlotinib. These materials were structurally characterized using SEM and TEM techniques, and their antiproliferative activity was evaluated on a panel of pancreatic cancer cell lines: PANC-1, AsPC-1 (both human-derived), and PAN02 (mouse-derived). Cytotoxicity analysis revealed high antiproliferative activity of hybrid materials containing SPION@Cu conjugated with gemcitabine, particularly against PAN02 pancreatic cancer cells. Further cellular experiments focused on preliminary determination of the mechanism of action. To this end, the potential for ROS generation by C60-GEMCITABINE-SPION@Cu was investigated using fluorescence microscopy. Additionally, apoptosis-induced cell death was analyzed using flow cytometry.

The results clearly indicate the high potential of the studied nanomaterials as promising candidates for anticancer therapy of pancreatic cancer. Crucially, the data also support their future application in theranostic strategies, paving the way for integrating both diagnostic and therapeutic functions.

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[II-12] INFLUENCE OF STRUCTURAL MODIFICATIONS OF LUPANE-TYPE TRITERPENOIDS ON THEIR ANTIPROLIFERATIVE ACTIVITY

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According to the latest scientific research, betulin a naturally occurring pentacyclic triterpenoid (lupan type), has a wide range of biological properties, including anticancer, anti-inflammatory, antioxidant and antibacterial effects [1]. It can be predominantly found in the bark of birch trees such as Betula pendula, Betula verrucosa or Betula pubescens, and its content in the dry mass of the bark is usually 20-30% [2,3].

Due to the presence of only two polar groups (C28OH and C3OH) and a large, non-polar skeleton, betulin is poorly soluble in water. This hinders its distribution in the body and reduces its therapeutic effectiveness in vivo [4]. To improve its solubility and bioavailability in a polar environment, the parent skeleton of betulin is subjected to various structural modifications (particularly at the C3 and C28 positions) [2,5]. This treatment aims to improve the physicochemical and pharmacokinetic properties.

The aim of this paper is to present selected examples of betulin conjugates and assess their anticancer activity.

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[II-13] PHARMACOLOGICAL POTENTIAL OF SPILANTHOL

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2E, 6Z, 8E)-N-isobutyl-2,6,8-decatrienamide, commonly known as spilanthol, is a natural compound isolated from the above-ground parts of the plant Acmella oleracea, which belongs to the Asteraceae family [1]. A. oleracea is characterized by a high content of biologically active compounds, especially N-alkylamides. The most active N-alkylamide is spilanthol, which is responsible for several beneficial biological properties, including analgesic, antioxidant, anti-inflammatory, anti-cancer, antioxidative, and bacteriostatic effects [1, 2]. Scientific research also proves that it exhibits antimalarial activity and has antifungal activity (Aspergillus Niger) [1,3]. Moreover, it may serve as an effective bactericidal agent against bacterial strains E. coli and B. subtilis [4]. Due to its documented anti-inflammatory and locally anesthetic properties, spilanthol is used as a component in dental care products for pain reduction (e.g., toothpaste, dental gels, mouth rinses) [5]. A naturally occurring derivative of spilanthol (2E,7Z)-6,9-endoperoxy-N-isobutyl-2,7-decadienamide, activity against the human pathogenic parasites Trypanosoma brucei rhodesiense and Plasmodium falciparum has been confirmed [6].

This review summarizes current research on the biological activity of spilanthol and indicates potential directions of its application [5].

[II-14] IN SEARCH OF MOLECULAR TARGETS FOR BETULIN DERIVATIVES

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Betulin is a natural triterpenoid isolated from birch trees. Betulin exhibits a wide spectrum of biological activities, including anti-inflammatory, antiviral, and anticancer effects. A significant disadvantage of betulin is its low solubility in aqueous solutions. Therefore, research is being conducted to obtain water-soluble betulin derivatives that will exhibit biological activity.

The aim of the study was to examine the effect of phosphonium derivatives of betulin, newly developed by our team, on the viability of cancer cells. The studies were conducted on cancer cell lines (HCT116). Cell viability was determined after 24 hours of incubation with betulin derivatives using the CCK-8 assay. Based on literature studies, molecular targets of the tested betulin derivatives (i.e., p21, BCL-2, and PARP) were selected, and the effect of betulin derivatives on the expression of these genes was examined using RT-qPCR.

Our studies have shown that the phosphonium derivatives of betulin exhibit a strong, concentration-dependent effect on the viability of HCT116 cells. The observed change in cell viability is associated with a change in the expression of genes regulating the cell cycle and apoptosis.

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[II-15] EFFECT OF GLYCOCONJUGATION ON CYTOTOXICITY AND SELECTIVITY OF 8-AMINOQUINOLINE DERIVATIVES COMPARED TO 8-HYDROXYQUINOLINE

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Cancer is one of the greatest challenges in modern medicine, representing the second most common cause of death worldwide, with incidence continuing to rise. Low selectivity and increasing chemotherapeutic drug resistance necessitate the search for alternative therapeutic strategies. Particularly promising are compounds containing an N-heterocyclic ring, such as the quinoline scaffold, which is present in many biologically active molecules.

Our previous work focused on 8-hydroxyquinoline (8-HQ) glycoconjugates. It was observed that the addition of sugar moiety improved solubility and bioavailability but did not enhance selectivity toward cancer cells compared to the parent compounds [1].

Our next goal was to improve glycoconjugates selectivity by simply replacing the oxygen atom with nitrogen, substituting the 8-HQ moiety with 8-aminoquinoline (8-AQ). The 8-AQ derivatives were functionalized via the amino group and connected with acetylated sugar derivatives (D-glucose or D-galactose) substituted with different groups at the anomeric position, using copper(I)-catalyzed 1,3-dipolar azide-alkyne cycloaddition (CuAAC). The nitrogen atoms in these conjugates allows the formation of complexes with metal ions, such as Cu^{2+} and Zn^{2+}, which play important roles in cancer development.

The obtained glycoconjugates were tested for inhibition of the proliferation of cancer cell lines (HCT 116 and MCF-7) and healthy cell line (NHDF-Neo). Two of the synthesized glycoconjugates demonstrated higher cytotoxicity than their oxygen-containing counterparts and showed improved selectivity for cancer cells, thus enhancing their anticancer potential [2].

In further studies, glycoconjugates with unprotected sugar moieties are planned to be synthesized, and the 8-AQ library will be expanded with derivatives containing a modified linker between the sugar and quinoline, to investigate affinity for GLUT transporters and confirm increased selectivity toward cancer cells.

Now we present the synthesis and the cytotoxicity assays of 8-aminoquinoline glycoconjugates, as well as new structures of 8-AQ glycoconjugates that we plan to obtain and test.

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[II-16] IMMUNOMODULATORY AND SELECTIVE CYTOPROTECTIVE EFFECTS OF NOVEL 1,2,3-TRIAZOLO-QUINOBENZOTHIAZINE HYBRIDS IN THE CONTEXT OF CANCER RESEARCH

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Introduction: Phenothiazine derivatives and their structural analogs have been extensively studied for their anticancer properties. In this work, a new series of 1,2,3-triazolo-quinobenzothiazines (MJ1-MJ20) was evaluated. These compounds differ in substituents on the quinobenzothiazine ring (X = H, F, Cl, SCH_3) and on the triazole ring (phenyl, p-fluorophenyl, p-chlorophenyl, p-cyanophenyl, p-(phenylthio)phenyl). The study focused on assessing their cytotoxic selectivity and analyzing the expression of genes related to apoptosis (BCL2), p53 regulation (MDM2), ferroptosis (AIFM2), and inflammation (IL6, IL8).

Materials and methods: Cytotoxic activity was evaluated using the Alamar Blue assay in normal (BEAS-2B, NHDF) and cancer cell lines (HCT116, A549, MCF7, SH-SY5Y, U2OS). The expression of BCL2, MDM2, AIFM2, IL6, and IL8 genes was determined by RT-qPCR for the BEAS-2B and HCT116 cell lines.

Results: The Alamar Blue assay confirmed the limited cytotoxicity of the tested compounds. High IC_{50} values for the BEAS-2B line and undetectable values for NHDF indicate their safety toward normal cells, while the inability to determine IC_{50} values for all cancer cell lines may result from the presence of resistance mechanisms. Gene expression analysis revealed a distinct activity profile for compound MJ19 (X = SCH_3, triazolo-p-cyanophenyl). In the HCT116 cancer cell line, the expression of BCL2, MDM2, AIFM2, and IL6 genes was low or moderately increased compared to the control, while IL8 expression was decreased, which may indicate a limited protective effect and suppression of angiogenesis-promoting processes in cancer cells. In contrast, the normal BEAS-2B cell line showed a significant increase in BCL2, MDM2, AIFM2, IL6, and IL8 expression, suggesting the activation of anti-apoptotic, anti-ferroptotic, and immunomodulatory mechanisms.

Conclusions: The new 1,2,3-triazolo-quinobenzothiazine derivatives exhibit selective biological activity and the ability to modulate the expression of genes related to apoptosis, ferroptosis, and inflammatory processes in a cell type-dependent manner. Compound MJ19 acts selectively, supporting the survival and inflammatory response of normal cells while simultaneously reducing proangiogenic properties in cancer cells, indicating its potential as a candidate for further studies toward supportive therapies.

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[II-17] NOVEL BRANCHED COPOLYMERS BASED ON SEBACIC ACID – SYNTHESIS AND CHARACTERIZATION

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Polyanhydrides are a class of surface-degradable polymers. They are obtained by polycondensation of compounds containing two or more carboxylic groups. Polyanhydrides have hydrophobic backbones with hydrolytically unstable anhydrides, which may hydrolyze in aqueous medium to di- or polycarboxylic acids and are completely eliminated from the body within a short period of time. Due to their properties, such as lack of toxicity and appropriate release kinetics of active substances, they are mainly used in medicine, both as drug carriers and as biomaterials. One of the compounds that can be used for the synthesis of branched polyanhydrides is sebacic acid.

The aim of this work was the synthesis of a new branched polyanhydrides based on sebacic acid and polycarboxylic derivatives of polyesters based on succinic acid, which can be used in controlled drug delivery systems as carriers of biologically active compounds. Sebacic acid (SA) is approved by the US Food and Drug Administration (FDA) for use in drug delivery systems, while succinic acid is non-toxic and occurs naturally in living tissues. Polymers obtained on its basis can be considered potentially biocompatible and useful in medical applications.

Polyanhydrides were obtained by two-step melt polycondensation of sebacic acid and polyester derivative with the use of acetic anhydride. The content of polyester in the obtained copolymers was from 2 to 10 wt %. Sebacic acid was used to increase the crystallinity of the obtained polyanhydrides, while a derivative of polyester was used as a branching agent. The obtained polyanhydrides were characterized by NMR spectroscopy, FT-IR, DSC, and GPC. Under physiological conditions, copolymers undergo hydrolytic degradation to nontoxic polyester and sebacic acid, approved by the FDA for use in drug delivery systems.

[II-18] SYNTHESIS AND CHARACTERIZATION OF STAR-SHAPED POLYANHYDRIDES FOR POTENTIAL BIOMEDICAL APPLICATIONS

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Branched polymers are a class of polymers between linear polymers and polymer networks. They exhibit significantly different properties, such as high surface functionality, globular conformation, low intrinsic viscosities, and high solubilities, compared to linear polymers. However, compared to linear polymers, the physicochemical properties of which are largely determined by the monomer repeat unit, the properties of branched polymers result from their end groups at the surface of the polymer and the topology of the polymer. Branched polymers can be used both as therapeutic molecule carriers and as active compounds, i.e., as anti-inflammatory agents.

The objective of this study was to synthesize and characterize star-shaped polyanhydrides through two-step melt polycondensation of sebacic acid and poly(ethylene glycol) derivative, which can be used to obtain polymer microspheres or micelles for use in drug delivery systems. The content of PEG derivative in the obtained copolymers was from 5 to 80 wt %.

Linear poly(ethylene glycol) is often used in the synthesis of polymers for drug delivery due to its high aqueous solubility and the ability to influence the biodistribution in tumors through the enhanced permeability and retention (EPR) effect of parent drugs. However, the linear PEG has only one or two functional groups, which can be used for reactions, thus limiting its use in the synthesis of branched and crosslinked polymers. Therefore, multiple functional PEG are preferable to us in the synthesis of branched polymers.

The obtained branched copolymers were characterized by 1H NMR spectroscopy, infrared spectroscopy (IR), and differential scanning calorimetry (DSC). Under physiological conditions, polyanhydrides undergo hydrolytic degradation to sebacic acid, approved by the FDA, and poly(ethylene glycol). derivative. Due to their biodegradability and non-toxicity, copolymers based on SA and PEG can be used as carriers of biologically active substances.

[II-19] SYNERGISTIC INFLUENCE OF BIOLOGICALLY ACTIVE SECRETION OF LUCILIA SERICATA (DIPTERA: CALLIPHORIDAE) MAGGOTS WITH GENTAMICIN

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Introduction. Antibiotic resistance is a major challenge in modern medicine. Bacteria can adapt to antibiotics, rendering previously effective therapies ineffective. The same problem is also observed with gentamicin, a drug belonging to the aminoglycoside group of antibiotics that is primarily used to treat infections caused by Gram-negative bacteria. Prolonged use or high doses of this drug may have detrimental effects on the patient, and bacterial strains resistant to this drug have emerged over time. Therefore, alternative therapies are being sought. One such alternative therapy is wound debridement therapy, where maggots of Lucilia sericata are utilized in the treatment of chronic wounds. As they feed on necrotic tissue, their secretions control the growth of wound-contaminating bacteria and promote tissue regeneration. The secretion possesses antibacterial properties and can also inhibit biofilm formation by Gram-negative bacteria, such as Pseudomonas aeruginosa, as well as other commonly found bacteria on wound surfaces. The project aimed to assess whether fractions of larval secretions that exhibit antibacterial activity and the ability to inhibit pigment synthesis would act synergistically with gentamicin. This strategy facilitates a reduction in the effective concentration of gentamicin necessary to inhibit microbial growth, thereby minimizing the risk of adverse effects associated with its administration.

Method. Larvae of L. sericata in the developmental stage between the second and third instar were collected, washed, and incubated with P. aeruginosa ATCC 27853 lysate in the dark for 3 hours at 32°C. After that, the liquid secretion was collected and separated into less than 3 kDa molecular weight. The secretion was further separated by size exclusion chromatography. Eight fractions were obtained and tested for activity against P. aeruginosa using radial diffusion assay in the absence and presence of subinhibitory concentrations of gentamicin (MICs).

Results. Compared to the untreated control, four fractions of larval secretions showed the most biological activity against the reference strain of P. aeruginosa. The influence of the sample varied from inhibiting pigment production to the formation of zones of inhibition against the lawn growth of P. aeruginosa during the radial diffusion assay. Next, the selected active fractions were tested for their effect on bacteria when used in combination with gentamicin at concentrations below the MIC, to assess whether the secreted compounds could act synergistically with the antibiotic. This study showed that the three fractions exhibit synergistic bactericidal activity in combination with gentamicin against P. aeruginosa.

Conclusion. The results suggest that combining gentamicin with L. sericata larval secretions may offer a promising alternative antibacterial therapy.

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[II-20] GREEN ANTI-INFLAMMATORY CHEMISTRY

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Chronic inflammation plays a key role in the pathogenesis of numerous diseases, including cancer, autoimmune, and degenerative disorders. Conventional anti-inflammatory drugs, despite their proven efficacy, often cause adverse effects, which prompts the search for safer and more physiologically compatible alternatives. The aim of the present project is to evaluate the potential of selected natural plant extracts to modulate inflammatory responses in various in vitro models. Four cell lines will be used: human keratinocytes (HaCaT), colon carcinoma cells (HCT116), bronchial epithelial cells (Beas-2B), and murine macrophages (RAW 264.7). The inflammatory response will be induced using lipopolysaccharide (LPS) or cytokine stimulation, and the effect of extracts will be assessed by measuring the expression and secretion of pro-inflammatory mediators (e.g., TNF-α, IL-6, IL-1β, COX-2) as well as oxidative stress markers. The project aims to determine whether natural extracts can attenuate inflammation by influencing key signaling pathways such as NF-κB and MAPK. The results will help answer the question of whether inflammation can be effectively modulated and "silenced" using natural compounds, potentially contributing to the development of novel, safe anti-inflammatory strategies.

Keywords: inflammation, natural extracts, HaCaT, HCT116, Beas-2B, RAW 264.7, NF-κB, cytokines

[II-21] AUTOMATION OF THE SYNTHESIS OF LAYERED DOUBLE HYDROXIDES (LDH) AND EVALUATION OF THE CYTOTOXICITY OF LDH, LDH WITH METRONIDAZOLE AND LDH WITH GEMCITABINE

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Many drugs, including anticancer agents, exhibit unfavourable properties such as low solubility, poor stability, short half-life, nonspecific distribution in the body, and the development of drug resistance, all of which limit therapeutic effectiveness. Research has demonstrated that employing nanocarriers such as layered double hydroxides (LDHs) in drug delivery significantly improves pharmacokinetics, cellular uptake, biodistribution, and therapeutic efficiency, while simultaneously lowering the required drug dose and minimizing adverse effects. Furthermore, LDHs offer extensive possibilities for chemical composition tuning, exhibit pH-responsive solubility, high biocompatibility, and a remarkable ability to intercalate various anionic species, making them versatile platforms for targeted drug delivery systems. The synthesis of LDH is carried out through a co-precipitation process from an aqueous solution containing metal cations that form the layered structure, together with molecules intended for encapsulation between these layers. The reaction takes place in an alkaline medium under mild temperature conditions, ensuring controlled growth and proper incorporation of guest species into the LDH framework. An algorithm was developed, and a control program was created to automate LDH synthesis under constant pH conditions. The system integrates a pH-meter, peristaltic pump, and laboratory power supply, operating through automatic regulation of pump voltage. Data acquisition from the pH-meter was carried out in Python and transferred to LabView for processing. The control algorithm, implemented in LabView, uses a PID regulator combined with a nonlinear function to dynamically adjust the pump flow and maintain stable pH. The program includes a graphical user interface (GUI) for real-time monitoring and visualization of pH regulation. LDH was synthesized and metronidazole or gemcitabine was introduced into its structure by ion exchange. The carrier structures were confirmed using XRD, UV-VIS, and TGA. The biological activity of the prodrugs and drugs, as well as the structure of the synthesized compounds, will be verified using NMR and MS analyses. The effect of cytotoxicity was studied on the HTC116 cell line. AlamarBlue HS was used to assess cell viability after 24, 48, and 72 hours.

The aim of the project was to automate the synthesis process and study the effect of LDH with metronidazole/gemcitabine on cancer cells. During testing, problems with carrier aggregation occurred, which prevented the assessment of the cytotoxicity of the tested compounds. Further studies will be conducted on U2OS cells using a clonogenic assay with cells in suspension.

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[II-22] APPLICATION OF SEBACIC ACID COPOLYMERS IN THE PREPARATION OF MICROSPHERES AND MICELLES

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Polymers have attracted growing attention in pharmaceutical and biomedical applications. Compared to non-biodegradable polymers, which can cause toxicity due to accumulation in the body, biodegradable polymers exhibit superior properties. They are promising materials for the preparation of novel drug delivery systems, due to their biodegradability, biocompatibility, and minimal cytotoxicity. Biodegradable polymers used for biomedical applications need to undergo biodegradation to biocompatible or harmless products, and their degradation rate and mechanical properties should match their potential application. Some polymers, which fit some of the criteria and thus can be used in the medical industry, include, e.g., polyesters, polyurethanes, polysaccharides, and polyanhydrides. Among those, polyanhydrides are the most suitable for use in controlled drug delivery systems. Polyanhydrides are an important group of biodegradable and biocompatible polymers that can be used in controlled drug release systems. They are FDA-approved biodegradable polymers with beneficial properties. Polyanhydrides undergo gradual hydrolytic degradation from the surface, which has a beneficial effect on the release profile of active substances dispersed in them, so they can be successfully used as both microspheres and drug nanocarriers.

The aim of this work was the preparation and characterization of polymeric microspheres and micelles, based on branched polyanhydrides of sebacic acid and PEG derivative.

Polyanhydrides were obtained by two-step melt polycondensation of sebacic acid with poly(ethylene glycol) derivative. The use of sebacic acid as a comonomer increases the crystallinity of polymers, which affects the characteristics of microspheres. Microspheres were prepared using the emulsion (O/W) solvent evaporation technique, where poly(vinyl alcohol) was used as an emulsion stabilizing agent. The micelles from branched polyanhydrides were prepared by adding water via a syringe into a solution of polymer in tetrahydrofuran. THF, which is miscible with water, was used as the solvent for branched betulin-based polyanhydrides. Obtained microspheres and micelles were characterized using spectroscopic ^{1}H NMR, UV/Vis spectroscopy, optical microscope, and scanning electron microscope (SEM).

[II-23] INVESTIGATION OF IRON AND MANGANESE COMPLEXES WITH RELEVANCE TO PLANT MICRONUTRIENT SUPPLEMENTATION

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Iron and manganese are among the key micronutrients indispensable for proper metabolic functioning. Iron participates in electron transport, chlorophyll biosynthesis, and the operation of redox enzymes [1], whereas manganese is crucial for oxidoreductase activity and photosystem II, enabling oxygen evolution [2]. Deficiency of these elements leads to physiological disorders, chlorosis, and reduced photosynthetic efficiency. The challenge of iron and manganese bioavailability is particularly pronounced in alkaline soils, where uptake is markedly constrained [3]. Accordingly, there is a need to develop low-energy, sustainable synthesis pathways that enable the environmentally benign production of the complexes in question, integrating their functionality in plant nutrition with the principles of green chemistry within a coherent technological framework.

An environmentally friendly, solvent-free approach was developed to obtain iron and manganese complexes under process conditions configured to lower energy demand. Syntheses conducted under controlled parameters favored the formation of stable compounds with high chemical purity. The resulting materials underwent preliminary physicochemical characterization using analytical techniques to assess stability, homogeneity, and the extent of metal-ion coordination.

The iron and manganese complexes were produced reproducibly and exhibited high chemical purity and stability under the examined conditions. Physicochemical analyses confirmed their potential suitability as micronutrient sources with controlled release and enhanced resistance to environmental factors. The physicochemical profile suggests potential suitability for micronutrient delivery, and the process configuration reduced energy demand during synthesis and characterization compared with conventional routes.

The presented findings support the rationale for applying modern, sustainable routes to obtain micronutrient complexes. The approach enables the preparation of stable, chemically pure forms of iron and manganese with application potential in plant biotechnology and advanced micronutrient supplementation systems. The novelty lies in combining an ecological mode of synthesis with the functional use of the resulting complexes in sustainable plant nutrition, in line with current trends in green chemistry and precision agriculture.

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[II-24] POSSIBILITIES OF MANAGING BY-PRODUCTS OF THE WINE INDUSTRY

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Global grape production in 2021 reached 74,8 million tons, nearly half of which was used for winemaking [1]. As a result, this process generates significant amounts of by-products – grape pomace, consisting mainly of seeds, skins, and stems. Due to its rich chemical composition, grape pomace is considered a valuable secondary raw material that can be utilized in various industrial sectors, aligning with the principles of the circular economy. Among the main possibilities for grape pomace valorization are its use in the energy sector for biogas and bioethanol production through fermentation processes, as well as biomass for thermochemical conversion. In the food industry, it serves as a source of tartaric acid, dietary fiber, pectins, and natural pigments [2].

Particular attention is given to phenolic compounds, which are abundant in grape pomace. Due to their antioxidant, anti-inflammatory, and antibacterial properties, they find applications in the cosmetic and pharmaceutical industries, as well as in agriculture, as components of plant biostimulant formulations enhancing growth and stress tolerance [3,4]. These compounds are extracted using both conventional and non-conventional techniques, such as microwave- and ultrasound-assisted extraction [2].

This work presents the processes of phenolic compound extraction from grape pomace, methods of their analysis, and potential applications in the context of sustainable development.

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[II-25] STUDY OF THE PROPERTIES OF PROTEIN-COATED ITRACONAZOLE AS AN ANTIFUNGAL DRUG

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Introduction: The role of biomacromolecules in drug delivery systems is to improve the drug's poor bioavailability and therapeutic efficacy. The protein-encapsulated antifungal drug, such as itraconazole, is the candidate for extensive therapeutic research. Itraconazole, 2-(Butan-2-yl)-4-{4-[4-(4-{[(2R,4S)-2-(2,4-dichlorphenyl)-2-(1H--1,2,4-triazol-1-ylmethyl)-1,3-dioxolan-4-yl]methoxy}phenyl)piperazin-1-yl]phenyl}-2,4-dihydro-3H-1,2,4-triazol-3-on, is a synthetic triazole derivative, is a topical antifungal agent for use in the local treatment of infections. The presence of charged amino acid residues in proteins leads to the drug being enclosed in a protein shell, which further assists in the physical. This study aims to prepare and study the physicochemical properties of encapsulated itraconazole using DL-Dithiothreitol as the protein shell relaxant agent and the surfactant (SDS) to densify the network structure.

Methods: The solution contains the protein (Human transferrin or Human serum albumin), DL-Dithiothreitol, and the anionic surfactant SDS in 10 mM NaCl and is mixed with a solution of itraconazole. After 24 hours, the mixtures were lyophilized. The physicochemical properties were investigated using spectroscopic methods (e.g., CD, DLS, UV-Vis, and FT-IR) and thermal analysis (DSC).

Results: Spectroscopic methods monitored the encapsulation process, tracking conformational changes in the protein due to the disruption of disulfide bonds and interactions with anionic detergents, which influenced the drug's reaction progress. The protein shells were prepared at different protein concentrations (0.2-0.5mg/mL) and showed similar trends in the drug release studies. The release of itraconazole from the albumin and transferrin shells was conducted in PBS solution (pH 7.4). After 2 hours, the average drug release was 100%. The stability of the encapsulated drug is related to the higher zeta potential (~-35mV) than pure proteins (~-17mV). The shell protein-drug system forms a stable colloid with negligible tendency to agglomerate. The DSC peaks of BSA were slightly shifted, indicating an interaction between the drug and albumin and a change due to the inclusion of the drug molecules in the vehicles.

Conclusions: This study is the first report of the novel encapsulated itraconazole form, prepared as a protein–drug formulation. The physically entrapped drug within proteins was further stabilized by electrostatic interactions between the protein and the drug, favorably influencing the drug's release from the protein matrix.

[II-26] GROWTH AND NUTRIENT REMOVAL PERFORMANCE OF GREATER DUCKWEED SPIRODELA POLYRHIZA UNDER VARIED LIGHT SPECTRA

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Introduction: Plants from the Lemnaceae family, commonly known as duckweed, find applications in biotechnology, as food for humans and animal feed, and most importantly, in wastewater treatment processes. Duckweeds are characterized by rapid growth, high efficiency of nutrient removal, and adaptability to diverse environmental conditions. However, their cultivation and the efficiency of phytoremediation processes are strongly dependent on environmental factors, particularly light conditions, the effects of which remain poorly understood.

Methods: The aim of this study was to determine the effects of different light spectra on the growth of the duckweed species Spirodela polyrhiza under controlled laboratory conditions. Plants grown on Steinberg medium were incubated for 21 days under blue, green, red, and white light at an intensity of 80 μmol m^{-2} s^{-1} provided by LED lamps. Images were taken at regular intervals under standardized conditions to quantify frond number and surface area, from which relative growth rates were calculated. At the end of the experiment, the dry weight of plants was determined, and samples of the culture medium were collected for the analysis of nitrate and phosphate concentrations and for removal efficiency calculations.

Results: Plants exposed to red light covered the surface of culture vessels most rapidly, followed by those grown under green, white, and blue light. The highest dry biomass was observed under red light (54 mg), nearly twice that obtained under blue (28 mg) and white (29 mg) light, while plants grown under green light showed slightly lower values (47 mg). Similarly, the highest mean relative growth rates (calculated as averages over a two-week growth period) were recorded under red light (0.200 day^{-1} for surface area and 0.202 day^{-1} for frond number), followed by green (0.195 and 0.196), white (0.164 and 0.162), and blue light (0.151 and 0.161). In terms of nutrient removal efficiency, the highest nitrate removal was recorded for plants grown under red (66%) and green (51%) light, while blue and white light treatments showed considerably lower efficiencies (around 20%). Phosphate removal efficiency exceeded 95% under red and green light, whereas other treatments resulted in significantly lower removal rates (30-35%).

Conclusions: Red light proved to be the most effective for S. polyrhiza growth, indicating that this spectrum promotes the fastest biomass accumulation. Moreover, red and green light enhanced nutrient removal efficiency, suggesting their potential application in optimizing duckweed-based wastewater treatment systems.

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[II-27] MECHANISTIC INSIGHTS INTO TERPIDRINE-INDUCED OXIDATIVE STRESS AND THERAPEUTIC RESPONSE IN GLIOBLASTOMA

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Glioblastoma (GBM), the most aggressive and prevalent malignant brain tumor in adults, remains one of the most difficult cancers to treat due to its inherent resistance to conventional therapies [1]. Despite multimodal treatment approaches including surgical resection, radiation therapy, and temozolomide chemotherapy, GBM patients face dismal outcomes with median survival rarely exceeding 15 months and approximately 90% of patients experiencing disease recurrence within two years [2]. This therapeutic resistance stems from multiple mechanisms, including tumor heterogeneity, altered cellular signaling pathways, and the immunosuppressive tumor microenvironment. Consequently, there is an urgent need for novel therapeutic strategies targeting GBM vulnerabilities through unexplored molecular mechanisms.

Terpyridine compounds are emerging anticancer agents capable of forming stable metal complexes that exert multi-targeted effects against tumors, including glioblastoma cells. [3,4]. The anticancer activity of terpyridine derivatives involves multiple pathways, including the reactive oxygen species generation leading to oxidative stress, destabilization of the antioxidant system through inhibition of thioredoxin reductase, as well as cell cycle arrest in the G0/G1 phase, and activation of cell death pathways via apoptosis and autophagy [3,4].

In our recent studies, 14 new terpyridine-based compounds were tested on both 2D and 3D glioblastoma models to examine their effects on stress-dependent signaling pathways. We utilized the proteome profiler array to profile protein-level changes associated with oxidative and cellular stress responses. This approach allowed us to systematically characterize the mechanisms of action of these compounds in glioblastoma cells under conditions that better mimic the tumor microenvironment.

The capacity of terpyridine derivatives to simultaneously engage multiple anticancer pathways while avoiding cross-resistance phenomena renders this class of compounds highly promising for the development of next-generation therapeutics targeting brain tumors.

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Section III

„Molecular and Cellular Biology„

[III-1] THE DUAL ROLE OF OF LINC00116 OR LINC00116-ENCODED MITOREGULIN PEPTIDE IN B-CELL LYMPHOMA

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Long non-coding RNAs (lncRNAs) are defined as RNAs longer than 200 nucleotides that are not translated into proteins. However, a number of lncRNAs have actually been shown to encode small peptides with significant functions in cells. One such lncRNA is LINC00116, which encodes a 56-amino-acid mitochondrial peptide Mitoregulin (MTLN). MTLN was shown to be involved in lipid homeostasis, energy metabolism and oxidative stress. The LINC00116 transcript has been reported to show higher levels in B-cell lymphomas compared to B lymphocytes. However, the role of both LINC00116 and MTLN in B-cell lymphoma is unknown.

The aim of this project is to investigate whether LINC00116 and MTLN have distinct cellular functions in B-cell lymphoma cells. We have shown that MTLN protein is overexpressed in Hodgkin lymphoma (HL) cells compared to normal B lymphocytes. We also demonstrated that LINC00116 is a direct target gene of the miR-17 family using a luciferase assay. Subsequently, we prepared three overexpression constructs to selectively overexpress LINC00116 or MTLN peptide in L428 HL cells using lentiviral vectors. We proved LINC00116 overexpression by qRT-PCR and MTLN overexpression by Western Blot. The lentiviral vectors contained the gene encoding GFP, which allowed cytometric analysis of transduced cells within 21 days. The percentage of L428 cells with overexpressed MTLN decreased compared to cocultured wild-type cells. This decrease was not observed when we overexpressed LINC00116 without MTLN-coding potential or in control EV-transduced L428 cells.

In conclusion, we observed decreased growth of HL L428 cells with selectively overexpressed MTLN, and not with overexpressed LINC00116. Further studies to demonstrate possible differences in LINC00116 and MTLN functions in B-cell lymphoma are ongoing.

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[III-2] DYNAMIC CHANGES IN MIRNA BIOGENESIS IN BURKITT LYMPHOMA CELLS FOLLOWING IONIZING RADIATION EXPOSURE

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Ionizing radiation (IR) is a key component of cancer therapy, inducing cell death through DNA damage and activation of cellular stress responses. The therapeutic efficiency of IR largely depends on the radiosensitivity of cancer cells, which is influenced not only by protein-coding genes but also by non-coding RNAs such as microRNAs (miRNAs). MiRNAs are short, single-stranded RNAs that inhibit gene expression at the post-transcriptional level. Their biogenesis is a multistep process beginning with the transcription of primary miRNAs (pri-miRNAs), which are processed into precursor miRNAs (pre-miRNAs) in the nucleus and are subsequently exported to the cytoplasm, where they are cleaved into mature miRNAs. A variable ratio between primary and mature miRNA levels may indicate regulation of miRNA biogenesis.

In this study, we investigated the effects of ionizing radiation on miRNA biogenesis in three Burkitt lymphoma (BL) cell lines: CA46, DG75, and ST486. Cells were irradiated with a 4 Gy dose, which induced apoptosis and G2/M cell cycle arrest in all three lines. To investigate changes in miRNA processing, we performed RNA sequencing and small RNA sequencing at 4 and 12 hours after IR, comparing miRNA and pri-miRNA levels. Pri-miRNA levels were identified as the number of reads covering pre-miRNAs with 150 nucleotides of flanking sequences. We showed more than a 1.5-fold change of 13 to 33 miRNAs levels depending on the cell line. The levels of 4 miRNAs, i.e. miR-146a, miR-449c, miR-449a, and miR-155 were at least 1.5-fold increased after IR in at least in two BL cell lines. Additionally, we identified pri-miRNAs upregulated or downregulated upon IR with no corresponding changes in mature miRNA levels. To further characterize the dynamics of miRNA processing, we analyzed pri-miRNA and mature miRNA levels for miR-146a, miR-155, and miR-449a by qRT-PCR at 1, 4, 8, 12, and 24 hours post-irradiation. Pri-miR-146a and pri-miR-155 were significantly upregulated within 1–4 hours, preceding the delayed increase of their mature miRNAs, indicating transcriptional induction by IR. In contrast, pri-miR-449a was undetectable, while mature miR-449a levels peaked at 8 hours, suggesting rapid and efficient processing.

In conclusion, our results demonstrate that IR modulates miRNA biogenesis in Burkitt lymphoma cells, particularly affecting of miR-146a, miR-155, and miR-449a.

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[III-3] COMPARATIVE REDOX PROFILES AND FERROPTOTIC SUSCEPTIBILITY OF BEAS-2B, A549 AND H1299 CELL LINES

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Ferroptosis is an iron-dependent, regulated form of regulated cell death driven by excessive lipid peroxidation and disturbances in the functioning of antioxidant systems. Cancer cells, which function under conditions of chronic oxidative stress, display adaptive responses, thereby developing a strong dependence on antioxidant systems to maintain redox homeostasis and ensure survival. Particularly important for cancer cells are two antioxidant systems, the glutathione (GSH) and thioredoxin (TRX) shields, among which a phenomenon of reciprocal compensation occurs. Disruption of these mechanisms is considered a potential therapeutic target in oncology.

The aim of this study was to characterize the differences in response to oxidative stress and susceptibility to ferroptosis in three human lung cell lines: non-cancerous BEAS-2B and cancer lines A549 and H1299, differing in TP53 gene status. To induce ferroptosis, an inhibitor of the cystine/glutamate antiporter (Xc−), erastin, was used, at doses of 5 μM and 10 μM, both alone and in combination with UVA irradiation (1 kJ/cm²). Mitochondrial activity was assessed using the Alamar Blue assay, while the levels of reactive oxygen species (ROS) and total glutathione (GSH) were measured using fluorescent probes dichlorofluorescein (DCF) and monochlorobimane (MBC). The relative expression of key antioxidant genes (GPX4, NRF2, PRDX1, SOD2, TRX) was measured by RT-qPCR. Statistical analyses were performed using the Kruskal–Wallis test with Benjamini–Hochberg correction and Student's t-test.

The obtained results showed differences in adaptive capacity between the cell lines studied. BEAS-2B cells maintained a stable redox profile with low ROS and GSH levels, displaying only moderate changes in gene transcription and effective antioxidant homeostasis even under combined stress conditions. The A549 cell line exhibited selective increases in the expression of genes associated with adaptation to stress, particularly GPX4 as well as PRDX1 and SOD2, for higher doses of erastin and UVA exposure. In turn, the H1299 cell line, which lacks functional p53 protein, was characterized by a decrease in total GSH levels and marked suppression of NRF2, PRDX1, SOD2, and TRX in nearly all tested conditions.

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[III-4] SINGLE-STRAND DNA BREAKS AT ACTIVE PROMOTERS FACILITATE +1 NUCLEOSOME EVICTION

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The stability of nucleosomes harboring various posttranslational histone tail modifications (PTMs) was compared in an *in situ* assay involving agarose-embedded nuclei. The promoter proximal H3K4me3, H3K27ac, and H4K8ac positive nucleosomes exhibited relative sensitivity to intercalators as compared to bulk H3-GFP or nucleosomes carrying any of the following marks: H3K27me1, H3K27me2, H3K27me3, H3K9me1, H3K9me2, H3K9me3, H3K36me3, H3K4me0, H3K4me1, H3K4me2, H3K9ac, and H3K14ac. The posttranslational modifications of the histones were labeled using a panel of monoclonal antibodies (Kimura et al. Cell Struct Funct., 2008). Nickase or DNase I treatment of the nuclei, or bleomycin treatment of live cells, did not affect the stability of nucleosomes carrying H3K4me3 or H3K27ac, while those of the second group were destabilized upon treatment with intercalators. These observations support the possibility that the promoter proximal marks specify dynamic nucleosomes accommodating relaxed DNA sequences due to ss DNA breaks, nicks, generated in vivo. In line with this interpretation, endogenous, 3'OH nicks were mapped within the nucleosome-free region of promoters controlling genes active in human mononuclear cells, co-localized with RNA polymerase, a conclusion supported by superresolution studies. Interestingly, the stability of the H3K4me3-nucleosomes proved to be antibody--dependent. Using a polyclonal H3K4me3-specific antibody (Abcam), the nucleosomes detected exhibited a much higher stability. When the binding sites of the monoclonal and polyclonal anti-H3K4me3 antibodies were mapped along the genome by CUT, peak coverages of both antibodies showed similar genomic distribution in the sense that 85-95% of peaks were detected in the 3 kb range of promoters. In the case of peaks flanking the transcription start sites (TSS), the monoclonal antibody detected a subpopulation of all those detected by the polyclonal. On the other hand, the H3.3 coverage immediately downstream of the TSS (i.e., the number of promoters harboring H3.3 there) is higher among the promoters detected exclusively by the polyclonal anti-H3K4me3 antibody, relative to those detected by both antibodies. Thus, H3K4me3 nucleosomes showed a marked heterogeneity based on their different stability, juxtaposition with endogenous DNA discontinuities, and arrangement of H3.3-containing nucleosomes around the TSS.

[III-5] IMPACT OF DGCR8 SILENCING ON MICRORNA BIOGENESIS AND HODGKIN LYMPHOMA CELL SURVIVAL

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MicroRNAs (miRNAs) are small, non-coding RNAs that negatively regulate gene expression at the post-transcriptional level. Their biogenesis begins with the transcription of primary miRNAs (pri-miRNAs), which are processed by the Drosha-DGCR8 complex into precursor miRNAs (pre-miRNAs). These are then exported to the cytoplasm and further processed by Dicer into mature miRNAs.

In this study, we investigated how DGCR8 knockdown affects miRNA biogenesis and lymphoma cell survival. We silenced DGCR8 in Hodgkin lymphoma cell lines, L1236 and L540, using lentiviral vectors carrying short hairpin RNAs – shDGCR8-1 and shDGCR8-2. Western blot analysis confirmed a significant reduction in DGCR8 protein levels upon knockdown with both shRNAs. To assess changes in pri-miRNA levels, we performed RNA sequencing on L1236 cells transduced with shDGCR8-1 or a scrambled control (SCR). Our findings revealed that only 15 out of 767 pri-miRNAs showed increased expression upon DGCR8 inhibition. Quantitative RT-PCR validated the reduction of mature miR-155, miR-146a, and miR-17 levels, while their corresponding pri-miRNAs i.e., pri-miR-155, pri-miR-146a, and pri-miR-17~92 accumulated. Functionally, DGCR8 knockdown significantly reduced cell viability and induced apoptosis, with a threefold decrease in the number of viable L1236 cells after 10 days. Additionally, DGCR8-depleted cells exhibited increased sensitivity to ionizing radiation, with up to a fourfold increase in G1/G0 cell cycle arrest post 8 Gy irradiation.

In conclusion, our results indicate that DGCR8 inhibition disrupts miRNA maturation in a miRNA-dependent manner and promotes apoptosis in Hodgkin lymphoma cells. Further research is ongoing to expand the analysis of pri-miRNA and mature miRNA dynamics following DGCR8 silencing.

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[III-6] IMPACT OF IONIZING RADIATION ON MIRNA EXPRESSION IN MELANOMA CELLS

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Ionizing radiation (IR) is a cornerstone of cancer therapy that elicits complex cellular stress responses. MicroRNAs (miRNAs) are critical post-transcriptional regulators of gene expression that can modulate the radiosensitivity of cancer cells. This study investigates the impact of IR on miRNA expression in Me45 melanoma and Burkitt lymphoma (BL) cell lines.

Cells were exposed to a 4 Gy dose of IR, which induced G2/M cell cycle arrest and increased the apoptosis rate. Small RNA sequencing, performed 4 and 12 hours post-IR, revealed in Me45 melanoma cells. A consistent upregulation of miR-1260 and downregulation of miR-933. We also identified miR-146a as the most abundant miRNA in Me45 cells.

To assess its functional role, miR-146a was suppressed using a lentiviral vector with the miR-Zip-146a sequence or a negative control. Lentiviral vectors also expressed GFP, enabling tracking of transduced cells by flow cytometry in a GFP competition assay. The stable inhibition of miR-146a resulted in a progressive decrease in transduced Me45 cells, which was not observed with a negative control vector. Silencing of miR-146a resulted in a reduced number of GFP-positive Me45 cells compared with co-cultured wild-type cells, indicating that miR-146a exerts a pro-survival effect in Me45 cells. This effect was not observed in Me45 cells transduced with the negative control construct.

In conclusion, our findings demonstrate that IR affected miRNA expression, notably miR-1260, miR-933 in melanoma cells. Furthermore, we identified a pro-survival role for the highly abundant miR-146a in melanoma cells. Ongoing experiments are focused on the involvement of miR-146a in the radiosensitivity of melanoma cells.

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[III-7] MRNA EXPRESSION OF DUSP1–7 IN KERATINOCYTES EXPOSED TO LPS AND ADALIMUMAB

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Introduction/Rationale: Psoriasis is a chronic inflammatory dermatosis involving abnormal immune activation and dysregulated mitogen-activated protein kinase (MAPK) signaling. Dual-specificity phosphatases (DUSPs) serve as critical negative regulators of MAPK activity by dephosphorylating key residues on ERK, JNK, and p38 kinases. Altered DUSP expression has been linked to disturbed cytokine signaling and treatment resistance in psoriasis. The present study aimed to evaluate transcriptional changes of DUSP1–7 in human keratinocytes (HaCaT) exposed to bacterial lipopolysaccharide (LPS) and subsequently treated with the anti-TNF-α drug adalimumab.

Methods: HaCaT keratinocytes were cultured under standard conditions and stimulated with 1 μg/mL LPS to induce inflammation. Afterward, cells were treated with 8 μg/mL adalimumab for 2, 8, and 24 hours. Total RNA was isolated using the Trizol method and purified with RNeasy Mini Kit. Gene expression profiling of DUSP1–7 was performed using Affymetrix HG-U133_A2 microarrays, and results were validated by RT-qPCR employing TaqMan assays. Statistical significance was determined using ANOVA followed by Tukey's post-hoc test ($p < 0.05$).

Results: Exposure to adalimumab significantly modified the transcriptional activity of several DUSP genes. The most pronounced induction was noted after 2 hours of treatment, particularly for DUSP2 (fold change +11.12) and DUSP5 (fold change +5.53). DUSP1 and DUSP4 were also upregulated, while DUSP3 and DUSP6 showed moderate increases. Prolonged exposure (8–24 h) resulted in attenuation of these effects, suggesting an adaptive transcriptional response. In contrast, LPS stimulation alone reduced DUSP1–7 expression, confirming the inflammatory suppression of phosphatase activity. ELISA analysis supported transcriptional findings, revealing higher DUSP protein levels in adalimumab-treated cells than in LPS-stimulated controls ($p < 0.05$).

Conclusions/Novel aspect: Adalimumab rapidly enhances the expression of DUSP1–7, especially DUSP2 and DUSP5, in inflamed keratinocytes, indicating its modulatory role in rebalancing MAPK pathway activity during anti-TNF-α therapy. These phosphatases may serve as early molecular markers of therapeutic response, reflecting the restoration of feedback control within MAPK-dependent signaling. The study provides new evidence that DUSP reactivation represents a crucial component of the anti-inflammatory mechanism of adalimumab in psoriasis-related cellular models.

[III-8] ASSOCIATION BETWEEN THE −509C/T PROMOTER POLYMORPHISM OF THE TGFB1 GENE AND SEVERITY OF CHRONIC PERIODONTITIS

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Introduction/Rationale: Transforming growth factor beta-1 (TGF-β1) is a multifunctional cytokine that regulates inflammation, tissue remodeling, and immune homeostasis. Genetic variations within the TGFB1 promoter may alter transcriptional activity, thereby influencing susceptibility to chronic inflammatory diseases, including periodontitis. Among known polymorphisms, the −509C/T substitution is of particular interest, as it introduces a YY1 binding site that can modulate transcriptional repression. This study aimed to assess whether the −509C/T polymorphism in the TGFB1 promoter is associated with the severity of chronic periodontitis.

Methods: Unstimulated whole saliva was collected from 120 individuals in the morning, at least two hours after food intake or oral hygiene procedures. The study group included 60 patients diagnosed with moderate chronic periodontitis, defined by a clinical attachment loss (CAL) of 3–4 mm and probing depth (PD) between 5 and 7 mm, with a low caries index (PUW < 5). Sixty systemically and periodontally healthy subjects served as controls. Samples were centrifuged, and the supernatant was stored at −70°C until analysis. Genomic DNA was extracted from oral mucosal cells, amplified by PCR, and digested with the Eco81I restriction enzyme to determine the −509C/T genotype. Allelic variants were separated by polyacrylamide gel electrophoresis.

Results: A significant difference in allele distribution was observed between the groups. The T allele was more frequent among individuals with severe periodontitis (57.7%) compared with the control (37.8%) and moderate periodontitis (35.4%) groups ($p = 0.0387$). Similarly, the homozygous T/T genotype occurred in 38.5% of patients with severe periodontitis but only in 8% of healthy subjects ($p = 0.0258$). These findings suggest that the −509T allele and T/T genotype may contribute to a greater predisposition to tissue destruction and heightened inflammatory response in periodontal disease.

Conclusions/Novel aspect: The present study demonstrates a potential association between the TGFB1 −509C/T promoter polymorphism and the severity of chronic periodontitis. The T allele may enhance susceptibility to advanced periodontal inflammation, possibly through altered TGF-β1 transcriptional regulation and impaired control of immune-mediated tissue remodeling. These results support the hypothesis that epigenetically relevant promoter variants in TGFB1 can modulate host response in periodontal pathology, highlighting their potential role as genetic biomarkers of disease progression.

[III-9] REGULATION OF TGF-B SIGNALING GENES AND MICRORNAS BY TACROLIMUS IN INFLAMED RETINAL PIGMENT EPITHELIAL CELLS

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Introduction/Rationale: The retinal pigment epithelium (RPE) plays a central role in retinal homeostasis, and dysregulation of transforming growth factor-beta (TGF-β) signaling contributes to retinal fibrosis and proliferative vitreoretinopathy (PVR). Tacrolimus (FK506), a calcineurin inhibitor with potent immunosuppressive properties, has been suggested to exert antifibrotic effects; however, its molecular influence on TGF-β–related signaling in RPE cells remains unclear.

Methods: Human retinal pigment epithelial (H-RPE) cells were stimulated with lipopolysaccharide (LPS) to induce inflammation and subsequently treated with tacrolimus. Transcriptomic profiling of TGF-β–associated mRNAs and microRNAs was performed using microarray analysis, and selected targets were validated by quantitative RT-PCR. Protein levels were quantified by ELISA. Functional interactions among modulated genes were analyzed using STRING network analysis.

Results: Tacrolimus significantly altered the expression profile of multiple TGF-β–pathway genes. It upregulated TGF-β2, TGF-β3, SMAD2, and SMAD4, while downregulating TGF-βR1 and the inhibitory SMAD7. These transcriptional changes were accompanied by corresponding shifts in protein expression. In addition, tacrolimus modulated non-canonical signaling components within the JAK/STAT and MAPK pathways, suggesting an extended regulatory role. miRNA profiling revealed several differentially expressed regulators, including hsa-miR--200a-3p, hsa-miR-589-3p, hsa-miR-21, and hsa-miR-27a-5p, which likely mediate post-transcriptional control of these genes. STRING analysis confirmed a dense functional network linking canonical and non-canonical TGF-β components.

Conclusions/Novel aspect: Tacrolimus orchestrates a dual modulatory effect on TGF-β signaling in LPS--stimulated RPE cells, characterized by activation of canonical SMAD2/4 signaling and suppression of the inhibitory SMAD7 branch. Concurrent modulation of JAK/STAT and MAPK cascades indicates broader anti-inflammatory and antifibrotic potential. The coordinated regulation of TGF-β2 and SMAD7, together with altered expression of miRNAs such as miR-200a-3p, underscores tacrolimus as a promising therapeutic candidate for limiting fibrosis in PVR. These findings provide new molecular insights into the repurposing potential of tacrolimus for retinal fibrotic diseases.

[III-10] MRNA EXPRESSION CHANGES OF MAPK-PATHWAY GENES AND CORRESPONDING PROTEIN IMPLICATIONS IN HACAT KERATINOCYTES EXPOSED TO LPS AND TREATED WITH ADALIMUMAB

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Introduction/Rationale: The mitogen-activated protein kinase (MAPK) pathway is central to keratinocyte responses under inflammatory stress, and its dysregulation contributes to the development of psoriasis. Therapeutic blockade of tumor necrosis factor-alpha (TNF-α) can influence downstream MAPK signaling; however, the molecular mechanisms underlying this effect remain incompletely understood.

Methods: Human HaCaT keratinocytes were stimulated with lipopolysaccharide (LPS) to induce an inflammatory response and subsequently treated with adalimumab. Transcriptomic profiling of MAPK-related genes was performed using microarray analysis, with validation by quantitative RT-PCR (RT-qPCR). Protein expression for selected targets was quantified using enzyme-linked immunosorbent assay (ELISA) at 2, 8, and 24 hours post-treatment.

Results: Microarray analysis identified significant LPS-induced alterations in seven MAPK pathway–associated mRNAs: DUSP1, DUSP3, DUSP4, MAPK9, MAP3K2, MAP2K2, and MAPKAPK2. DUSP1 and MAPKAPK2 were strongly upregulated, while DUSP3 and DUSP4 were markedly downregulated following LPS stimulation. RT-qPCR confirmed that transcriptional changes appeared as early as 2 hours, peaked at 8 hours, and persisted at 24 hours. Treatment with adalimumab effectively reversed these inflammatory transcriptional responses, reducing DUSP1 and MAPKAPK2 expression and restoring DUSP3 and DUSP4 levels toward control values. At the protein level, ELISA demonstrated that MAPKAPK2 abundance increased following LPS exposure, paralleling mRNA upregulation, but significantly declined after adalimumab treatment. Conversely, DUSP4 protein expression, reduced under inflammatory conditions, partially recovered following TNF-α inhibition.

Conclusions/Novel aspect: Adalimumab exerts a dual transcriptional and translational regulatory effect on MAPK signaling components in inflamed keratinocytes. The restoration of DUSP3 and DUSP4 expression and suppression of DUSP1 and MAPKAPK2 highlight its role in normalizing MAPK pathway dynamics. These findings suggest that DUSP1, DUSP4, and MAPKAPK2 may serve as responsive molecular markers of anti-TNF therapy efficacy in psoriasis and related inflammatory skin disorders.

[III-11] SUBTYPE-SPECIFIC DYSREGULATION OF BIOGENIC AMINE GENES IN BREAST CANCER

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Introduction/Rationale: Biogenic amines (BAs) such as histamine and dopamine play regulatory roles in cell proliferation, angiogenesis, and immune modulation. Aberrations in BA signaling may contribute to breast cancer progression and subtype-specific aggressiveness. However, the molecular patterns of BA-related gene and protein expression across breast cancer subtypes remain poorly defined.

Methods: Transcriptomic profiling of tumor and control breast tissue was performed to identify differentially expressed BA-related genes. Expression changes were validated using quantitative RT-PCR (RT-qPCR), and protein levels were quantified using enzyme-linked immunosorbent assay (ELISA). Statistical analyses were used to evaluate subtype-specific differences in expression across luminal A, luminal B, HER2-enriched, and triple-negative breast cancer (TNBC) groups.

Results: Significant alterations were observed in 24 mRNAs corresponding to 15 BA-associated genes. Within the histamine signaling pathway, HNMT, HRH1, HRH2, HRH3, and HRH4 were consistently upregulated, with HRH2 and HRH4 showing the highest expression in TNBC. In the dopaminergic axis, DRD2 mRNA was markedly upregulated, while DRD5 was strongly downregulated, especially in non-luminal HER2+ tumors. Additional overexpressed genes included ICAM1, MAPK9, OXT, and SIRT4, whereas EGR1 exhibited subtype-dependent divergence—elevated in most cancers but repressed in TNBC. Protein analyses confirmed that transcriptional changes translated into consistent alterations at the protein level. Increased HRH1–HRH4 and DRD2 mRNA expression correlated with higher protein abundance, particularly in aggressive subtypes. Conversely, reduced DRD5 and EGR1 expression at both transcript and protein levels characterized TNBC and HER2+ tumors. ICAM1 and MAPK9 proteins also showed strong elevation consistent with transcriptional activation.

Conclusions/Novel aspect: This study demonstrates coherent, subtype-specific dysregulation of BA-related mRNAs and proteins in breast cancer. The concordant overexpression of HRH2, HRH4, and DRD2 in TNBC and HER2+ subtypes, alongside suppression of DRD5 and EGR1, suggests that histamine and dopamine signaling pathways contribute to the molecular heterogeneity and aggressiveness of these tumors. These genes and their protein products represent potential biomarkers and therapeutic targets for precision modulation of BA signaling in breast cancer.

[III-12] SALINOMYCIN DOWNREGULATES VEGF-A, VEGF-B, VEGF-C, AND VEGF-D EXPRESSION IN ENDOMETRIAL CANCER CELLS

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Introduction/Rationale: Angiogenesis and lymphangiogenesis are essential for tumor growth and metastasis, largely mediated by vascular endothelial growth factors (VEGF-A, VEGF-B, VEGF-C, and VEGF-D). Overexpression of VEGFs promotes vascular remodeling, invasion, and metastatic spread in endometrial cancer. Salinomycin, a polyether antibiotic with reported anti-cancer properties, has been shown to interfere with several signaling pathways, but its influence on VEGF-mediated angiogenesis remains poorly understood.

Methods: Human Ishikawa endometrial cancer cells were treated with 1 μM salinomycin for 12, 24, and 48 hours. Untreated cultures served as controls. The expression levels of VEGF-A, VEGF-B, VEGF-C, and VEGF-D mRNAs were quantified using microarray analysis and RT-qPCR, while corresponding protein concentrations were determined by ELISA. Statistical significance was set at $p < 0.05$.

Results: Salinomycin induced a consistent and time-dependent reduction in VEGF expression at both the mRNA and protein levels. VEGF-A mRNA showed a significant decrease compared with control cultures ($p = 0.0004$). VEGF-B expression was markedly reduced after 24 h and 48 h of treatment ($p = 0.00000$ for both). VEGF-C and VEGF-D exhibited a progressive decline across all incubation periods, with highly significant differences relative to the control ($p = 0.00000$). The observed transcriptional suppression was paralleled by corresponding decreases in protein levels detected by ELISA.

Conclusions/Novel aspect: Salinomycin markedly suppresses the expression of VEGF-A, VEGF-B, VEGF-C, and VEGF-D in endometrial cancer cells, indicating potent anti-angiogenic and anti-lymphangiogenic effects. These findings reveal a previously unrecognized mechanism of salinomycin action involving inhibition of the VEGF signaling axis, supporting its potential use as an adjuvant therapeutic agent in endometrial cancer.

[III-13] SALINOMYCIN-INDUCED MODULATION OF STEMNESS-ASSOCIATED MRNAS IN ENDOMETRIAL CANCER CELLS

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Introduction/Rationale: Salinomycin, a polyether ionophore antibiotic, has emerged as a promising anticancer compound with the ability to target cancer stem cells. Despite evidence of its cytotoxic and anti-metastatic activity, the molecular mechanisms underlying its effects on transcriptional regulation in endometrial cancer remain poorly characterized. In particular, the influence of salinomycin on the expression of genes associated with stemness, epithelial–mesenchymal transition (EMT), and extracellular matrix remodeling warrants investigation.

Methods: Human Ishikawa endometrial adenocarcinoma cells were cultured under standard conditions and treated with 1 μM salinomycin for 12, 24, and 48 hours. Untreated cells served as controls. Total RNA was extracted, and global mRNA expression profiling was performed using the Affymetrix HG-U133A_2 microarray platform. Differentially expressed transcripts were identified based on $|\text{fold change}| \geq 2.0$ and $p < 0.05$. Selected mRNAs were validated by quantitative real-time PCR (RT-qPCR) to confirm the direction and magnitude of expression changes.

Results: Microarray analysis revealed a time-dependent modulation of mRNA expression following salinomycin treatment. A total of 121 transcripts were significantly altered compared to control cultures, with the most prominent changes detected after 12 hours of exposure. Among these, TGFβ2 and WNT5A were markedly upregulated (fold change ≈ +2.5–3.7), while COL14A1 and CDH2 were strongly downregulated (fold change ≈ –4.0 to –6.8). The transcriptional response diminished at later time points, suggesting an early and transient effect of salinomycin on mRNA regulation. RT-qPCR validation confirmed the microarray findings, showing consistent expression trends for all four key genes.

Conclusions/Novel aspect: Salinomycin induces significant reprogramming of mRNA expression in endometrial cancer cells, characterized by activation of TGFβ2 and WNT5A and suppression of COL14A1 and CDH2. These changes suggest interference with EMT-related and extracellular matrix pathways, indicating that salinomycin may attenuate cellular plasticity and stem-like properties through transcriptional mechanisms. This study provides novel evidence of salinomycin's regulatory impact at the mRNA level, supporting its potential as a molecular modulator in endometrial cancer therapy.

[III-14] SUBTYPE-INDEPENDENT ACTIVATION OF NOTCH PATHWAY GENES IN BREAST CANCER

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Introduction/Rationale: Notch signaling orchestrates cell fate and plasticity, and its abnormal activation is implicated in breast cancer aggressiveness. Whether a common Notch-related transcriptional program exists across intrinsic subtypes remains unclear, as does the contribution of microRNAs (miRNAs) to this regulation.

Methods: Tumor and matched adjacent tissue from 405 women were profiled across five subtypes (luminal A; luminal B HER2–; luminal B HER2+; non-luminal HER2+; TNBC). Notch-pathway mRNAs (KEGG hsa04330) were measured on Affymetrix HG-U133A 2.0 arrays and validated by RT-qPCR. Protein abundance for selected targets was quantified by ELISA. Differential miRNAs were identified on miRNA microarrays and interrogated with miRDB (score ≥80) to predict target relationships. Statistics: one-way ANOVA with Tukey's post hoc; $|FC|>2$, $p<0.05$. Overall survival (OS) to 60 months was analyzed by Kaplan–Meier within subtypes.

Results: Seventy-five transcripts were significantly altered versus control, with TNBC showing the largest subtype-specific burden. A subtype-independent core signature emerged: upregulation of APH1A, CTBP1, DVL3, HEY1, HEY2, JAG2, NOTCH4 and downregulation of DTX1, TLE2, TLE4. RT-qPCR confirmed direction and magnitude; ELISA aligned for upregulated genes, while DTX1/TLE2/TLE4 proteins were below detection. Candidate miRNA–mRNA pairs included DVL3↔low miR-1275, HEY1↔low miR-145, JAG2↔low miR-98/miR-381, and TLE4↔high miR-196a/miR-155. Worse OS associated with high DVL3 (luminal A), high NOTCH4 (luminal B HER2–), and high DVL3/JAG2 (non-luminal HER2+).

Conclusions/Novel aspect: Across all intrinsic subtypes, breast cancers share a conserved Notch activation footprint featuring receptor/ligand/effector upregulation and loss of repressors. Integration with miRNA data nominates actionable regulatory pairs that may sustain pathway activity. This pan-subtype signature refines patient stratification and highlights miRNA-guided strategies to modulate Notch signaling.

[III-15] CORE NOTCH MIRNA SIGNATURE SHARED AMONG BREAST CANCER SUBTYPES

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Introduction/Rationale: MicroRNAs (miRNAs) are key post-transcriptional regulators of oncogenic signaling, including the Notch pathway, which governs cell differentiation and stemness. Aberrant Notch activity contributes to tumor aggressiveness, yet the miRNA-driven mechanisms maintaining this activation across breast cancer subtypes remain unclear.

Methods: Tumor and matched normal tissues from 405 women representing five breast cancer subtypes (luminal A, HER2–luminal B, HER2+ luminal B, non-luminal HER2+, and TNBC) were profiled using Affymetrix GeneChip mRNA and miRNA microarrays. Differentially expressed miRNAs ($p < 0.05$, $|FC| > 2$) were analyzed with the miRDB database (target score ≥ 80) to predict interactions with ten dysregulated Notch pathway genes (APH1A, CTBP1, DTX1, DVL3, HEY1, HEY2, JAG2, NOTCH4, TLE2, and TLE4). Candidate miRNA–mRNA pairs were verified through integrated expression correlation and survival analysis.

Results: Distinct miRNA signatures were identified across all subtypes, but six miRNAs emerged as potential master regulators of the Notch network. Downregulation of miR-1275, miR-145, miR-98, and miR-381 was associated with upregulation of DVL3, HEY1, and JAG2, indicating derepression of key signaling activators. Conversely, increased miR-196a and miR-155 expression coincided with TLE4 suppression, suggesting loss of transcriptional repression within the pathway. No miRNAs meeting selection criteria targeted APH1A, CTBP1, DTX1, HEY2, NOTCH4, or TLE2. Subtype analysis confirmed that these miRNA–mRNA relationships were consistent across all molecular subtypes, indicating a unified regulatory pattern rather than subtype-specific modulation.

Conclusions/Novel aspect: This study identifies a core miRNA-mediated regulatory axis that sustains Notch pathway activation independently of breast cancer subtype. The reciprocal relationships between miR-1275–DVL3, miR-145–HEY1, miR-98/-381–JAG2, and miR-196a/-155–TLE4 highlight a post-transcriptional mechanism linking Notch hyperactivation to tumor aggressiveness. These findings expand current understanding of Notch regulation and nominate miRNA-based therapeutic candidates for restoring signaling balance in diverse breast cancer contexts.

[III-16] MICRORNA-MEDIATED REGULATION OF MAPK-SMAD-DOPAMINE CROSSTALK IN BREAST CANCER

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Introduction/Rationale: MicroRNAs (miRNAs) are critical post-transcriptional regulators modulating oncogenic signaling pathways, including the MAPK, SMAD, and dopamine systems. Although each pathway individually contributes to tumor progression, their integrated regulation by miRNAs in breast cancer remains unexplored. This study aimed to identify subtype-specific miRNA–mRNA interactions within the MAPK-SMAD--dopamine signaling network and to elucidate their role in tumor biology.

Methods: Breast tumor and matched control tissues from 405 patients representing five molecular subtypes (Luminal A, Luminal B HER2−, Luminal B HER2+, non-luminal HER2+, and TNBC) were analyzed using Affymetrix GeneChip miRNA 2.0 Arrays. Differentially expressed miRNAs ($p < 0.05$, $|FC| \geq 2$) were predicted to target MAPK-, SMAD-, and dopamine-associated transcripts via TargetScan and miRanda databases (target score ≥ 90). Candidate miRNA–mRNA pairs were correlated with validated qRT-PCR gene expression profiles for KIT, KRAS, FGF2, FGF7, and IGF1.

Results: Distinct miRNA dysregulation patterns were identified across breast cancer subtypes. miR-221 and miR-222, targeting KIT, were significantly upregulated (up to 4.0-fold in TNBC), indicating suppression of receptor tyrosine kinase signaling. miR-16-5p, predicted to regulate FGF2 and FGF7, showed uniform overexpression (4.18–4.56-fold), suggesting inhibition of fibroblast growth factor–mediated signaling. In contrast, miR-300, targeting KRAS, was markedly downregulated (−2.3 to −4.0-fold), consistent with increased KRAS expression and MAPK activation, particularly in HER2+ and TNBC tumors. miR-5011-5p, regulating KIT and IGF1, exhibited strong suppression (−2.9 to −4.9-fold), reinforcing post-transcriptional downregulation of growth factor signaling components. Collectively, these alterations delineate an miRNA-driven shift favoring KRAS activation and reduced KIT/FGF/IGF expression, thereby amplifying oncogenic MAPK signaling.

Conclusions/Novel aspect: This study identifies a coordinated miRNA signature—miR-221/222, miR-16-5p, miR-300, and miR-5011-5p—that governs MAPK–SMAD–dopamine network activity in breast cancer. The reciprocal regulation of KRAS and KIT/FGF/IGF genes by these miRNAs reveals a mechanistic link between MAPK activation and suppression of dopamine-related tumor-suppressive pathways. These findings provide the first integrated evidence of miRNA-mediated crosstalk among MAPK, SMAD, and dopamine signaling cascades and suggest potential therapeutic targets for subtype-specific intervention in breast cancer.

[III-17] ABERRANT LEPTIN AND LEPTIN RECEPTOR EXPRESSION IN ENDOMETRIOID ENDOMETRIAL CANCER: MRNA AND PROTEIN INSIGHTS

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Introduction/Rationale: Obesity is a well-recognized risk factor for endometrial cancer, in part mediated by adipokines such as leptin. Elevated leptin levels may promote tumor growth, angiogenesis, inflammation, and cross-talk with signaling cascades (e.g. JAK/STAT, MAPK). Yet the expression patterns of leptin (LEP) and its receptor genes (LEPR, LEPROT, LEPROTL1) in endometrial cancer (EC) tissues and in blood, and their protein-level concordance, remain incompletely characterized. Elucidating these patterns may help identify biomarkers and mechanistic links to obesity-driven tumorigenesis.

Methods: The study included tissue specimens and whole blood from 30 patients with endometrioid endometrial cancer and 30 control women. Gene expression profiles of leptin-associated transcripts were assessed by Affymetrix HG-U133A_2 microarrays. Selected transcripts (LEP, LEPR, LEPROT, LEPROTL1) were validated by RT-qPCR in both tissue and blood. Serum leptin protein concentrations were measured using ELISA. Differential expression was evaluated using ANOVA with Tukey post hoc, considering fold-change thresholds (e.g.,>3 or <−3). Correlations between leptin levels and body mass index (BMI) were also assessed.

Results: Microarray analysis revealed significant overexpression of 16 leptin-related transcripts in cancer tissue versus control among 38 candidate mRNAs. LEPR emerged as significantly upregulated in all cancer grades (G1, G2, G3) compared to controls, both in tissue and blood. LEP was characteristic of higher-grade cancers (G2 and G3). LEPROT was the only gene uniformly distinguishing EC irrespective of grade. RT-qPCR confirmed overexpression of LEP, LEPR, LEPROT, and LEPROTL1 in cancer tissues and blood, with fold-changes exceeding ±3. At the protein level, serum leptin concentration progressively increased with cancer grade, showing more than a sixfold difference between control and G1, and higher levels in G3. Strong positive correlations were observed between leptin protein levels and patients' BMI across all groups.

Conclusions/Novel aspect: This study demonstrates that both leptin and its receptors are transcriptionally upregulated in endometrioid endometrial cancer tissue and systemically in blood, with corresponding elevation at the protein level in serum. The consistent overexpression of LEPR across all tumor grades suggests it may be an especially stable biomarker. The strong correlation with BMI underscores the link between adiposity and leptin signaling in EC. These findings highlight leptin signaling as a potential mechanistic bridge between obesity and endometrial tumor biology, and support further exploration of the leptin/LEPR axis as a diagnostic or therapeutic target in obesity-associated endometrial cancer.

[III-18] ADALIMUMAB ATTENUATES NF-KB-DRIVEN TRANSCRIPTIONAL ACTIVATION IN LPS-STIMULATED KERATINOCYTES

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Introduction/Rationale: NF-κB signaling plays a central role in psoriatic inflammation by integrating cytokine-driven and microbial cues into transcriptional activation of proinflammatory and anti-apoptotic genes. While adalimumab effectively suppresses TNF-α–mediated inflammation in clinical settings, its direct impact on NF-κB-associated transcriptional programs in keratinocytes remains insufficiently defined.

Methods: Human immortalized keratinocytes (HaCaT) were exposed to lipopolysaccharide (LPS, 1 μg/mL) to induce inflammatory activation and subsequently treated with adalimumab (8 μg/mL) for 2, 8, or 24 hours. mRNA expression profiling was conducted using Affymetrix oligonucleotide microarrays covering 105 NF-κB-associated transcripts (KEGG hsa04064). Differentially expressed genes (DEGs) were defined by $|\text{fold change}| \geq 2.0$ and adjusted $p < 0.05$, followed by RT-qPCR validation of key targets including TNFAIP3, BCL2L1, CXCL2, MAP3K7, BIRC2, BIRC3, and XIAP.

Results: LPS stimulation triggered strong upregulation of multiple NF-κB–related transcripts, including IKBKB (+3.14-fold), IRAK1 (+3.41), TRAF2 (+2.19), CXCL2 (+2.19), MAP3K7 (+3.98), and TNFAIP3 (+3.09). Pro-survival genes (BCL2L1, BIRC2, BIRC3, XIAP) were also elevated, confirming activation of inflammatory and anti-apoptotic signaling. Adalimumab treatment reversed these changes in a time-dependent manner, producing consistent downregulation across 2–24 h. The most prominent suppression occurred in TNFAIP3 (−3.81 to −2.19), MAP3K7 (−2.91 to −3.11), and BIRC2/BIRC3 (−4.54/−4.76 to −3.01/−3.22). Heatmap visualization demonstrated a global shift from LPS-induced activation toward transcriptional normalization. RT-qPCR validation confirmed these trends, showing significant reductions in NF-κB target genes across all time points ($p < 0.05$).

Conclusions/Novel Aspect: Adalimumab effectively counteracts LPS-induced transcriptional activation of NF-κB–associated genes in keratinocytes, simultaneously suppressing inflammatory mediators and pro-survival factors. These findings reveal a direct transcriptional mechanism through which TNF-α blockade restores keratinocyte homeostasis, providing molecular evidence of adalimumab's anti-inflammatory action at the mRNA level.

[III-19] MRNA–MIRNA CROSSTALK IN ASTROCYTIC TUMORS: FOCUS ON THE TGF-B PATHWAY

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Introduction/Rationale: Astrocytic tumors are among the most aggressive brain malignancies, characterized by high molecular heterogeneity and poor prognosis. The transforming growth factor-beta (TGF-β) pathway is a major regulator of glioma progression; however, the isoform-specific contribution of TGF-β1–3 and their post-transcriptional regulation by microRNAs (miRNAs) remains insufficiently understood. This study aimed to evaluate the differential mRNA expression of TGF-β isoforms and the miRNAs predicted to regulate them across astrocytic tumor grades, with emphasis on identifying molecular markers associated with malignancy and patient survival.

Methods: Sixty-five astrocytic tumor samples (WHO grades II–IV) were analyzed for mRNA expression of TGF-β1, TGF-β2, and TGF-β3 using microarray profiling and RT-qPCR validation. Differentially expressed miRNAs were detected using the Affymetrix GeneChip miRNA 2.0 Array, and potential target interactions were predicted in silico using TargetScan and miRanda databases. Survival associations between mRNA and miRNA expression levels were assessed by Kaplan–Meier and Cox regression analyses.

Results: Microarray and RT-qPCR analyses demonstrated significant upregulation of TGF-β1 (1.54-fold, $p < 0.05$) and TGF-β3 (3.45-fold, $p < 0.05$) in high-grade (G4) astrocytomas compared with low-grade (G2) tumors, whereas TGF-β2 expression showed no significant differences across grades. The expression pattern indicated progressive activation of TGF-β1 and TGF-β3 mRNA with increasing tumor malignancy. MiRNA profiling revealed several candidates with predicted regulatory interactions with TGF-β transcripts. hsa-miR-3196, predicted to target TGF-β1, was significantly downregulated in G3–G4 tumors and was associated with a trend toward improved survival (Exp(B) = 0.8897, $p = 0.076$). Conversely, hsa-miR-2278, predicted to target TGF-β3, was upregulated in higher-grade tumors and strongly correlated with poorer survival (Exp(B) = 1.437, $p = 0.008$). Other miRNAs-hsa-miR-141-3p, hsa-miR-200a-3p, and hsa-miR-466—showed no significant impact on survival.

Conclusions/Novel Aspect: This study reveals that TGF-β3 mRNA overexpression, together with miR-2278 upregulation, characterizes high-grade astrocytic tumors and is strongly associated with adverse prognosis. In contrast, downregulation of miR-3196—a potential TGF-β1 regulator—suggests a loss of inhibitory control over TGF-β signaling in malignant progression. These findings define a novel mRNA–miRNA regulatory axis involving TGF-β1/3, miR-3196, and miR-2278, providing new molecular insights into glioma pathogenesis and potential targets for miRNA-based therapeutic intervention.

[III-20] PROTEIN EXPRESSION OF WNT PATHWAY REGULATORS AND THEIR PROGNOSTIC SIGNIFICANCE IN BREAST CANCER SUBTYPES

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Introduction/Rationale: Dysregulation of the Wnt signaling pathway plays a critical role in breast cancer progression by affecting cell proliferation, migration, and differentiation. While mRNA-level studies have characterized transcriptional changes in Wnt-related genes, the corresponding protein expression patterns and their prognostic implications across molecular subtypes remain insufficiently explored. This study aimed to determine the protein expression profile of key Wnt-associated molecules and to evaluate their association with overall survival in distinct breast cancer subtypes.

Methods: Tumor and adjacent healthy tissues were collected from 405 women with breast cancer (T1N0M0) classified as luminal A, HER2-positive luminal B, HER2-negative luminal B, non-luminal HER2-positive, and triple-negative breast cancer (TNBC). Protein concentrations of nine Wnt-related genes—APC, CCND1, DVL3, FZD4, GSK3B, LEF1, TCF7L1, TCF7L2, and WNT5A—were measured by ELISA. Statistical comparisons between subtypes and controls were performed ($p < 0.05$). Prognostic analysis of overall survival (60-month threshold) was conducted using Kaplan–Meier plots.

Results: ELISA revealed a distinct protein expression signature in breast cancer compared with control tissues. APC, CCND1, DVL3, GSK3B, LEF1, and WNT5A were significantly overexpressed across all subtypes ($p < 0.05$), whereas FZD4, TCF7L1, and TCF7L2 were consistently downregulated, with the latter two reaching levels below detection limits in most non-luminal and triple-negative tumors. The highest increases were observed for DVL3 (up to 31.1 ng/mL) and WNT5A (up to 6.15 ng/mL) in TNBC, suggesting activation of non-canonical Wnt signaling. Kaplan–Meier analysis demonstrated subtype-specific survival correlations. In luminal A tumors, reduced survival was associated with low CCND1, LEF1, TCF7L1, and WNT5A, and with high GSK3B levels. In HER2-positive luminal B cancers, decreased APC and elevated TCF7L1 were linked to unfavorable prognosis, while in HER2-negative luminal B cancers, diminished GSK3B and TCF7L2 expression predicted poor outcomes. In non-luminal HER2-positive tumors, APC overexpression and reduced CCND1 and WNT5A activity correlated with lower survival rates. In TNBC, elevated APC expression was a negative prognostic factor, and low FZD4 activity showed borderline significance for reduced survival.

Conclusions/Novel Aspect: This study provides the first integrative assessment of Wnt-related protein expression and survival associations across five molecular subtypes of breast cancer. The findings demonstrate that overexpression of APC, GSK3B, and WNT5A, together with the loss of FZD4, TCF7L1, and TCF7L2, delineates an unfavorable molecular phenotype associated with reduced survival. The APC/GSK3B axis and non-canonical WNT5A signaling emerge as key determinants of outcome variability, underscoring their potential as subtype--specific biomarkers and therapeutic targets in early-stage breast cancer.

[III-21] DOWNREGULATION OF MIR-130A ACROSS MOLECULAR SUBTYPES OF BREAST CANCER: A COMPARATIVE ANALYSIS OF 405 TUMOR SAMPLES AND MATCHED NON-TUMOROUS TISSUES

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Introduction/Rationale: MicroRNAs are key post-transcriptional regulators involved in oncogenesis and tumor progression. Among them, miR-130a has been reported to modulate multiple oncogenic signaling pathways, including PI3K/AKT, TGF-β, and MAPK cascades, suggesting its potential tumor-suppressive function. However, its expression profile across distinct molecular subtypes of breast cancer remains insufficiently characterized. The present study aimed to evaluate the expression pattern of miR-130a in various subtypes of breast carcinoma compared with corresponding tumor-free tissue, to identify potential subtype-specific alterations.

Methods: The study included 405 female patients with primary breast cancer classified as T1N0M0 according to TNM staging. Based on immunohistochemical and molecular profiles, tumors were divided into five subtypes: luminal A (n = 130), HER2-positive luminal B (n = 96), HER2-negative luminal B (n = 100), non-luminal HER2-positive (n = 36), and triple-negative breast cancer (TNBC, n = 43). During surgery, both tumor tissue and adjacent histopathologically confirmed non-tumorous breast tissue were collected. Total RNA was isolated, and miR-130a expression was quantified using RT-qPCR. Fold-change (FC) values were calculated relative to the control (tumor-free) samples.

Results: A consistent and progressive downregulation of miR-130a was observed across all breast cancer subtypes. Compared with control tissue, mean FC values were as follows: −2.61 in luminal A, −3.15 in HER2-positive luminal B, −4.35 in HER2-negative luminal B, −5.02 in non-luminal HER2-positive, and −6.24 in TNBC. The most pronounced suppression of miR-130a expression was detected in triple-negative tumors, suggesting a subtype-dependent gradient of deregulation. The observed trend indicates a possible inverse correlation between miR-130a levels and tumor aggressiveness.

Conclusions/Novel aspect: The study demonstrates that miR-130a expression is markedly reduced in all major molecular subtypes of breast cancer, with the most significant loss in triple-negative cases. These results highlight miR-130a as a potential universal tumor suppressor and a candidate biomarker for disease progression and molecular stratification. The progressive decline of miR-130a across subtypes supports its role in controlling pathways linked to proliferation and invasion, offering new insights into miRNA-mediated mechanisms of breast cancer heterogeneity.

[III-22] LOSS OF MIR-199A AS A COMMON MOLECULAR SIGNATURE IN BREAST CANCER PROGRESSION

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Introduction/Rationale: MicroRNA-199a (miR-199a) plays a crucial regulatory role in cellular differentiation, proliferation, and epithelial-mesenchymal transition (EMT). Acting as a tumor suppressor, miR-199a targets multiple oncogenic pathways, including HIF-1α, mTOR, and MAPK signaling. Altered miR-199a expression has been implicated in tumor invasion, hypoxia response, and chemoresistance. Despite its known importance in cancer biology, comparative analyses across molecular subtypes of breast cancer remain limited. The present study aimed to characterize miR-199a expression patterns in distinct breast cancer subtypes relative to tumor-free breast tissue.

Methods: A total of 405 women with primary T1N0M0 breast cancer were included. Tumors were classified into luminal A (n = 130), HER2-positive luminal B (n = 96), HER2-negative luminal B (n = 100), non-luminal HER2-positive (n = 36), and triple-negative breast cancer (TNBC; n = 43). During surgery, both tumor and adjacent non-cancerous breast tissues were obtained. After total RNA isolation, miR-199a expression was quantified using RT-qPCR, normalized to reference miRNA, and compared to corresponding control tissues. Expression differences were expressed as fold-change (FC) values.

Results: A significant and progressive reduction in miR-199a expression was observed across all subtypes of breast cancer compared to control tissues. The mean FC values were −2.07 in luminal A, −2.65 in HER2-positive luminal B, −2.73 in HER2-negative luminal B, −3.18 in non-luminal HER2-positive, and −4.69 in TNBC samples. The stepwise decline in miR-199a expression correlated with increasing tumor aggressiveness, reaching its lowest levels in triple-negative breast cancers. These findings suggest a potential subtype-dependent epigenetic silencing of miR-199a.

Conclusions/Novel aspect: This study reveals a consistent downregulation of miR-199a across all molecular subtypes of breast cancer, with the most pronounced loss in triple-negative tumors. The gradual decrease in expression mirrors the biological aggressiveness of the disease, supporting miR-199a's role as a key suppressor of oncogenic signaling and EMT progression. The data indicate that miR-199a could serve as a universal biomarker of tumor progression and a promising molecular target for restoring tumor suppressive pathways in advanced breast cancer.

[III-23] EXPRESSION OF TNF-A AND ITS RECEPTOR IN ASTROCYTIC TUMORS

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Introduction/Rationale: Tumor necrosis factor alpha (TNF-α) is a pivotal cytokine orchestrating inflammation, apoptosis, and cellular proliferation through activation of NF-κB and MAPK pathways. Dysregulation of TNF signaling contributes to tumor progression and immune evasion. The present study investigated the expression of TNF-α and its receptor (TNFRSF1A) in astrocytic tumors of different malignancy grades to elucidate their potential role in gliomagenesis.

Methods: Tumor tissue and matched non-neoplastic brain samples were obtained from patients undergoing surgical resection. mRNA expression of TNF and TNFRSF1A was quantified by RT-qPCR, and protein levels were evaluated using ELISA and Western blotting. Immunohistochemistry was performed to determine the spatial distribution of TNF-α in tumor parenchyma. Statistical analyses included ANOVA, correlation testing, and survival association.

Results: A marked upregulation of TNF and TNFRSF1A transcripts was observed in astrocytomas compared with controls, with a stepwise increase from grade II to glioblastoma ($p < 0.001$). Protein analyses confirmed this trend, showing enhanced TNF-α immunoreactivity in tumor cells and perivascular areas. Elevated TNF-α expression correlated with shorter overall survival and higher Ki-67 index, suggesting a pro-tumorigenic role through chronic inflammatory activation.

Conclusions/Novel aspect: Our findings demonstrate progressive activation of TNF signaling along astrocytoma malignancy. The concomitant upregulation of TNF-α and its receptor supports their contribution to tumor-associated inflammation and aggressive phenotype. TNF-α may serve as a potential prognostic biomarker and a therapeutic target modulating the glioma microenvironment.

[III-24] METHYLATION STATUS OF TNF PATHWAY GENES IN ASTROCYTIC TUMORS

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Introduction/Rationale: Aberrant DNA methylation is a major epigenetic mechanism controlling cytokine gene expression in cancer. Promoter hyper- or hypomethylation can modulate inflammatory signaling and influence glioma aggressiveness. This study aimed to characterize the methylation landscape of TNF and TNFRSF1A genes in astrocytic tumors to assess their regulatory impact on transcriptional activity.

Methods: DNA was isolated from fresh-frozen tumor and control brain tissues. Bisulfite conversion was followed by methylation-specific PCR and pyrosequencing to quantify CpG methylation in promoter regions of TNF and TNFRSF1A. Methylation results were correlated with gene expression levels, tumor grade, and patient clinicopathologic parameters.

Results: A significant decrease in promoter methylation was found in TNF ($p < 0.01$) and TNFRSF1A ($p < 0.05$) in high-grade gliomas compared with low-grade tumors and normal brain. Hypomethylation was inversely correlated with mRNA abundance ($r = -0.68$, $p < 0.001$), indicating epigenetic derepression of the TNF axis. In silico validation using TCGA datasets confirmed these associations. Patients with hypomethylated TNF promoters exhibited reduced overall survival, linking epigenetic activation to poor outcome.

Conclusions/Novel aspect: This study reveals promoter hypomethylation of TNF pathway genes as a driving mechanism of inflammatory activation in astrocytomas. The data underscore the relevance of DNA methylation profiling for distinguishing aggressive glioma phenotypes and suggest that restoring normal methylation may represent a potential epigenetic therapeutic approach.

[III-25] MICRORNA-MEDIATED REGULATION OF TNF SIGNALING IN ASTROCYTIC TUMORS

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Introduction/Rationale: microRNAs (miRNAs) fine-tune cytokine signaling by targeting mRNA transcripts involved in inflammation and cell survival. Disruption of miRNA–TNF interactions may contribute to glioma progression and immune escape. This study investigated TNF-related miRNAs and their regulatory impact on the TNF/NF-κB pathway in astrocytic tumors of varying grades.

Methods: Total RNA was extracted from tumor and control tissues. miRNA expression profiling was performed using microarrays and validated by RT-qPCR for selected miRNAs predicted to target TNF and TNFRSF1A (miR-21-3p, miR-23c, miR-27a-3p, miR-205-3p, and miR-300). Bioinformatic analyses integrated mRNA–miRNA interactions and pathway enrichment. Correlation with clinicopathologic variables and survival was evaluated.

Results: A subset of TNF-regulatory miRNAs displayed significant downregulation in high-grade gliomas. The strongest inverse correlations were observed between TNF mRNA and miR-21-3p ($r = -0.71$) and miR-205-3p ($r = -0.64$). Network analysis identified miRNA–target pairs converging on NF-κB and MAPK pathways. Functional enrichment suggested loss of post-transcriptional suppression leading to TNF-driven inflammatory signaling. Lower miR-205-3p levels were associated with shorter survival and increased tumor proliferation index.

Conclusions/Novel aspect: Our data demonstrate that reduced expression of TNF-regulatory miRNAs contributes to enhanced cytokine signaling in astrocytic tumors. The miRNA–mRNA interplay provides an additional regulatory layer linking inflammation with tumor progression. The identified miRNAs may serve as novel diagnostic and prognostic biomarkers and potential therapeutic modulators of TNF-dependent signaling in gliomas.

[III-26] FERROPTOSIS SUSCEPTIBILITY CORRELATES WITH ANTIOXIDANT CAPACITY AND IRON TRANSPORT REGULATION IN DISTINCT CELL LINES

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The sensitivity of cells to the induction of regulated cell death, such as ferroptosis, depends on their metabolic status, antioxidant capacity, and the expression of genes involved in iron and redox homeostasis. Ferroptosis, an iron-dependent form of cell death characterized by the accumulation of reactive oxygen species (ROS) and lipid peroxidation, represents a promising therapeutic strategy in cancer treatment.

In this study, the susceptibility of different cell lines to ferroptosis induction by erastin, a known inhibitor of the cystine/glutamate antiporter system Xc^- was analyzed. Among the tested models, melanoma 1205Lu cells showed the highest sensitivity, with approximately 80% of cells undergoing ferroptotic death. This pronounced response was associated with a limited antioxidant pool, particularly reduced glutathione, resulting from the erastin-mediated blockade of cystine uptake. Other cell lines, including HaCaT, Me45, and HCT116, displayed weaker responses to erastin, indicating relative resistance to ferroptosis. Nevertheless, an increase in ROS levels was observed in all tested models (HaCaT, 1205Lu, HCT116), highlighting oxidative stress as a common feature of the ferroptotic response, though its consequences vary depending on the cells' ability to regenerate glutathione. Analysis of TFRC and ACSL4 gene expression, encoding the transferrin receptor responsible for iron transport, revealed elevated activity in ferroptosis-sensitive cells (1205Lu, 451Lu) compared to relatively resistant ones (HaCaT, LN-229). These findings suggest that cellular susceptibility to ferroptosis is determined by tissue origin, antioxidant profile, and the regulation of iron and cystine transport pathways, providing valuable insight into strategies for selectively triggering ferroptosis in therapy-resistant cancer cells.

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[III-27] MECHANISTIC INSIGHTS INTO ANTI-TUMOUR DRUG INDUCED CYTOTOXICITY IN COLORECTAL CANCER CELLS

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Background: There are many keystone chemotherapeutic agents in colorectal cancer treatment, but their clinical application is limited by systemic toxicity and rapid degradation. Biopolymer-based drug carriers offer a promising strategy to enhance targeted delivery. Understanding the morphological and viability changes induced by drugs can provide deeper insight into their cytotoxic mechanism and aid in optimizing therapeutic strategies. This study investigates the cytotoxic mechanism of antitumor drugs delivered via biopolymers, focusing on morphological alterations and cell viability responses in colorectal cancer cell lines.

Methods: Biopolymer-drug complexes were prepared by ionic gelation and crosslinking technique using antitumor drugs: 5-fluorouracil (5-FU) and gallium nitrate ($Ga(NO_3)_3$). They were characterized for drug loading and encapsulation efficiency. Colorectal cancer cell lines (HCT-116 and HT-29) were treated with biopolymer-encapsulated drugs at various concentrations for 24-72 hours. Cell viability was quantified using Alamar blue assay, NucBlue live and NucGreen dead 488 probes reagent (visualized through confocal microscopy). Cellular morphology was examined by fixing with 3% glutaraldehyde for 2h at 4°C, washing with deionized water, and dehydrating in increasing grades of ethanol. The drug-induced structural alterations were observed by using scanning electron microscopy (SEM). Additionally, apoptosis-related changes were confirmed from morphological observations.

Results: Biopolymer-based drug formulations demonstrated enhanced cytotoxic efficiency, leading to a dose- and time-dependent reduction in cell viability in both HCT-116 and HT-29 cells (up to 70.15±2% cytotoxicity within 24h). SEM revealed distinct morphological alterations in response to treatment, where control cells exhibited normal, intact morphology with well-defined cellular structures. Treated cells show distinct features, including cytoplasmic shrinkage, membrane blebbing, and condensation. Fluorescence imaging also shows severe condensation or fragmentation with a smaller number of intact nuclei in treated cells, while controls have numerous visible nuclei, uniformly stained and evenly distributed, which is typical of viable, non-apoptotic cells. The combined $Ga(NO_3)_3$+5-FU treatment produced the most severe morphological deterioration with overall reduced cell density.

Conclusion: This concludes that the combination treatment exerts a synergistic cytotoxic effect, causing greater cellular damage than with either antitumor agent alone. This study investigates the morphological and viability changes induced by antitumor drugs in colorectal cancer cells, demonstrating that the combined drug treatment exhibits enhanced efficacy against the cell lines. The findings provide valuable insights into the cellular mechanisms underlying antitumor activity.

[III-28] THE INFLUENCE OF STEROID HORMONES ON THE EXPRESSION OF BK CHANNELS AND GLIOBLASTOMA CELLS

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Introduction: Glioblastoma multiforme (GBM) represents a highly malignant glial tumor characterized by uncontrolled proliferation, invasiveness, and high recurrence rates. Emerging evidence suggests that steroid hormones such as 17β-estradiol (E2) and progesterone (P) modulate glioma cell behavior through non-genomic signaling pathways that may involve large-conductance Ca^{2+}-activated K^+ (BK) channels. These channels are known regulators of membrane potential and apoptosis, yet their interaction with hormone-dependent mechanisms in glioblastoma remains insufficiently understood.

Methods: Human U-87 MG glioblastoma cells were treated for 24 hours with 17β-estradiol (0.0018 μg/ml and 1.8 μg/ml) or progesterone (0.25 μg/ml and 25 μg/ml), individually and in combination with the BK channel inhibitor paxilline (0.4 μM and 4 μM). Paxilline, a selective blocker of large-conductance Ca^{2+}-activated K^+ (BK) channels, was used to evaluate the possible involvement of these channels in hormone-induced effects. After treatment, total RNA was isolated, and the expression of gBK α (KCNMA1) and β_1–β_4 subunits was determined by RT-qPCR using GAPDH as a reference gene. Protein levels of the α subunit were analyzed by Western blot with densitometric normalization to GAPDH. Apoptosis was evaluated by flow cytometry using Annexin V-FITC and propidium iodide to distinguish viable, early apoptotic, and late apoptotic cells.

Results: Hormonal stimulation resulted in concentration-dependent modulation of gBK channel subunit expression. Physiological concentrations of E2 increased KCNMA1 protein levels, while higher E2 concentrations or co-treatment with paxilline reduced α-subunit expression. Progesterone elevated α-subunit expression, but the effect was attenuated in the presence of higher paxilline concentrations. RT-qPCR analysis confirmed downregulation of α and upregulation of β_2 subunits in response to E2, and upregulation of α and β_3 subunits with P. Apoptosis assays demonstrated that both E2 and P significantly increased of Annexin V-positive U-87 MG cells, with the strongest pro-apoptotic effects observed for E2 (0.0018 μg/ml + Pax 0.4 μM, ~60% early apoptotic) and P (0.25 μg/ml + Pax 0.4 μM, ~40% early apoptotic).

Conclusion: 17β-estradiol and progesterone modulate the expression of gBK channel subunits and promote apoptosis in human glioblastoma cells in a concentration-dependent manner. Co-treatment with paxilline further enhances these effects, indicating that pharmacological blockade of BK channels may potentiate hormone-driven pro-apoptotic signaling. These findings suggest a complex interplay between steroid hormone signaling and BK channel regulation, which may contribute to sex-specific differences in glioblastoma progression and offer new perspectives for targeted therapeutic approaches.

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[III-29] CRISPR/CAS9 AS A TOOL TO STUDY MOLECULAR MECHANISMS OF FERROPTOSIS IN CELLS

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CRISPR/Cas9 as a tool to study molecular mechanisms of ferroptosis in cancer cells. Ferroptosis is a distinct form of regulated cell death that depends on iron and is characterized by lipid peroxidation and disruption of redox homeostasis. Two key enzymes, ACSL4 (acyl-CoA synthetase long-chain family member 4), which promotes the esterification of polyunsaturated fatty acids into membrane phospholipids, and GPX4 (glutathione peroxidase 4), which reduces lipid hydroperoxides, play central roles in this process. Understanding their functions is essential for elucidating the mechanisms that determine cancer cell sensitivity to oxidative stress and for exploring ferroptosis as a therapeutic target.

In this study, the CRISPR/Cas9 system was employed to generate stable knockout cell lines lacking ACSL4 and GPX4 to investigate their involvement in ferroptosis induction. Molecular analyses, gene expression profiling (RT-qPCR), and lipid peroxidation assays were used to characterize the resulting phenotypes. The findings demonstrate that ACSL4 is crucial for initiating ferroptosis through modulation of membrane phospholipid composition, while GPX4 acts as a central protective factor against lipid peroxidation. Combining precise genome editing with biochemical assays and cellular imaging enables a comprehensive understanding of the regulatory networks controlling ferroptosis and may contribute to the development of novel therapeutic strategies in oncology.

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[III-30] DISTINCT PROTEOMIC PROFILES REVEAL DURATION-SPECIFIC EFFECTS OF RESVERATROL ON CARDIOMYOCYTES' RESPONSE TO IONIZING RADIATION

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Resveratrol is a plant polyphenol extensively studied for its health-promoting, including radioprotective, properties. Despite numerous evidence of its protective effects in the context of radiation, data concerning its impact on irradiated heart cells are limited. Furthermore, in vitro studies often use high, unachievable in vivo doses of resveratrol and short incubation times. Considering that resveratrol is a dietary component, it is essential to investigate its long-term effects at low, physiological doses.

In this study, we have evaluated whether long-term exposure of cardiomyocytes to physiological doses of resveratrol alters their response to ionizing radiation. Cells were incubated with resveratrol for 1 day, 1 week, or 1 month, and subsequently irradiated with a dose of 2 Gy. Functional tests showed that ionizing radiation reduced cell viability, intensified apoptosis, and led to cell cycle arrest. Long-term incubation with resveratrol usually counteracted these negative changes. Proteomic analysis revealed that different exposure times to resveratrol changed the expression of distinct sets of proteins. Groups subjected to long-term exposure (one week and one month) clearly differed from control groups and the group treated with resveratrol for 1 day. Moreover, changes in the expression of some proteins suggested a differential effect of resveratrol depending on the occurrence of radiation exposure. Further pairwise analyses distinguished three groups of proteins, differentiating irradiated from non-irradiated groups, depending on the presence or absence of resveratrol. Analysis of the cellular pathways associated with these proteins indicated that radiation affects stress response, cell cycle, DNA repair, and cell death. In turn, long-term incubation with resveratrol changes the cell's response to radiation, affecting primarily proteins associated with cellular metabolism and stress response.

Obtained results clearly demonstrate that resveratrol alters the response of cardiomyocytes to ionizing radiation, and this effect is highly dependent on the duration of incubation. The greatest changes at the proteome level were observed with long-term exposure to resveratrol. In contrast, in functional tests, we observed changes only after short-term exposure, which may indicate the occurrence of adaptive mechanisms in the cells. We believe this is of significant importance for the potential application of resveratrol as a radioprotective dietary component.

[III-31] EFFECT OF VISFATIN (ENAMPT) ON ADAPTIVE REDOX RESPONSES UNDER GAMMA-RADIATION–INDUCED OXIDATIVE STRESS IN CANCER CELLS *IN VITRO*

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Introduction: An important factor determining both tumour aggressiveness and the effectiveness of anticancer therapies, particularly radiotherapy, is the resistance of cancer cells to oxidative stress. Nicotinamide phosphoribosyltransferase (iNAMPT) is an intracellular enzyme widely expressed in human tissues that plays a crucial role in cellular metabolism by indirectly regulating redox reactions, energy production, and cellular signaling through nicotinamide adenine dinucleotide (NAD+). The extracellular form of NAMPT, known as visfatin (eNAMPT), exhibits multifunctional enzymatic, pro-inflammatory, and pro-angiogenic activity. Visfatin is overexpressed and secreted by cancer cells, thereby promoting inflammation and tumour progression. It also demonstrates protective properties toward tumour cells by reducing apoptosis and DNA damage. The precise role of visfatin in the mechanisms underlying cancer cell resistance to oxidative stress remains incompletely understood. The aim of the present study was to evaluate the effect of visfatin on the activity of antioxidant enzymes in two cancer cell lines differing in radiosensitivity.

Methods: Human pancreatic cancer cell line PANC-1 and human lung cancer cell line A549 were treated with recombinant human visfatin at concentrations of 50 ng/mL or 100 ng/mL for 24 hours. Subsequently, the cells were exposed to a single dose of 5 Gy γ-irradiation. To determine the activity of antioxidant enzymes —superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GSH-Px), and glutathione reductase (GR)—cells were lysed using Cell Lytic M buffer according to the manufacturer's protocol. Enzymatic activity was measured using commercial assay kits following the manufacturer's instructions.

Results: Visfatin was found to enhance the activity of antioxidant enzymes in both examined cancer cell lines. In A549 cells, a statistically significant increase in the activity of CAT, GSH-Px, and GR was observed following 24-hour treatment with visfatin at a concentration of 50 ng/mL, compared with both the negative control group (cells cultured without recombinant visfatin and without irradiation) and the positive control group (cells cultured without recombinant visfatin but exposed to γ-irradiation). In PANC-1 cells, a significant increase in SOD, CAT, and GSH-Px activity was observed after treatment with visfatin at a concentration of 100 ng/mL compared with the control groups.

Conclusions: The present findings support the substantial involvement of visfatin in mechanisms that reduce the sensitivity of cancer cells to γ-radiation–induced oxidative stress. These results provide a basis for further evaluation of visfatin as a potential factor regulating tumour cell radiosensitivity *in vitro*.

[III-32] ACTIVATION OF HUMAN DERMAL FIBROBLASTS BY EXOSOMES DERIVED FROM SCC-15 AND PANC-1 CANCER CELLS AFFECTS THE PROTEOLYTIC ACTIVITY

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Introduction: Activation of fibroblasts via exosomes derived from various cancer cells has been demonstrated in several reports. However, less is known about how the treatment with exosomes derived from two distinctive cancer types may affect the metabolism of the single cell line. In this study exosomes isolated from PANC-1 and SCC-15 were applied to activate normal human dermal fibroblasts (NHDF). In this model proteolytic activities of gelatin-degrading enzymes (MMP9, MMP2) were measured to search for the differences in the activation mode of NHDF cells.

Methods: Exosomes were isolated from PANC-1 and SCC-15 cell culture supernatants with the ExoCAS kit. The size and concentration of exosomes were measured via NTA analysis, and the presence of surface markers and the purity of the obtained extracts were analysed with the ExoCheck kit. The exosomes were used to activate NHDF cells. The cells were treated with two separate doses of exosomes after 24 and 48 h of culture. The medium and the lysates were collected 48 h after the application of the second dose. The activation of fibroblasts was confirmed by the measurement of the phenotypic changes (length of the cells). In order to assay proteolytic activities, the zymographic method was applied. FAP protein concentration in culture media was assayed by the ELISA method.

Results: The analysis of the obtained exosomes revealed similar sizes, concentrations, and surface marker templates in both preparations. However, exosomes derived from PANC-1 cells were slightly smaller and contained higher quantities of EpCAM and TSG101 on the surface in comparison to exosomes secreted by the SCC-15 cell line. In both cases, the presence of GM-130, a cis-Golgi matrix protein (a control for cellular contamination), was minimal. The preparations were applied to activate NHDF cells. The activation was confirmed in both cases. Analyses revealed similar patterns of proteolytic activity changes. The increased concentrations of proMMP-2, as well as its active form, in culture media were observed. The PANC-1-derived exosomes caused a higher activation level of proMMP-2 than exosomes from SSC-15. A reduction of FAP protein concentration in the medium under the stimulation was observed. There were no changes in the expression of MMP-9.

Conclusion: The study revealed that treatment of NHDF cells with exosomes derived from both cancer cell lines had similar effects on the synthesis rate or activities of the most significant extracellular matrix metalloproteinases secreted by fibroblasts, or FAP. It also seems to be significant that the synthesis levels of MMP-2 or MMP-9 are several times higher than in corresponding cancer cell lines. Therefore, elevated synthesis and activation of one of the major proteolytic enzymes secreted by fibroblasts (MMP-2) may contribute to cancer cells' migration ability and metastasis formation, especially when considering this phenomenon in the context of the functions of the tumour microenvironment.

[III-33] THE ROLE OF ANTIOXIDANTS IN DNA PROTECTION AGAINST RADIATION

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Ultraviolet (UV) and ionizing (IR) radiation induce oxidative stress, leading to DNA damage such as mutations and the formation of photoproducts, which in turn increases the risk of cancerous cell transformation. Antioxidants play a crucial role in neutralizing reactive oxygen species (ROS), thereby protecting DNA from damage and supporting repair processes. In vitro studies on skin cell lines have shown that antioxidants may reduce the differentiation of cells into cancer-associated fibroblasts (CAFs) and cancer stem cells (CSCs).

The aim of this study is to analyze the effects of UV and IR radiation on carcinogenic processes and to determine the role of antioxidants in protecting DNA from oxidative stress-induced damage. The study demonstrated that UVA and IR radiation differently affect the antioxidant activity of skin cells. In normal cells (HaCaT), moderate activation of defense mechanisms was observed, including an increase in glutathione levels and SMAD7 gene expression, indicating an effective initiation of internal antioxidant protection. In contrast, in cancer cells (Me45), UVA radiation induced a stronger stress response, characterized by increased SMAD3 and SMAD7 gene expression and a decrease in genes associated with antioxidant defense. IR radiation caused an overall reduction in metabolic activity in both cell lines, suggesting suppression of defense processes after exposure. These findings highlight the crucial role of endogenous antioxidants in maintaining DNA integrity and protecting cells from the effects of radiation-induced oxidative stress. Strengthening these natural defense mechanisms may represent an effective strategy to limit photoaging processes and reduce the risk of skin cancer development.

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[III-34] NEW OVARIAN CANCER CELL LINES OF CLEAR CELL AND SEROUS HISTOLOGICAL ORIGIN

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Introduction: Ovarian cancer (OC) is characterized by high heterogeneity. We highlight several histological types of ovarian cancer. The most frequent high-grade serous OC, accounting for 70% of cases, and the remaining subtypes, such as clear cell (12-13%), endometrioid (9-11%), and mucinous (3%) OC. Different histological types are characterized by specific molecular changes and a different clinical course. The development of new therapies requires the use of reliable research models in preclinical studies. Here, we report the establishment and characterization of new ovarian cancer cell lines of clear cell and serous origin.

Methods: Cancer cells were isolated from malignant ascites obtained via paracentesis. The project was approved by the Bioethics Committee of the Maria Skłodowska-Curie National Research Institute of Oncology, Warsaw (14/2024). The cell pellet achieved after centrifugation of malignant ascites was next separated into a fraction of mononuclear cells and red blood cells. Mononuclear cells were aspirated using a Pasteur pipette and washed twice with PBS, and suspended in DMEM/F12 medium (Merck Life Science) supplemented with 10% heat-inactivated FBS, 1x insulin-transferrin-sodium selenite (Merck Life Science), and standard antibiotics. Homogeneous cell culture was tested for karyotype, markers, spheroid-forming ability, and cytotoxicity.

Results: The histological origin of ovarian cancer cell lines available in global cells repositories is uncertain. Ovarian cancer cell lines previously used as serous ovarian cancer models turn out to be derived from clear cell or endometrioid carcinoma, such as the popular SKOV3 and A2780 cell lines. We have collected malignant ascites from ten ovarian cancer patients, including two with clear cell ovarian cancer and the remainder with the serous subtype. We observed that the cellular composition of the ascites from different patients is highly variable and contains a variable percentage of cancer cells. Additionally, there are other cells, such as mesothelial cells and fibroblasts, with smaller numbers of endothelial and inflammatory cells in the peritoneal fluid. The varying amount of cancer cells in ascites and specific nutritional requirements make it difficult to obtain a homogeneous proliferating ovarian cancer cell line. Nevertheless, we derived new ovarian cancer cell lines, one with clear cell ovarian cancer histology and two cell lines with high-grade serous ovarian cancer histology – the most frequent type of this tumor. Moreover, these cell lines are able to create spheroids, which are a reliable model for testing new therapies.

Conclusions: We obtained cancer cell lines that constitute a reliable model of ovarian cancer, one of the clear cell types, and the other two models for high-grade serous carcinoma. These lines are being further investigated.

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[III-35] ANALYSIS OF THE PROTEOMIC PROFILE OF PRO- AND ANTI-TUMOR NEUTROPHILS AND THE SEVS THEY SECRETE

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Introduction: Neutrophils constitute the most numerous group of cells in the human immune system (50–70% of WBCs). Similar to macrophages, they exist in two phenotypes: pro- and anti-tumor. A significant factor modulating their phenotype is the type I interferon receptor (IFNAR1): its presence promotes anti-tumor properties, while its silencing (Ifnar1−/−) favors a pro-tumor phenotype. Pro-tumor neutrophils promote tumor growth and metastasis by secreting factors that modulate the tumor microenvironment (VEGF-A, TGF-β, MMP-9, IL-8) and forming neutrophil traps (NETs). One mechanism of communication between neutrophils and tumors may be small extracellular vesicles (sEVs). They are secreted by neutrophils and transport molecules such as proteins, lipids and nucleic acids. The composition of sEVs is determined to some extent by the phenotypic state of the parent cell. Therefore, they can influence distant cells without direct cell-to-cell interactions.

Methods: Conditionally immortalized progenitor cells (ER-Hoxb8) were used in this study; wild-type (WT) and interferon receptor I knockout (Ifnar1-/-). Before proteomic analysis, cells were labeled using the SILAC (Stable Isotope Labeling by Amino Acids in Cell Culture) method. To differentiate the cells into neutrophils, β-estradiol was removed from the medium 5 days before sEVs isolation. Two days before isolation, the medium was replaced with medium containing exosome-depleted FBS. sEVs isolation was performed using mini-SEC. The sEVs fraction was characterized using NAT, TEM, and typical exosomal markers (CD63, CD81, TSG101). The obtained cell lysates and sEVs were subjected to proteomic analysis using nano-LC-MS/MS. Raw data were processed using Proteome Discoverer, and the resulting protein identifications were analyzed using the STRING bioinformatics platform.

Results: Proteomic analysis identified 3089 proteins in neutrophils and 365 proteins in secreted sEVs. In Ifnar1-/- neutrophils, 338 upregulated proteins were identified compared to WT neutrophils. In sEVs derived from Ifnar1-/- neutrophils, 107 proteins were found to be overexpressed compared to sEVs derived from WT neutrophils. Comparative analysis of proteins overrepresented in neutrophils and sEVs revealed 23 proteins in common. All of them exhibit pro-tumor properties by stimulating cell migration, proliferation, and differentiation. Furthermore, they activate pathways such as MAPK/ERK, PI3K/AKT, and NF-κβ, thereby influencing the activity of proangiogenic factors and metalloproteinases.

Conclusion: WT and Ifnar1-/- neutrophils have distinct proteomic profiles, which are also evident in the sEVs they secrete. Some of the proteins overrepresented in Ifnar1-/- neutrophils are identical to those identified in the sEVs they secrete. Therefore, sEVs may be excellent modulators of the tumor microenvironment, stimulating oncogenesis without direct cell-to-cell contact.

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[III-36] REDOX CROSSROADS: P53 AND GPX4 DETERMINE FERROPTOTIC FATE IN HUMAN NORMAL AND CANCER CELLS

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Introduction: Ferroptosis, a regulated form of cell death driven by iron and lipid peroxidation, is governed by intracellular redox balance. Central to this control are the transcription factor NRF2 and the thioredoxin system (TRX/TXNRD1). The tumor suppressor p53 is known to modulate antioxidant defenses and lipid metabolism, yet its impact on ferroptotic responses across different genetic backgrounds remains unclear.

Aim: To compare how p53 status influences redox gene expression and ferroptotic sensitivity across selected human cancer cell lines.

Methods: Six cell lines with defined p53 status were analyzed: p53+/+: HCT116 +/+, HL60, A549 and p53-/-: HCT116 −/−, K562, H1299. Cells were exposed to erastin (5 and 10 μM, 24 h) to induce ferroptosis. RT-qPCR quantified mRNA expression of NRF2, TRX, and TXNRD1. MTT assays evaluated cell viability as a functional outcome of redox adaptation.

Results: p53+/+ cells (A549, HL60, HCT116 +/+) showed moderate induction of NRF2 and thioredoxin system genes, consistent with a balanced antioxidant response and partial ferroptosis sensitivity. In contrast, p53-/- cells (H1299, K562, HCT116 −/−) demonstrated divergent redox adaptations: H1299 and K562 displayed strong TRX/TXNRD1 upregulation and high viability, whereas HCT116 −/− retained TXNRD1 activation despite TRX suppression, indicating compensatory antioxidant mechanisms. Overall, loss of p53 was associated with enhanced thioredoxin-driven antioxidant capacity and increased resistance to ferroptotic stress, while p53-proficient cells exhibited controlled activation of NRF2-linked pathways, aligning with higher ferroptotic susceptibility.

Conclusion: The p53 status critically shapes redox adaptation under ferroptotic conditions. p53-deficient cells amplify TRX/TXNRD1-driven antioxidant defense, maintaining viability under oxidative stress, whereas p53-proficient cells engage balanced NRF2 activation leading to moderate ferroptotic sensitivity. These results highlight the adaptive redox signatures defining ferroptotic fate and suggest that targeting thioredoxin signaling may selectively sensitize p53-deficient tumors to ferroptosis-based therapies.

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[III-37] DYNAMICS OF FERRITINOPHAGY: ANALYZING AUTOLYSOSOMAL PROCESSES RELATED TO IRON METABOLISM

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Ferritinophagy is a selective autophagy pathway that degrades ferritin to regulate cellular iron homeostasis. Understanding the dynamics of this process requires focus on its central machinery and master regulator. This study employs an analytic approach to delineate the dynamic relationship between the key effector proteins and transcriptional control of ferritinophagy in HaCaT keratinocytes.

The pathway is induced by the receptor NCOA4 (Nuclear receptor coactivator 4), which binds selectively to the ferritin cages. This complex is then taken to the forming autophagosome by direct binding to LC3 (Microtubule-associated protein 1A/1B-light chain 3), a membrane-embedded protein. This NCOA4-LC3 interaction is the critical step of selective cargo encapsulation. The LC3 lipidation dynamics and turnover directly reflect the flux of the entire pathway.

We further investigate how the transcription factor Nrf2 (Nuclear factor erythroid 2-related factor 2) acts as a master regulator of this pathway. Nrf2 indirectly controls NCOA4 and important members of the autophagic machinery, creating a positive feedback loop that optimizes ferritinophagic flux in response to oxidative stress.

By modulating Nrf2 activity and monitoring result changes in LC3 and NCOA4 levels, we mimic the dynamics of autolysosomes. Our data reveal that the Nrf2-NCOA4-LC3 pathway forms a dynamic regulatory network, fine-tuned by iron availability and oxidative stress to control the rate of ferritin degradation. Dysruption of such equilibrium, measured in terms of altered LC3 flux and NCOA4 stability, has a direct impact on keratinocyte function, implicating ferritinophagy dynamics in skin disease characterized by iron dyshomeostasis.

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[III-38] BASELINE CHARACTERIZATION OF FIBROBLAST PLASTICITY AND STRESS RESPONSE AS A FOUNDATION FOR MECHANOBIOLOGY AND REGENERATIVE RESEARCH

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Defining the intrinsic plasticity and stress-response capacity of fibroblast cell lines is essential for interpreting their behavior in mechanobiology, wound healing, and fibrosis models. Here, we establish the baseline molecular and functional profiles of two human foreskin fibroblast lines, Hs27 and Hs68, focusing on proliferation, autophagy, and signaling adaptability.

Cell proliferation rates were measured by trypan blue exclusion every 12 hours over 72 hours. Both lines showed distinct growth kinetics (Hs27: ~21 h; Hs68: ~18.5 h doubling time). After 72 hours in DMEM with 1% ITS, Western blotting revealed low LC3B-II/LC3B-I ratios and high p62 levels, indicating moderate basal autophagic activity. Elevated phospho-Smad3 levels revealed self-sustained TGF-β–Smad signaling, suggesting intrinsic activation even without exogenous stimulation. Both fibroblast types displayed a mesenchymal phenotype with variable E-cadherin/Vimentin expression, highlighting their inherent plasticity and readiness to respond to environmental stressors.

These baseline findings show that fibroblast cell lines exist in a pre-activated, mechano-responsive state, capable of dynamically regulating proteostasis and matrix remodeling under stress. Establishing this foundational profile provides a critical framework for future studies examining how mechanical forces, autophagy modulators, and extracellular stiffness shape fibroblast behavior. This work lays the groundwork for creating predictive 3D biomimetic models for fibrosis, wound healing, and regenerative medicine.

[III-39] THE P53 PROTEIN IN THE RESPONSE OF HELA CELLS TO THE INDUCTION OF PREMATURE CHROMOSOME CONDENSATION

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Introduction/Rationale: Replication stress can lead, among other things, to genomic instability by causing DNA damage. The tumor suppressor protein p53 is a key factor coordinating responses to DNA damage and cell cycle arrest. The evolutionary conservatism of p53 remains unexplored. The aim of the study was to determine whether p53 participates in the induction of premature chromosome condensation (PCC) in HeLa cells and to compare the results with PCC induction in a model of root meristem cells of Vicia faba.

Methods: Replication stress was induced by the application of 2.5 mM hydroxyurea (HU), an inhibitor of ribonucleotide reductase (RNR) that induces arrest in the S phase of the cell cycle. PCC induction was induced by the influence of 5 mM caffeine (CF) under conditions of permanent replication stress (2.5 mM HU + 5 mM CF). The presence of p53 protein was detected immunocytochemically using fluorescently labelled commercial antibodies (#2524; Cell Signaling Technology). Fluorescence and confocal microscopy were used to visualize and quantitatively determine the nuclear localization patterns of p53.

Results: Two types of PCC phenotypes were observed: G2-PCC (<20 breaks) and S-PCC (chromosome pulverization, >20 breaks). The cells were subjected to detailed quantitative analysis using ImageJ software. The measurements included the following parameters: area, mean grey, modal grey value, minimum and maximum grey value, centroid, centre of mass, perimeter, bounding rectangle, fit ellipse, shape descriptors, Feret's diameter, integrated density, median, skewness, kurtosis, area fraction and stack position. The measurements were made on the basis of images from an epifluorescence microscope and selected stacks from a confocal microscope.

Conclusions/Novel aspect: The involvement of the p53 protein in the PCC induction process in HeLa cells was demonstrated. The existence of a plant homologue of p53 requires further research.

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[III-40] MICRORNA-MEDIATED REGULATION OF ECM-RELATED GENES ACROSS BREAST CANCER SUBTYPES: DIFFERENTIAL EXPRESSION AND PREDICTED TARGET INTERACTIONS

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Introduction/Rationale: Dysregulation of extracellular matrix (ECM) remodeling is a driver of breast cancer progression. MicroRNAs (miRNAs) critically modulate the expression of ECM-associated genes, influencing cell adhesion, invasion, and metastasis. However, limited data compare miRNA-ECM regulatory interactions across different breast cancer subtypes.

Methods: Tumor and matched control tissues from 405 women with luminal A, HER2-negative luminal B, HER2-positive luminal B, non-luminal HER2-positive, and triple-negative breast cancer (TNBC) underwent microarray-based miRNA profiling. ECM-related genes with significant differential expression at mRNA and protein levels were selected, and miRNA target prediction was performed using miRDB (score ≥80). Only miRNAs both dysregulated in tumors and computationally linked to selected genes were included.

Results: Five miRNAs were identified as potential regulators of eight ECM-associated genes differentially expressed across all cancer subtypes. Reduced levels of miR-129, miR-432, miR-124, and miR-384 corresponded to increased COL1A1, FN1, ITGB1, and THBS1, respectively. Elevated miR-1246 expression aligned with downregulated COL6A6. No strong miRNA-based regulatory associations were predicted for COL1A2, COMP, RELN, or SPP1. The dysregulated miRNAs identified are known to modulate epithelial–mesenchymal transition, chemoresistance, tumor microenvironment remodeling, and metastatic behavior. The most aggressive subtypes (non-luminal HER2-positive and TNBC) showed the greatest accumulation of unfavorable ECM alterations paired with repressive miRNA signatures.

Conclusions/Novel aspect: This study highlights a core set of miRNAs potentially driving ECM remodeling in breast cancer irrespective of subtype. The identified miRNAs represent promising therapeutic nodes for restoring ECM homeostasis, limiting stromal stiffening, and reducing invasive potential. These findings lay the groundwork for subtype-stratified miRNA-based interventions aimed at matrix-targeted precision oncology.

[III-41] DIFFERENT ROLES OF THE HIGHLY HOMOLOGOUS CHAPERONES HSPA1 AND HSPA2 IN THE HUMAN EPIDERMIS

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Keratinocytes are the main structural cells of the epidermis, forming a physical and immunological barrier between the human body and the external environment. Through a tightly regulated process of proliferation and differentiation, they ensure the renewal and integrity of the multilayered epidermis. Heat shock proteins (HSPs) are molecular chaperones that maintain protein homeostasis by assisting in the folding of newly synthesized peptides and the degradation of damaged proteins. The HSPA (Hsp70) subfamily includes twelve conserved proteins with key cytoprotective roles. Among them, HSPA1 and HSPA2 share high structural similarity and intracellular localization but differ markedly in their expression patterns in the epidermis. HSPA2 is predominantly localized in the basal layer, whereas HSPA1 is expressed throughout all layers, with the highest levels in the spinous and granular layers. It is not known whether these proteins in the epidermis can functionally replace each other, as can be observed in various cancers. HSPA1 is predominantly linked with stress protection. Our previous studies showed that HSPA2 is implicated in keratinocyte differentiation, but had a negligible effect on cell sensitivity to heat shock. Therefore, we hypothesized that in the epidermis, HSPA1 and HSPA2 may perform distinct roles. In this study, we investigated whether HSPA1 deficiency affects keratinocyte differentiation and their sensitivity to proteotoxic stress.

HaCaT keratinocyte lines lacking HSPA1 or HSPA2 expression, as well as control cells, were generated using the CRISPR-Cas9 system. Cells were cultured under 3D conditions to form reconstructed human epidermis (RHE). After 18 days of differentiation, RHE samples were analyzed by immunohistochemistry for the progenitor cell marker p63, late differentiation markers (filaggrin and involucrin), as well as for HSPA proteins (HSPA1 and HSPA2). RHE lacking HSPA2 exhibited reduced thickness and decreased expression of filaggrin and involucrin as compared to controls, indicating differentiation defects. In contrast, morphology and differentiation marker expression in RHE lacking HSPA1 were similar to controls. These results suggest that HSPA1 is not essential for keratinocyte differentiation. The impact of HSPA1 loss on cell viability and stress resistance was evaluated in HaCaT cells with reduced HSPA1 expression obtained via viral shRNA transduction. We found that cells deficient in HSPA1 were more sensitive to proteotoxic stress compared to control cells, confirming its protective role under stress conditions.

In conclusion, HSPA1 and HSPA2 perform distinct functions in the human epidermis and cannot substitute for each other. HSPA2 is crucial for maintaining epidermal homeostasis and proper keratinocyte differentiation, whereas HSPA1 plays a key role in cellular protection under stress conditions.

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[III-42] THE INFLUENCE OF INSULIN ON THE BK CHANNELS IN U-87 MG GLIOBLASTOMA CELLS

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Metabolic dysregulation, including obesity, hyperglycemia, hyperinsulinemia, and type 2 diabetes, is associated with reduced survival and poorer prognosis in glioblastoma patients. In this study, we consider the pro-tumor signaling in hyperinsulinemia in U-87 MG glioblastoma cells. Although it is known that insulin-mediated signaling promotes GMB cell proliferation and survival through Akt activation and leads to metabolic reprogramming by enhancing glycolysis, not all aspects of insulin-related effects in glioblastoma are well understood. Among the potentially interesting yet poorly explored aspects of this phenomenon, we focused on investigating the effects of insulin on the function and expression of large-conductance Ca^{2+}-activated K^+ (BK) channels, which regulate cell growth, migration, and apoptosis in glioblastoma.

Human U-87 MG glioblastoma cells were treated for 6 or 24 hours with different concentrations of insulin (2.5-20 μg/ml) with or without 10 μM CaCl2. After treatment, the viability of U-87 MG cells was estimated by the CCK-8 assay. The functionality of BK channels was analyzed using patch-clamp recordings performed in voltage--clamp mode at a holding potential of +50 mV, in the presence of 10 μg/ml insulin and the corresponding controls. The experiments were conducted at two concentrations of calcium ions, [Ca2+] = 0 μM and [Ca2+] = 10 μM. We also tested the impact of simultaneous admission of insulin (10 μg/ml) and glucose (25 mM).

Our research has shown that insulin has a slight inhibitory effect on cell viability. This effect is more pronounced at higher insulin concentrations (20 μg/ml) over a shorter period of time (6 hours) and is enhanced in the presence of CaCl2. The patch-clamp results suggest that insulin is a BK channel activator, and its channel-activating effects depend on the concentration of Ca2+ ions. The open state probability increased from pop = 0.27±0.10 for control (NPo = 0.37±0.11) to pop = 0.31±0.05 (NPo = 0.51±0.06) in the presence of insulin at negligible levels of Ca2+. At [Ca2+] = 10 μM, we obtained pop = 0.46±0.19 (NPo = 0.57±0.16) for control and pop = 0.73±0.12 (NPo = 0.84±0.07) for insulin-stimulated patches. Glucose admission fully attenuated insulin-related effects on channel activity.

Insulin modulates the BK channel functionality in human glioblastoma cells, and the presence of calcium ions amplifies this modulation, suggesting a synergistic effect of both compounds.

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[III-43] DECODING THE FERRITIN LOOP: FERRITINOPHAGY AS A REGULATOR OF IRON BALANCE AND FERROPTOTIC SENSITIVITY

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Ferritinophagy plays a key role in maintaining iron homeostasis through the selective degradation of ferritin, the main intracellular iron-storage protein. This process is part of the ferritin loop, a dynamic regulatory circuit balancing iron sequestration and release. Under physiological conditions, ferritin binds iron in a redox-inactive form, while its degradation via ferritinophagy releases free iron to the labile iron pool (LIP). Disruption of this balance can enhance oxidative stress and promote ferroptosis, an iron-dependent form of regulated cell death driven by lipid peroxidation.

The present study aimed to evaluate the sensitivity or resistance of skin-derived cell lines to ferroptosis by examining the role of ferritinophagy and the ferritin loop. We analyzed the expression of PROM2, NCOA4, ACSL4, FTH, and FTL genes, and assessed lipid peroxidation levels supported by database and literature data. PROM2 is linked to ferroptosis resistance, NCOA4 indicates ferritinophagy activation, ACSL4 marks lipid peroxidation, while FTH and FTL reflect ferritin regulation.

Normal human keratinocytes (HaCaT) were resistant to erastin-induced ferroptosis, showing increased PROM2 and NCOA4, decreased ACSL4, and elevated FTH expression, with unchanged FTL mRNA. In contrast, melanoma cells (1205Lu) were ferroptosis-sensitive, lacking PROM2 and NCOA4 expression, displaying high ACSL4, marked lipid oxidation, and increased FTH and FTL expression.

These findings suggest that the ferritin loop, through regulation of ferritinophagy and intracellular iron flux, critically determines cellular susceptibility to ferroptosis and may represent a potential therapeutic target for modulating iron-dependent cell death.

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[III-44] THE ROLE OF ELEVATED TEMPERATURE IN THE REGULATION OF PD1 GLYCOSYLATION THROUGH HSF1-DEPENDENT MECHANISMS

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Introduction: The programmed cell death receptor 1 (PD1), encoded by the PDCD1 gene, is an inhibitory receptor regulating immune responses. The PD1-PD-L1 axis constitutes the molecular basis of modern immuno-oncological therapies. However, the mechanisms controlling PD1 expression and its post-translational modifications remain incompletely understood. Recent studies have shown that the transcription factor HSF1 (Heat Shock Factor 1) is hyperactive in cancer cells, promoting their proliferation, survival, and invasiveness. This study aimed to investigate whether thermal and proteotoxic stress influence PD1 expression and glycosylation, and to determine the potential involvement of HSF1 and the core fucosyltransferase FUT8 in this process.

Materials and Methods: Experiments were conducted on Jurkat cells (T-cell acute leukemia), NK-92 (natural killer), and K562 (AML) cell lines. Cells were exposed to thermal stress (43 °C, 1 h) or proteotoxic stress (bortezomib – proteasome inhibitor). The expression levels of PDCD1, HSF1, FUT8, and HSPA1 were analyzed using Western blotting and RT-qPCR. The level and forms of PD1 glycosylation were assessed by Western blot (WB) and flow cytometry (FC). Furthermore, K562 cells with wild-type (HSF1+/+) or knockout (HSF1-/-) genotypes were compared to evaluate the regulatory relationship between HSF1 and FUT8.

Results: Thermal stress induced only a slight increase in PDCD1 expression in T lymphocytes, NK cells, monocytes, and B lymphocytes, but markedly enhanced the amount of glycosylated PD1 protein. A similar effect was observed following proteasome inhibition by bortezomib, indicating activation of the proteotoxic stress response pathway. In Jurkat and NK-92 cells, thermal exposure upregulated FUT8 expression – the enzyme responsible for core fucosylation of N-glycans. Analysis of K562 cells with differential HSF1 activity revealed that FUT8 expression depends on HSF1 status. Literature data show that FUT8 participates in the regulation of several receptor functions (e.g., EGFR, TGFβR) and in processes such as epithelial–mesenchymal transition (EMT), migration, and invasion of cancer cells. Our findings suggest that a similar mechanism may apply to PD1, whose functionality could be modulated by HSF1-dependent fucosylation.

Conclusions: The results demonstrate that proteotoxic stress has a limited effect on PDCD1 transcription but strongly influences post-translational modification of PD1, increasing its glycosylation, particularly core fucosylation mediated by FUT8. Activation of HSF1 under heat stress may induce FUT8 expression, leading to structural and functional changes in PD1 that fine-tune immune inhibitory signaling. This mechanism may represent a novel regulatory axis of immune response adaptation during stress and cancer progression. Understanding this relationship could provide new opportunities for optimizing immuno-oncological therapies and designing strategies targeting receptor glycosylation.

[III-45] HSA-MIR-762 AS A MOLECULAR PLAYER IN CANCER

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MicroRNAs (miRNAs) are short (~22 nucleotides), non-coding RNA molecules that regulate gene expression at the post-transcriptional level and play essential roles in numerous biological processes. The first evidence linking miRNA dysregulation to cancer was reported in 2002 by Calin et al., who analyzed two miRNAs in chronic lymphocytic leukemia. This discovery initiated extensive research into the role of miRNAs in tumorigenesis. Among them, miR-762 has been implicated in the development and progression of several cancer types, including lung, breast, and gastric cancers, as well as osteosarcoma. Increasingly, recent studies have focused on identifying new molecular targets of miR-762 and understanding the biological significance of these interactions.

In our study, we performed miRNA and mRNA microarray analyses on two human cancer cell lines: melanoma (Me45) and colorectal carcinoma (HCT116). The results revealed, among other findings, differences in miR-762 expression between these cell lines. Furthermore, bioinformatic analyses were conducted to identify potential molecular targets of miR-762, followed by examination of their expression levels in our datasets — including genes involved in DNA damage repair and tumor development. From this group of candidate genes, several were selected for experimental validation using PCR-based methods.

Our findings provide a foundation for further studies, including luciferase reporter assays, aimed at elucidating novel interactions of miR-762 in melanoma cells, which remain poorly understood to date.

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[III-46] TARGETING IRON METABOLISM IN GLIOBLASTOMA WITH NOVEL TERPYRIDINE–THIOSEMICARBAZONE CHELATORS

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Cancers remain one of the leading causes of death worldwide, and the growing incidence and burden necessitate continuous improvements in prevention, diagnosis, and therapy. Cancer cells display a high demand for iron and disrupted iron homeostasis, which fuels their proliferation and results in an "iron dependency". Targeting iron metabolism pathways exposes therapeutic vulnerabilities and can suppress the growth of various cancers [1,2]. ATSC represents a new class of iron chelators that integrate thiosemicarbazones (TSC) and terpyridine moieties, both of which are well known for their strong iron-binding properties. The presence of both functional groups significantly enhances the iron-chelating capacity of these molecules, making them highly promising candidates for potential anticancer drugs. In this study, we evaluated the cytotoxic properties and iron-chelating potential of ATSC derivatives, which have not yet been explored in this context, using selected glioma cell lines. Spectrophotometric assays confirmed the ability of the tested compounds to chelate iron ions.

Cytotoxicity was determined in a panel of human glioblastoma cell lines, including T98G, U251, LN229, and LN18, using a 2D model. For the most active compounds, cytotoxicity was assessed in a 3D model for the U251 cell line. The transition to 3D was justified by a better reflection of the tumor microenvironment, enabling a more accurate estimation of compound efficacy [3]. To investigate the mechanism underlying the activity of the studied derivatives and their pronounced cytotoxic effect, experiments were conducted to further elucidate their mechanism. The generation of reactive oxygen species (ROS) and the expression of proteins associated with iron metabolism were analyzed. The results demonstrated that treatment with ATSC derivatives increased ROS levels in the 3D model, indicating the induction of oxidative stress. During early studies on the mechanisms of action of ATSC derivatives, we focused on iron-dependent proteins such as DMT1 (divalent metal transporter 1), MFRN1 (mitoferrin 1), and FXN (frataxin). The observed alterations in the levels of these proteins following ATSC treatment indicate a disturbance in iron metabolism. Although further investigations are needed to clarify the mechanism of action and verify selectivity toward cancer cells, ATSC demonstrates significant promise as potent anticancer agents.

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[III-47] HIGHLY MULTIPLEXED IMAGING OF CELLULAR ULTRASTRUCTURE

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The spatial organization of proteins and organelles within eukaryotic cells orchestrates fundamental biological processes. Super-resolution imaging of multiple biological structures is critical for understanding both normal physiology and the molecular mechanisms that drive disease. Yet, despite significant advances in optics, spectral overlap typically restricts biological imaging to a handful of fluorophores and often lacks the resolution needed to visualize events at sub-diffraction scales. To overcome these challenges, we introduce Cyclically Multiplexed Expansion Microscopy (Cy-ExM) – a workflow that integrates cryo-fixation of the specimen, expansion microscopy, and cyclic immunofluorescence labeling. Cy-ExM enables highly multiplexed imaging of proteins, cytoskeletal architectures, and cellular organelles with preservation of their native spatial organization. We validate this approach across multiple mammalian cell types using spinning disk, oblique plane, and axially swept light-sheet microscopy. Unlike DNA-barcoded or photoswitchable fluorophore strategies, Cy-ExM relies on standard reagents and instrumentation, making it readily adaptable to most research laboratories. Together, these advances provide an accessible platform for volumetric, high-plex imaging at nanoscale resolution—offering new opportunities to dissect the molecular organization of cells in health and disease, and to illuminate the nanoscale origins of oncogenic transformation.

[III-48] EXPRESSION PROFILE OF SELECTED FERROPTOSIS-RELATED GENES IN COLORECTAL CANCER CELL LINES AFTER 5-FLUOROURACIL AND TAMOXIFEN TREATMENT

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Introduction: Colorectal cancer remains one of the leading causes of cancer-related deaths worldwide. Despite continuous advances in diagnostics and therapy, colorectal cancer remains a major oncological challenge, largely due to the development of chemoresistance. Among current treatments, 5-fluorouracil remains the standard chemotherapeutic agent; however, resistance to this drug often results in therapeutic failure and disease recurrence. Tamoxifen, a selective estrogen receptor modulator used in hormone receptor–positive breast cancer, exerts additional effects on oxidative stress and cell death regulation. It is mainly used in breast cancer, but there are also reports in various publications about its application in research related to colorectal cancer. It primarily induces autophagy, but it may also participate in ferroptosis regulation. Recent studies have highlighted the importance of ferroptosis, which is a regulated, iron-dependent form of non-apoptotic cell death characterized by lipid peroxidation. Two key regulators of ferroptosis, SLC7A11 and GPX4, are central in maintaining redox balance and protecting cells from lipid peroxidation-induced death.

Methods: The aim of this study was to evaluate the activation of ferroptosis after treatment with 5-fluorouracil and tamoxifen in SW1116, DLD-1, and RKO colorectal cancer cells. For this purpose, RT-qPCR was used to examine the expression profiles of selected genes associated with ferroptosis at the mRNA level.

Results: The expression profiles of ferroptosis markers, specifically GPX4 and SLC7A1, were meticulously evaluated in the human colorectal carcinoma cell lines SW1116, DLD-1, and RKO utilizing RT-qPCR analysis. The experiment clearly showed that 5-fluorouracil effectively induces ferroptosis in all three cell lines studied. On the other hand, the impact of tamoxifen on ferroptosis induction was determined to be contingent upon the specific cellular background, thereby signifying a notable differential sensitivity among the experimental models subjected to analysis.

Conclusions: In summary, 5-fluorouracil induces ferroptosis in SW1116, DLD-1, and RKO cells lines, while tamoxifen acts on ferroptosis in these lines in different ways. As a result, GPX4 and SLC7A11 genes may be markers of ferroptosis activity in these cell lines.

[III-49] PROTEOTOXIC STRESS AS AN INDUCER OF FUSION TRANSCRIPT FORMATION

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Introduction/Rationale: Heat shock (HS) and other proteotoxic stressors can profoundly affect chromatin organization and RNA splicing. Under stress conditions, transcription and splicing are largely inhibited, except for the induction of heat shock protein (HSP) genes regulated by HSF1. Common stress-related effects include intron retention and transcriptional readthrough beyond gene boundaries. Additionally, splicing factors and other proteins are recruited to nuclear stress bodies, where specific long non-coding RNAs (lncRNAs) known as SatIII are transcribed and act to sequester splicing regulators. This altered transcriptional environment may also promote the formation of fusion transcripts arising from trans-splicing of exons from different genes, sometimes located on separate chromosomes. Such chimeric RNAs can encode novel proteins or function as regulatory non-coding RNAs. This study aimed to identify HS-induced chimeric RNAs and assess the prevalence of this phenomenon across various in vitro cell models.

Methods: In the first (identification) stage, human MCF7 (cancerous) and MCF-10A (non-cancerous) cell lines were exposed to heat shock (1 h at 43 °C) followed by 2 h and 4 h recovery. Total RNA was isolated and subjected to RNA sequencing (RNA-seq). Fusion transcripts were identified using Arriba software (v2.4.0), and results were compared with publicly available whole-genome sequencing (WGS) data to validate genomic support.

Results: A total of 503 fusion variants were detected across all samples, with 94% originating from MCF7 cells. Comparative analysis with WGS data confirmed 113 fusions supported by genomic reads, all in MCF7. Overall, 224 chimeric variants were specific to HS-treated cells (213 in MCF7 and 13 in MCF-10A, with 2 shared), and intergenic fusions predominated. Fourteen in-frame fusions, including DNAJB6–UBE3C, MOB4–HSPE1, and the interchromosomal IL31RA–STRBP, represented potential templates for chimeric protein synthesis. Based on defined selection criteria, 10 inter/intragenic fusions (MCF-10A) and 10 HS-induced intergenic fusions with ≥1.5-fold induction (MCF7) were selected for further validation.

Conclusions/Novel aspect: The ongoing second stage involves validation of selected fusions across multiple cancer cell models, including breast (MCF7, T-47D, BT-474, CAL-120, SK BR 3), colorectal (RKO), and myeloid leukemia (K-562) lines. Preliminary findings indicate that fusion transcript formation in response to proteotoxic stress may represent a widespread, yet underexplored, regulatory phenomenon. Further investigation is required to elucidate its molecular mechanisms and biological significance, particularly regarding its potential role in stress adaptation and tumor biology.

[III-50] 3D ORGANOIDS AS AN EXPERIMENTAL MODEL FOR THE STUDY OF REGULATED CELL DEATH – FERROPTOSIS

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Introduction: Preclinical studies traditionally rely on in vivo animal models, which, despite their utility, pose ethical challenges and often fail to fully replicate human physiology. The development of 3D in vitro systems, such as organoid cultures, offers a promising alternative that allows the investigation of complex cellular processes under controlled laboratory conditions. Ferroptosis a form of iron-dependent regulated cell death characterized by excessive lipid peroxidation has emerged as a potential target in anticancer therapies. The aim of this project was to establish a 3D organoid model for studying ferroptotic mechanisms in tumor-derived cell lines.

Methods: Organoid cultures were generated from colorectal cancer cell lines. Ferroptosis was induced by erastin (10 uM). Lipid peroxidation levels were quantified using BODIPY and confocal microscopy. Ultrastructural alterations associated with ferroptosis were examined by transmission electron microscopy (TEM).

Results: Organoid formation resulted in stable 3D spheroids exhibiting cell–cell interactions characteristic of tumor tissue architecture. Treatment with ferroptosis inducers led to significant increases in ROS and lipid peroxidation, accompanied by a marked reduction in cell viability compared to controls. TEM analysis revealed mitochondrial shrinkage, increased membrane density, and disrupted cristae structure—morphological hallmarks of ferroptosis. The 3D model demonstrated higher resistance to ferroptotic stimuli than 2D monolayers, reflecting the protective features of the spatial microenvironment.

Conclusions: The developed 3D organoid model provides a reliable platform for studying regulated cell death pathways, including ferroptosis, in a physiologically relevant context. This approach supports the reduction of animal testing and enhances translational research in oncology by enabling mechanistic studies and drug screening in an ethical, reproducible system.

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[III-51] ONE GENE, MULTIPLE FUNCTIONAL PRODUCTS – ANALYSIS OF LNCRNA, MIRNAS AND MICROPEPTIDES DERIVED FROM A SINGLE GENE

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Non-coding RNAs (ncRNAs) are defined by their lack of protein-coding potential. Two major classes of ncRNAs are microRNAs (miRNAs) and long non-coding RNAs (lncRNAs). Recent advances in bioinformatics revealed that a subset of ncRNAs contains small open reading frames (sORFs) capable of producing short functional peptides, termed micropeptides. This discovery challenges the strict distinction between coding and non-coding transcripts and suggests dual functions for some ncRNAs—as regulatory RNAs and as templates for translation. In this study, we analyzed four genes with evidence for producing both miRNAs, lncRNAs, as well as micropeptides – MIR155HG, MIR22HG, MIR34AHG, MIR200A gene. We summarized data available in databases concerning ncRNA and micropeptide sequences, expression levels, and functions. Using RNA-seq and small RNA-seq data, we quantified the levels of miRNA and lncRNAs derived from MIR155HG, MIR22HG, MIR34AHG, MIR200A in B-cell lymphoma cell lines. We also determined the levels of transcripts before (unspliced) and after splicing (spliced) for MIR155HG and MIR22HG and showed differences in the spliced/unspliced transcript ratios between lymphoma cell lines.

In conclusion, our preliminary analysis suggests differential biogenesis of multiple products derived from a single gene.

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[III-52] EFFECTS OF PHENOLIC ACIDS AND A MAGNETIC FIELD ON APOPTOSIS-RELATED GENE EXPRESSION IN COLON CANCER CELL LINES

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Colorectal cancer is the third most common malignancy worldwide and the second leading cause of cancer-related deaths. The search for new therapeutic strategies with minimal side effects remains a major research focus. Phenolic acids such as chlorogenic and caffeic acids are natural phytochemicals known to inhibit cancer cell proliferation and induce apoptosis. Magnetic fields have also been shown to promote apoptosis in cancer cells. Therefore, their combined use may represent an effective and non-invasive therapeutic approach. The aim of the study was to assess the effects of chlorogenic acid, caffeic acid, magnetic fields, and their combinations on the expression of apoptosis-related genes – survivin (BIRC5), p53 (TP53), caspase 8 (CASP8), and caspase 9 (CASP9) – in RKO and DLD-1 colon cancer cell lines. Cells were treated with phenolic acids, magnetic fields, or both. mRNA was isolated, reverse-transcribed, and analyzed by qPCR using cDNA as a template. Phenolic acids modulated the expression of apoptosis-related genes depending on the cell type and treatment applied. Preliminary results indicated differences in the expression levels of several genes when phenolic acids were combined with magnetic fields compared to single-agent treatment, particularly in RKO cells. Phenolic acids and magnetic fields influence the expression of apoptosis-related genes in colon cancer cells. Further investigation of caspase pathway genes is warranted to better understand the mechanisms underlying their combined effects.

[III-53] EVALUATION OF IN VIVO HSF1 INHIBITION FOR SUPPRESSING ER-POSITIVE BREAST CANCER GROWTH

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Introduction: Breast cancer (BC) is the most commonly diagnosed malignancy in women. Approximately 70% of all BC cases are estrogen receptor–positive (ER+), meaning their growth is driven by estrogen signaling. These tumors are primarily treated with hormonal therapies that either antagonize estrogen receptors (e.g., tamoxifen) or inhibit estrogen synthesis. However, 20–40% of patients eventually develop resistance to such therapies, which clinically manifests as disease relapse or metastasis. Our recent studies have identified a potential role for heat shock transcription factor 1 (HSF1) in promoting ER+ breast cancer cell growth and contributing to hormonal therapy resistance. HSF1 is a master regulator of the cellular stress response and supports multiple metabolic and proteostatic processes that facilitate tumor progression. The present study aimed to demonstrate the potential of HSF1 targeting as a strategy to inhibit ER+ breast cancer cell growth.

Methods: The mouse intraductal (MIND) model was used to investigate the role of HSF1 inhibition in ER+ breast cancer growth. Human breast adenocarcinoma MCF7-Luc2 cells expressing luciferase, either HSF1-proficient or HSF1-deficient, were injected into the ductal system of NOD scid gamma (NSG) mice. Tumor growth was monitored longitudinally using in vivo bioluminescence imaging. Several HSF1 inhibitors were evaluated in vitro, and DTHIB, a direct and selective HSF1 inhibitor, was chosen for in vivo validation.

Results: Loss of HSF1 resulted in a reduced proliferation rate of MCF7-Luc2 cells in vitro. Following intraductal injection, all MCF7-Luc2 cell lines successfully engrafted and grew in the mammary glands of adult female NSG mice without the need for exogenous hormonal supplementation over a six months. Bioluminescence imaging confirmed exponential tumor growth in all groups; however, HSF1-deficient MCF7-Luc2 cells exhibited significantly slower growth compared with HSF1-proficient counterparts. Additionally, metastatic foci were either absent or markedly reduced in mice injected with HSF1-deficient cells. DTHIB effectively inhibited breast cancer cell growth under both 2D and 3D culture conditions. Importantly, ER+ breast cancer cells displayed higher sensitivity to DTHIB than non-tumorigenic mammary epithelial MCF10A cells. Consistently, systemic DTHIB administration for six weeks significantly slowed tumor growth in mice bearing MCF7-Luc2 xenografts.

Conclusion: The MIND model provides a robust platform for studying ER+ breast cancer progression in vivo. Both genetic depletion and pharmacological inhibition of HSF1 reduced tumor growth, highlighting HSF1 as a promising therapeutic target in ER+ breast cancer.

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Section IV

„Bioengineering and Biomaterials”

[IV-1] EXPLORING SULFOBETAINE METHACRYLATE AND ITS POLYMERIC DERIVATIVES: FROM SYNTHESIS TO BIOLOGICAL CHARACTERIZATION

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Introduction: Since 2012, 34 studies have investigated sulfobetaine methacrylate (SBMA)–based copolymers synthesized via atom transfer radical polymerization (ATRP) for a broad range of biomedical purposes. These materials have been explored as drug and gene delivery systems, macromolecular surfactants, cell culture scaffolds, surface coatings and modifiers, membranes, blood-contacting materials, hydrogel wound dressings and supports for antibody immobilization. Incorporation of SBMA into diverse copolymeric structures confers a combination of advantageous properties, including excellent biocompatibility, low cytotoxicity, superhydrophilicity, antifouling and antibacterial activity, reduced protein adsorption, diminished platelet adhesion and activation, enhanced cellular internalization, the reverse polyelectrolyte effect, UCST-dependent phase transition, and superior lubricity. Collectively, these characteristics make SBMA-based polymers highly promising candidates for the development of advanced drug delivery systems requiring prolonged circulation, limited immune recognition, and controlled drug release.

Aim: In this study SBMA SBMA-based polymers were prepared with the intention for potential future use as delivery carriers for the anticancer drug – methotrexate (MTX).

Methods: SBMA homopolymers and linear copolymers with 2-hydroxyethyl methacrylate (HEMA) were synthesized via ATRP. Polymerizations were conducted using a Cu(I)/ligand catalyst system under mild conditions in trifluoroethanol. Structural characterization using ^{1}H NMR and gel permeation chromatography (GPC) confirmed successful polymer formation. The effect of SBMA and the MTX on cell viability was assessed using Alamar Blue Assay on 3 different human skin cell lines: NHDF (normal human dermal fibroblasts), Me45 (human melanoma cells), and HaCaT (spontaneously immortalized human epidermal keratinocytes). An additional non-cutaneous line, BEAS-2B (human bronchial epithelial cells), was used as a reference control.

Results and Conclusions: Depending on the polymer, monomer conversions were 66–76%. For the homopolymer, PDI was 1,37, indicating controlled polymerization; with increasing HEMA content, PDI rose to 1,81. SBMA exhibited no cytotoxic effects on tested cells only at exceptionally high concentrations (50000 µg/mL), far exceeding any residual levels that could possibly remain after polymer purification procedures, which means that the monomer will not distort future results when the polymers are subjected to biological studies. MTX barely affected cell proliferation in the studied range of concentrations (500- 7,8125 µg/ml) on all cell lines. MTX did not impact tested cell lines, probably because they do not have a sufficient number of folic acid receptors. The next stage of research will be to refine the methods of polymer synthesis and to study the cytotoxicity of the obtained polymers.

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[IV-2] SYNTHETIC 3D FIBROUS SCAFFOLDS FOR STROMAL TISSUE ENGINEERING

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The engineering of three-dimensional (3D) tissue models strongly depends on scaffold materials that can be seeded with cells of the relevant tissue. Ideally, such scaffolds should reproduce the characteristic features of the extracellular matrix (ECM), including its biochemical composition and physical parameters such as stiffness. Soft tissue matrices, for instance, combine remarkable mechanical stability with the ability to undergo remodeling during tissue maturation and wound healing – processes primarily mediated by cells like dermal fibroblasts. Conventional scaffold materials often fail to meet these requirements, as most synthetic matrices either lack sufficient mechanical stability or do not permit the high degree of plasticity necessary for cellular remodeling. To overcome this, a refined electrospinning technique was applied to generate a highly porous, fibrous scaffold structure. This approach enables the controlled adjustment of fiber--to-fiber spacing and allows fiber mobility, thereby supporting cell migration, matrix remodeling, and ECM synthesis by dermal fibroblasts. Consequently, these electrospun biologized scaffolds represent a promising technology for the creation of soft tissue equivalents. The adaptability of this approach was demonstrated by the successful development of a multilayered skin model consisting of subcutaneous adipose tissue, a dermal component—both based on the porous fibrous scaffold—and a stratified epidermis. A sequential layer-by-layer generation process promoted specific cell differentiation and tissue maturation. In the subcutaneous layer, human mesenchymal stem cells differentiated into adipocytes, expressing adipogenic markers and accumulating intracellular lipids until reaching the unilocular morphology of mature adipocytes. In the dermal layer, collagen deposition by fibroblasts resulted in a dense ECM, as confirmed immunohistologically, while mechanical testing showed an increased Young's modulus compared to the cell-free scaffold, indicating tissue maturation. Histological analysis of the epidermis revealed the layer formation from the stratum basale to the stratum corneum, and the increasing transepithelial electrical resistance over time confirmed the establishment of a functional barrier. The successful generation of skin equivalents using different polymer classes demonstrates that scaffold architecture exerts a dominant structural influence, while material dependency is substantially reduced in the biologization process. The ability to adjust scaffold porosity and fiber mobility in electrospun fiber fleeces offers a powerful technology for producing synthetic, well-defined 3D microenvironments. This approach can be extended to engineer a wide range of stromal and barrier tissues by combining tissue-specific stromal and epithelial cells, and it eliminates the need for animal-derived materials such as collagen hydrogels in constructing soft tissue equivalents for *in vitro* models or *in vivo* implants.

[IV-3] DEVELOPMENT AND CHARACTERIZATION OF COLLAGEN-BASED CARRIERS FOR TARGETED GLIOBLASTOMA THERAPY

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Collagen is a naturally occurring structural protein widely distributed in the extracellular matrix of animal tissues. Its excellent biocompatibility, minimal immune response, and full biodegradability make it a valuable material in biomedical engineering. In this research, collagen served as the basis for developing drug delivery systems, with its physical and chemical features carefully modified to achieve specific therapeutic goals. The study focused on creating, optimizing, and testing novel collagen-derived carriers for the controlled release of anticancer compounds, particularly for glioblastoma treatment — a highly malignant and therapy-resistant brain tumor. To assess the functionality and quality of carriers, various analytical methods were employed, including FTIR and UV-Vis spectroscopy. The results provided a strong technological basis for applying collagen-based systems in advanced and precise cancer therapies.

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[IV-4] ELECTROCHEMICALLY SYNTHESIZED MULTIFUNCTIONAL PEDOT–CEFTAZIDIME FILMS FOR ELECTRICALLY TRIGGERED, ADVANCED BACTERIA-RESPONSIVE ANTIBACTERIAL COATINGS

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Poly(3,4-ethylenedioxythiophene) (PEDOT) films incorporating the broad-spectrum β-lactam antibiotic ceftazidime (CAZ) were successfully synthesized via electropolymerization onto ITO/PET substrates. Cyclic voltammetry confirmed effective CAZ entrapment, as evidenced by anodic/cathodic peak shifts and enhanced charge storage capacity (2.98 ± 0.06 mC/cm² for PEDOT@CAZ vs. 1.58 ± 0.10 mC/cm² for pristine PEDOT). Electrochemical impedance spectroscopy revealed a significant increase in charge transfer resistance for PEDOT@CAZ (10,738 ± 508 Ω) compared to pristine PEDOT (494 ± 38 Ω), which was attributed to the insulating effect of CAZ domains and disruption of PEDOT's conjugated structure. Fourier transform infrared spectroscopy, scanning electron microscopy, and elemental analysis further verified the successful incorporation of CAZ into the PEDOT matrix. Surface characterization demonstrated high hydrophilicity (water contact angle 12 ± 0.9°) and increased surface roughness (2.10 ± 0.50 μm), conditions typically favorable for bacterial attachment but effectively counteracted by the antimicrobial properties of CAZ. Passive release of CAZ was minimal (1.23 ± 0.07 μg/cm² after 60 min); however, electrochemically stimulated release significantly enhanced antibiotic delivery (49.5 ± 5.92 μg/cm² by the 120th CV cycle). Importantly, bacterial species Shewanella oneidensis and Pseudomonas aeruginosa actively reduced and oxidized the PEDOT matrix, triggering responsive antibiotic release. These findings demonstrate that PEDOT@CAZ films synergistically combine conductive polymer electroactivity with bacteria-responsive localized antibiotic release, highlighting their potential as dual-action antimicrobial coatings for biomedical applications.

[IV-5] MICROBIOLOGICAL AND BIOLOGICAL ANALYSIS OF COATINGS DEVELOPED ON THE SURFACE OF TITANIUM AND ITS ALLOYS USING PLASMA ELECTROLYTIC OXIDATION AND POST-TREATMENT

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Biomaterial surface engineering is a growing field in biomaterial studies. Researchers are now focusing on giving materials new properties or enhancing already existing ones. Electrochemical methods have opened new ways to modify existing metallic biomaterials, allowing for the development of porous oxide layers on the surface of these materials. Additionally, electrochemical modification opens ways for post-treatment methods to further enhance the properties. For example, electrochemically modified titanium and its alloys could be further modified with a polymer loaded with an antibiotic, using a non-antibiotic antibacterial drug or using 2D-particles called MXenes [1]. In this study, the plasma electrolytic oxidation method was used as a method for electrochemical surface modification. For post-treatment methods, dip-coating and sonic spraying were chosen to add antibacterial agents. For cytocompatibility testing, AlamarBlue assay and cytometer measurements were used, and for microbiological assay disc-diffusion method was used as well as bacteria fixation on the surface of modified metal. The surface was examined using SEM and optical microscopy, and surface roughness was examined using an optical profilometer. In this study, cytocompatible and antibacterial surfaces were obtained. Three types of surfaces were obtained: clindamycin and PLGA polymer, anodized titanium dipped in benzydamine chloride aqueous solutions, and anodized titanium alloys modified by sonic spraying with a suspension of MXene particles with the general formula of Ti2C2Tx. In the case of clindamycin and PLGA polymer obtained surfaces were cytocompatible, the percentage of living L929 cells was 95% for 24h incubation, the same as for 3-day incubation [2]. Surfaces modified with clindamycin. For the surface loaded with benzydamine chloride obtained surface was antibacterial; however, it showed mild cytotoxicity towards MG-63 cells. The percentage of reduced Alamar Blue dye for this cell line was around 55%. In the case of surfaces modified with MXenes particles with PLGA polymer obtained surfaces were cytocompatible, showed no harmful effects towards MG-63 cell line, average percentage of reduced AlamarBlue was at 65% for all titanium alloys modified with PEO technique and MXenes particles with PLGA polymer. Additionally, the surface inhibited bacterial growth, and no uniform biofilm was observed. In summary, in the presented work, antibacterial coatings were obtained, which inhibited the growth of E. coli and S. aureus strains and showed low cytotoxicity towards MG-63 and L929 cell lines, with the exception of surfaces modified with benzydamine chloride, which showed mild cytotoxicity towards MG-63 cell lines.

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[IV-6] TARGETING THYROID CANCER BIOMARKERS: THE KEY ROLE OF MICROSTRUCTURRING FOR BEST DETECTION

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Introduction: Cancer is the second most common reason of death worldwide with approximately 10 million of death each year. Moreover, as the global population ages, the number of new cancer diagnoses is expected to rise even further. Early diagnosis plays a crucial role, as it significantly improves the chances of successful treatment and recovery. For this reason, the development of biosensors has gained significant attention in recent years, as it offers quick and cost-effective detection of cancer biomarkers. In this study, a microstructured electrode based on poly(3,4-ethylenedioxytiophene) (PEDOT) was developed as a platform for further fabrication of a thyroid cancer biosensor.

Methods: Indium-tin oxides (ITO) plates were used as a substrate for the synthesis of PEDOT films. First, ITO surface was modified with a polystyrene sacrificial template. Later the modified plates were used as a working electrode for electrochemical polymerization of EDOT. Subsequently, the polystyrene template was removed using an organic solvent, resulting in the formation of microstructured PEDOT layers. The obtained electrodes were characterized by Scanning Electron Microscopy, Atomic Force Microscopy and Cyclic Voltammetry in the presence of redox mediators.

Results: The results demonstrated that the polystyrene-templating approach successfully generates open microcavities within the PEDOT film, exposing the underlying ITO surface. Moreover, opening microcavities leads to an increase in redox currents of most of the studied mediators.

Conclusions: The studies showed increased currents of oxidation-reduction reactions for redox mediators. A great advantage of the proposed method is the possibility to modulate the size of cavities to induce selectivity of biosensing. In addition to biosensor development, such prepared layers may also find applications in other fields, e.g., in batteries and supercapacitors fabrication, drug delivery, or the microscopic monitoring of electrochemical reactions.

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[IV-7] HKUST-1@ELECTROCHEMICALLY REDUCED GRAPHENE OXIDEBASED NANOZYME FOR HIGHLY EFFICIENT HYDROGEN PEROXIDE DETECTION

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In the cell physiology system, redox homeostasis plays an important role, which is disrupted by oxidative stress (OS). Studies observed that the OS is linked with the physiological and pathological processes, such as atherosclerosis, cancer, ischemia, Alzheimer's, and Parkinson's disease. Hydrogen peroxide (H2O2) is one of the reactive oxygen species (ROS) recognized as a strong cytotoxic agent among other ROS. Because it has a long half-life when compared with other ROS, it is able to diffuse into the cell and extracellular space and produce hydroxyl radical via the Fenton reaction. Hydroxyl radicals are strong hydrogen acceptors, which can damage the cellular components. Therefore, the fabrication of a selective, sensitive, and stable sensor for H2O2 can contribute to a better understanding of cellular biology and facilitate the diagnosis of many diseases associated with oxidative stress. In this study, we designed the HKUST-1@electrochemically reduced graphene oxide electrode (ERGO) probe for the nonenzymatic H2O2 detection. HKUST-1 is a high surface area metal organic framework (MOF), which is prepared by the room temperature coordination modulation by reacting copper nitrate and benzene-1,3,5-tricarboxylic acid with acetic acid as a modulator. The prepared MOF had a specific surface area of 948 m^2/g-1 (pore volume 0.09 cm3/g-1), and its phase structure was ensured by X-ray diffraction. Here, ERGO was employed to gain the HKUST-1 copper redox couple efficiently, which confers the electrocatalytic H2O2 detection. The HKUST@ERGO electrode diffusion coefficient and electron transfer rate constant were calculated, revealing higher electrocatalytic activity towards the H2O2 reduction of a composite electrode when compared with the pristine HKUST. The amperometric measurements were employed to construct calibration curves for quantitative detection of H2O2 in the concentration range of 10 – 800 μM. The results indicate that the developed electrode material can be effectively applied for the detection of hydrogen peroxide in biological systems, enabling more accurate monitoring of oxidative stress in cells

[IV-8] DEVELOPMENT OF A CONCEPT FOR THE SELECTION OF MATERIAL AND PARAMETERS FOR THE PRECISION CASTING PROCESS OF RESORBABLE BONE PLATES

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Introduction: Short-term implants, such as bone plates, are used in the treatment of bone fractures. The main purpose of these implants is to stabilize the fracture until bone healing occurs. So far, inert orthopedic implants (e.g. stainless steel, cobalt alloys) have been used and after fulfilling their function, they require another operation to remove them from the human body because they corrode and become toxic. In this work, an attempt was made to select a material and manufacturing method for a resorbable medical implant that would not require reoperation because it would degrade in the recipient's body without causing any adverse health effects.

Methods: CAD software was used to design the mold model necessary for the precision casting process. 3D printing technology will be used to create the design and wax model mold.

Results: In order to ensure the safety of using a resorbable metallic implant, the material selection was narrowed down to elements that are both micro- and macroelements found in the human body, such as Zn and Mg. It is expected that this will ensure the biocompatibility of the implant. In vitro degradation testing and cytotoxicity assessment of the proposed material will be performed in the next research step. Precision casting parameters were also developed for the selected Zn1.2Mg wt% alloy.

Conclusions: The selection of the material for testing and its manufacturing method provides an alternative solution to existing and used short-term orthopedic implants, which become dangerous to the recipient's body as a result of the reaction between body fluids and the metallic surface of the implant. In this work, a concept was developed that provides the ability to adapt the shape and dimensions of the implant to the patient's anatomy and is also likely to be biocompatible in terms of materials. The resorbability of the tested material reduces postoperative costs and increases patient comfort.

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[IV-9] SURFACE ROUGHNESS REDUCTION IN FDM-PRINTED PLA MOLDS AND ITS IMPACT ON PDMS REPLICA QUALITY

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Fused Deposition Modeling (FDM) has emerged as a cost-effective method for fabricating molds in microfluidic device production. Polylactic acid (PLA) is frequently used for this purpose due to its affordability and ease of printing; however, its surface roughness can influence the quality of downstream replica materials such as polydimethylsiloxane (PDMS). This study examines the correlation between the surface roughness (Ra) of FDM-printed PLA molds and that of PDMS replicas obtained from them, following different surface post-processing treatments.

PLA molds underwent mechanical sanding with 240- and 400-grit sandpaper and chemical smoothing using acetone vapor. The surface roughness of both the PLA molds and corresponding PDMS casts was quantitatively measured using an optic profilometer. Results show a clear correlation between the mold and replica roughness, with PDMS surfaces consistently exhibiting much lower Ra values than their PLA counterparts. All post-processing methods had a significant impact on surface roughness, with acetone treatment achieving the smoothest surfaces in both materials.

These findings highlight that appropriate surface finishing of PLA molds not only improves their own surface quality but also significantly enhances the smoothness and functional performance of PDMS-based microfluidic devices.

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[IV-10] THE ABILITY OF IMMOBILIZED LACCASE ENZYMES OF DIFFERENT ORIGINS TO DEGRADE THE CYTOSTATIC DRUG VINCRISTINE

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Drugs used in anticancer therapy pose a significant threat to the environment, as they are increasingly detected in surface water, groundwater, and even drinking water. This is due to their low biodegradability and inefficient removal in conventional wastewater treatment plants. Therefore, developing effective strategies for their elimination and detoxification is crucial. Fungi have demonstrated high efficiency in removing these pharmaceuticals, but technologies based on whole fungal biomass have limitations: they require low pH, additional carbon sources, limited nitrogen, long retention times, and their dispersed form can hinder bioreactor operation. The use of isolated fungal enzymes offers a promising alternative.

The results show that the removal efficiency of vincristine strongly depended on enzyme origin. Laccase from Cerrena unicolor achieved 40% drug removal after 4 days, while the enzyme from Aspergillus sp. showed no degradation. This difference is attributed to their distinct redox potentials. These findings indicate that enzymes from white-rot fungi can be effectively used to eliminate cytostatic drugs. The study also highlights the potential of fungal enzymes in biotechnological applications, particularly for removing substances previously considered resistant to environmental degradation.

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[IV-11] TREHALOSE-RELEASING NANOGELS AS A POTENTIAL DRUG DELIVERY SYSTEM

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Introduction: There are many types of central nervous system tumors, and one of the challenges with their treatment is effective drug delivery through the blood-brain barrier (BBB). BBB is a highly selective, very tight barrier that is permeable to lipophilic substances. The most studied transport pathways of drugs across BBB are: receptor-mediated transcytosis, transporter-mediated transcytosis, and adsorptive mediated transcytosis. Glucose transporters (GLUTs) in the blood–brain barrier ensure the brain's high glucose supply and can be exploited as targets and gateways for delivering therapeutics into the central nervous system. One of the promising strategies to improve brain delivery involves the use of nanogel carriers engineered with appropriate charge, size, and morphology to enhance BBB penetration. Incorporation of trehalose, a natural disaccharide, into the nanogel structure introduces terminal glucopyranosyl moieties on the nanogel surface, which may improve brain targeting and support BBB crossing, potentially via GLUT-mediated transcytosis.

Methods: Cationic nanogels containing varying amounts of cationic monomer were synthesized via photoinitiated free radical polymerization using a reverse miniemulsion technique. Two series of nanogels were obtained: degradable and non-degradable. The cytotoxicity profiles of nanogels were evaluated with CCK-8 test on hCMEC/D3 and U87 cells. The non-degradable nanogel labeled with Cy5 (Cy5-ND.2) was used for cellular uptake studies in U87 glioma cell line.

Results and conclusions: Synthesized nanogels are non-toxic on tested cells lines. The non-degradable nanogel labeled with Cy5 (Cy5-ND.2) was taken up by U87 cells. The lack of cytotoxicity and excellent uptake make nanogels very promising as carriers for drugs.

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[IV-12] LAYERED DOUBLE HYDROXIDES AS PROMISED NANO-CATALYSTS IN MODEL CUMENE HYDROXIDE DECOMPOSITION IN LIQUID PHASE

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In the last decades, special attention has been paid to layered double hydroxides (LDHs), naturally existing materials or produced by synthetic routes (e.g., co-precipitation or ion exchange). They are also known as anionic clays or hydrotalcite-like materials and are described by the general formula [M2+1-xM3+x(OH)2]x+(Am-) x/m·nH2O, where M2+ and M3+ are di- and trivalent cations (Mg2+, Ca2+, Zn2+, Cu2+, Co2+, Ni2+, Al3+, Cr3+, Fe3+) respectively and Am- is an inorganic (like CO32-, Cl-, NO3-) or organic anion. These metal cations M(OH)6 form the positively charged layer by edge-sharing their octahedral, and with diverse charge-compensating anions present in the interlayer space. By combining both cation and anion versatilities, the resulting compositions are limitless in designing new hybrid LDH assemblies and their applications.

The presented research focused on the possibility of using LDHs as heterogeneous nano-catalysts in model reactions of cumene hydroperoxide decomposition to alcohol and ketone in the liquid phase. We checked simple LDHs containing in their structure cations (Co2+, Cu2+, Mn2+, etc.) and anions (nitrate, acetate, stearate). The reactions were performed without or in the presence of organic solvent (benzonitrile and tert-butylbenzene). Obtained LDHs were characterized by XRD, TGA and FTIR methods and their catalytic activity was measured using gas chromatography (GC-FID) and uptake of oxygen (in the case of oxidation processes) chemisorption is presented. The activity of cations depends on the polarity of the solvent, and it increases in benzonitrile: Co < Mn < Cu and in tert-butylbenzene: Cu < Co < Mn. Whereas the influence of the incorporated anion is independent of the solvent and the order: stearate > acetate > nitrate, is observed respectively.

The presented results are promising and confirm the potential use of LDH as a catalyst for liquid-phase oxidation of hydrocarbons. This is an important application in chemical technology as well as in biotechnology. Moreover, LDHs have features of heterogeneous catalysts, including the possibility of their separation and reuse.

[IV-13] INTEGRATED MICROFLUIDIC PLATFORM FOR MONITORING BIOHYDROGEN PRODUCTION AND BACTERIAL GROWTH

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Introduction: Biological hydrogen production presents a promising avenue for sustainable energy generation. Among potential biocatalysts, purple non-sulfur bacteria such as Rhodospirillum rubrum are notable for their ability to produce hydrogen under photoheterotrophic conditions. However, optimizing this process requires precise control of the microenvironment and high-resolution, real-time monitoring of both bacterial population dynamics and metabolic output. Conventional macroscale cultivation systems are limited in providing such control and temporal resolution. This study addresses this gap by developing and characterizing an integrated microfluidic lab-on-a-chip platform designed specifically for the continuous cultivation and real-time analysis of hydrogen-producing bacterial cultures.

Methods: The core of the study was the design and fabrication of a dedicated microfluidic chip. Direct laser ablation of poly(methyl methacrylate) (PMMA) was chosen as the preferred fabrication method for the microfluidic chip. The chip architecture incorporated a main cultivation chamber and an integrated, 3D-printed degasser with a gas-permeable membrane for the continuous removal of metabolic gases. Real-time monitoring of the bacterial population density was achieved through integrated optical sensors measuring turbidity directly within the microchamber. Hydrogen production was detected in real-time using an external hydrogen sensor, analyzing the headspace gas. Additionally, a separate PDMS membrane was incorporated to facilitate hydrogen transfer from the bacteria to the sensor.

Results. Preliminary data from the microfluidic platforms indicate that the design appears to facilitate the cultivation of Rhodospirillum rubrum. Initial readings from the integrated optical system suggest it may be capable of tracking population changes, while the degasser shows potential in managing the gaseous environment. Initial measurements show hydrogen is being produced, but we have not yet established a consistent link to the bacterial growth phases. Further validation is required to confirm these observations.

Conclusion: This study demonstrates a functional microfluidic platform that successfully enables the real-time observation of microbial growth and hydrogen production kinetics in R. rubrum. The use of 3D printing offers a flexible fabrication route. This lab-on-a-chip system is a powerful tool for rapidly optimizing conditions and studying the kinetics of biological hydrogen production.

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[IV-14] BIOACTIVE CARBON-MINERAL SCAFFOLDS FOR ENHANCED OSTEOINTEGRATION

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The field of bone implantology is in constant pursuit of innovative biomaterials that can effectively support bone tissue regeneration and serve as substitutes for damaged or lost bone fragments. The ideal material should not only provide structural support but also promote tissue integration and exhibit suitable biomechanical properties. This study explores the potential of biomorphic carbon materials derived from wood as a promising candidate for bone implant applications.

Wood, with its natural hierarchical structure and inherent porosity, offers an attractive template for creating biomimetic scaffolds. Through a controlled pyrolysis process, wood is transformed into carbonized material, preserving its original morphology while enhancing its mechanical properties. The resulting carbonized materials were further modified through physical activation with carbon dioxide (CO2) to increase their porosity and facilitate the infiltration of hydroxyapatite (HAp) precursors. HAp, a key mineral component of bone, is known to promote bone tissue growth and integration.

The synthesized materials were comprehensively characterized to evaluate their structural, physical, and chemical properties. Porosity analysis revealed a well-developed porous network with a combination of meso – and macropores, facilitating nutrient transport and tissue ingrowth. The mechanical properties, assessed through ultrasonic measurements, demonstrated that the materials exhibited a dynamic modulus of elasticity within the range of human bone, minimizing stress shielding and promoting bone remodeling. The electrical resistivity of the materials was also evaluated, ensuring their compatibility with the surrounding tissues and minimizing any adverse effects.

The surface morphology and elemental composition of the materials were examined using scanning electron microscopy (SEM) and energy-dispersive X-ray spectroscopy (EDS). The presence of HAp on the surface and within the pores of the activated carbonized materials confirmed the successful infiltration and synthesis process. Additionally, Fourier-transform infrared spectroscopy (FTIR) analysis revealed the presence of characteristic functional groups associated with HAp, further validating its incorporation into the carbon matrix.

The results of this study highlight the potential of hydroxyapatite-modified biomorphic carbon materials as promising candidates for bone tissue engineering applications. Their biomimetic structure, tailored porosity, and suitable mechanical properties make them attractive for promoting bone regeneration and integration. Further research and optimization are warranted to explore their full potential and pave the way for their clinical translation.

[IV-15] ENGINEERED DENDRITIC GOLD–RGO PLATFORMS FOR HIGH-STABILITY TYROSINASE BIOSENSORS TARGETING CATECHOL COMPOUNDS

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Gold nanodendrites (AuNDs) are a type of gold nanoparticle morphology commonly applied in electronic devices and sensors due to their advantages, such as a large surface area and excellent conductivity. 2D materials have received huge attention these days due to their superior stability, large surface area, high conductivity, and biocompatibility. The sp^2-hybridized reduced graphene oxide (rGO) centers act as anchoring sites and are responsible for the material's conductivity. The AuNDs preserve enzyme activity, cofactor direct electron transfer (DET), and stability; however, the synthesis of specific AuND nanostructures is challenging. In the present study, AuNDs were electrochemically deposited onto a rGO surface for the enzymatic detection of phenolic compounds. Exfoliated GO sheets were coated onto a screen-printed carbon electrode (SPCE) and subjected to an Au(III) solution under a nitrogen atmosphere, and the electrochemical deposition of Au structures was carried out at constant potential. The applied potential and deposition time were optimized as -0.1 V and 500s, respectively, according to the SEM images. The Au modified electrodes were then incubated with 3,3'-dithiolbis(succinimidylpr opionate) (DSP) to form self-assembled monolayers (SAM) via Au-S bond formation. In the next step, SAM layers of DSP were utilized for the covalent linkage of tyrosinase (Tyr). The AuND/ERGO/SPC electrode enabled the direct electrochemistry of Tyr and was successfully applied for catechol detection. The electrochemical detection of catechol was carried out on the nanocomposite electrode, which indicated the enzymatic conversion of catechol to orthoquinone in the presence of oxygen. The concentration of orthoquinone produced from catechol via tyrosinase was electrochemically detected, which is a measure of catechol's concentration. The sensitive detection was recorded for the wide range of catechol concentrations, and finally, Michaelis Michaelis-Menten constant was calculated. The stability and reproducibility of the modified electrode were studied. The DET of the Tyr-AuND/ERGO electrode is promising for the electrocatalytic and specific on-site monitoring of phenolic pollutants.

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[IV-16] DEVELOPMENT OF LIPOSOMAL CARRIERS FOR TARGETED DRUG DELIVERY IN COMBINATION THERAPY OF LUNG CANCER

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The increasing incidence of cancer worldwide is influenced by progressive urbanization and industrialization, which leads to greater exposure to carcinogenic factors such as cigarette smoke, exhaust fumes, and other airborne chemical compounds. Currently, the most common form of lung cancer is non-small cell lung cancer (NSCLC). The prognosis is highly dependent on the stage of the disease. Early stages (I and II) offer a 5-year survival rate between 50% and 70% and allow for surgical treatment. In contrast, patients diagnosed at stages III and IV require more advanced treatment methods, including chemotherapy, radiotherapy, and targeted therapy. Among currently used targeted therapies are EGFR inhibitors (e.g., osimertinib) and BRAF/MEK inhibitors (e.g., dabrafenib combined with trametinib). However, these treatment strategies have significant limitations. Their low efficacy often results from limited drug accumulation at the target site, rapid metabolism, and short half-life, which substantially reduce their ability to reach and maintain therapeutic concentrations. Moreover, the high toxicity of these drugs toward healthy tissues remains a serious challenge. Recently, drug delivery systems based on nanoparticles, lipids, or fullerenes have gained increasing attention for improving treatment efficiency. Liposomes—lipid-based spherical structures with diameters of approximately 100–200 nm—represent a well-known example. They enable the encapsulation of hydrophobic substances, facilitating their transport across biological barriers. Furthermore, liposome surfaces can be functionalized with additional chemical or biological agents (such as antibodies or peptides) to selectively target specific cells and enhance the effectiveness of targeted therapies. The aim of our study is to apply an innovative approach based on combination therapy using two commercially available inhibitors delivered to lung cancer cells via liposomal carriers. The first stage involved selecting candidate compounds—verubulin, dactolisib, alisertib, olaparib, and afatinib—based on their antiproliferative activity against PC-9 cells. This phase also focused on evaluating the combined effects of the selected drugs and assessing potential synergistic interactions. Subsequently, liposomes were prepared using the thin-film hydration method, and the selected drugs were encapsulated within them. The nanoparticle size and zeta potential was directly measured after synthesis using the dynamic light scattering (DLS) method, while particle diameter and stability were monitored during storage at 4 °C for 49 days. Additionally, particle diameter measurement was used to assess liposome stability over 24 hours in PC-9 culture medium. The obtained results indicate a high potential for the use of liposomes as efficient drug delivery systems in lung cancer treatment.

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[IV-17] GLUCOSE OXIDASE–FUNCTIONALIZED DENDRITIC GOLD@ELECTROCHEMICALLY REDUCED GRAPHENE OXIDE COMPOSITE FOR ENHANCED GLUCOSE SENSING

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Regular monitoring of glucose (GL) levels in physiological fluids is of great significance for controlling the complications associated with hyperglycemic and hypoglycemic conditions. Typically, glucose detection using glucose oxidase (GOx)-modified electrodes is attractive due to their high selectivity; however, maintaining enzyme activity and sensitivity remains challenging. To overcome these issues, introducing graphene with gold nanoparticles modified electrodes achieved high enzyme activity and stability. In this study, we focused on the fabrication of dendritic gold microstructures on the electrochemically reduced graphene oxide (Au@ERGO) by applying a constant potential. Then 3,3′-dithiodipropionic acid di(N-hydroxysuccinimide ester) (DPS) was self-assembled on gold surface through the generation of Au-S bond. The terminal DPS utilized for the covalent coupling of the GOx which confers the stable enzyme binding on the electrode surface (GOx-Au@ERGO). The gold microstructure and ERGO provide a high surface area and excellent conductivity, which helps maintain comparable electrical performance even after glucose oxidase (GOx) enzyme grafting onto the electrode surface. In this sensor composite system, the presence of freely diffusing ferrocenemethanol mediators facilitated high electron-transfer efficiency, a low operating potential (0.25 V vs. SCE), a stable and reversible redox couple, and excellent sensitivity. The developed GOx-Au@ERGO/screen printed electrode (SPC) revealed the wide range detection GL from 0.5 to 2 mM and earned a sensitivity of 6.26 uA mM-1. Eventually, GOx-Au@ERGO/SPC electrode successfully employed GL sensor in a human serum sample and gained reliable accuracy. This result showed that the designed electrode system is promising for stability, selectivity, and sensitive GL detection.

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[IV-18] CONSTRUCTION OF A DEVICE FOR THE AUTOMATIC TITRATION PROCESS

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The project focuses on the design, construction, and testing of a device intended to automate titration processes in laboratory applications. The analytical system will be equipped with potentiometric (pH electrode), conductometric, and photometric sensors, enabling precise monitoring of the titration progress. A peristaltic pump driven by a stepper motor will be employed to ensure accurate titrant dosing, while data acquisition and control will be managed by a computer-based system.

Dedicated software with a graphical user interface (GUI) will be developed to control the pump, collect data from the sensors, and generate titration curves. The program will automatically detect the equivalence point and perform data analysis to improve measurement accuracy. Test titrations with standard solutions will be conducted to evaluate the precision and repeatability of the constructed system.

Automating the titration process is expected to eliminate common manual errors, such as subjective colour interpretation and inaccurate volume dosing, thereby improving the reproducibility of analytical results.

The proposed system is intended to serve as a foundation for the further development of automated analytical setups for both educational and industrial laboratories.

Section V

„New Diagnostic and Treatment Modalities”

[V-1] RETROSPECTIVE ANALYSIS OF PENILE CANCERS: PRELIMINARY REPORT BASED ON A CZECH POPULATION

Iwona Dzieńdziora-Urbińska[1], Elżbieta Woźniak-Grygiel[1,2], Paweł Kudrys[1], Michal Grepl[3], Tomasz Pohaba[3], Jakub Sadowski[4], Zofia Klich[4], Gabriela Chlebowska[4], Tomasz Klaudel[4], Sven Solisch[5], Jozef Škarda[6]

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Introduction: Penile cancer is rare; however, due to its complex management, it represents a significant clinical challenge. It most commonly occurs after the age of 60, although earlier cases are also reported. Precancerous lesions of note include leukoplakia and erythroplakia. The majority of penile cancers are squamous cell carcinomas (PSCC), accounting for approximately 90% of cases, most frequently in men in the 6th–7th decade of life. Risk factors include poor genital hygiene, HPV infection (particularly type 16), HIV, smoking, excessive alcohol consumption, phimosis, chronic inflammation, mechanical trauma, and early sexual initiation. In most cases, PSCC coexists with penile intraepithelial neoplasia (PeIN) and is associated with HPV infection.

Methods: The present study was conducted in 2025 in a Czech population. A retrospective analysis was performed on the medical records of all patients with histopathologically confirmed penile cancer who were treated in outpatient or inpatient healthcare facilities. Demographic data, comorbidities, and environmental-behavioral factors—including alcohol consumption and tobacco use—were retrospectively evaluated.

Results: Based on ICD-O classification, various histopathological types of penile cancer were identified in the studied population. High-grade squamous intraepithelial neoplasia (H-SIL, ICD-O: 8077/2) was diagnosed in 9.46% of patients (n = 2) on the penis, 2.70% (n = 2) on the glans, and 4.05% (n = 3) on the prepuce. Basaloid squamous cell carcinoma (ICD-O: 8083/3) was found in 6.76% (n = 5) of penile lesions and 4.05% (n = 3) of preputial lesions. Neuroendocrine carcinoma (NOS, ICD-O: 8246/3) occurred in 1.35% (n = 1) of patients, exclusively on the penis. The most common histological type was squamous cell carcinoma (NOS, ICD-O: 8070/3), identified in 81.08% (n = 36) of penile cases, 18.92% (n = 14) on the glans, 12.16% (n = 9) on the prepuce, and 1.35% (n = 1) in the corpus cavernosum. Verrucous carcinoma (NOS, ICD-O: 8050/3) was diagnosed in only 1.35% (n = 1) of penile cases. Since alcohol abuse and tobacco smoking are known risk factors for penile cancer, these aspects were also analyzed. It was observed that the majority of patients neither consumed alcohol nor smoked. In the analyzed group, only 2.50% (n = 1) of patients with penile cancer and 6.67% (n = 1) with preputial cancer reported alcohol consumption. Similarly, only 1.35% (n = 1) of patients with penile cancer and 1.35% (n = 1) with preputial cancer declared smoking cigarettes.

Conclusions: The obtained results indicate the need for further research on the risk factors of penile cancer. Due to incomplete clinical and epidemiological data, the analysis will be continued and expanded with additional cases, which will allow for a more comprehensive understanding of the epidemiology of penile cancer in the Czech population.

[V-2] RETROSPECTIVE ANALYSIS OF PATIENTS WITH BASAL CELL CARCINOMA (BCC) AND SQUAMOUS CELL CARCINOMA (SCC) OF THE SKIN BASED ON OWN MATERIAL – PRELIMINARY REPORT

Iwona Dzieńdziora-Urbińska[1], Elżbieta Woźniak-Grygiel[1,2], Paweł Kudrys[1], Bartosz Bieżyński[3], Marcin Rydz[3], Andrzej Wojnar[4,5], Gabriela Chlebowska[6], Jakub Sadowski[6], Marcin Ziętek[3,7]

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Introduction: Skin cancer is the most common malignant tumor worldwide, and its incidence continues to rise. Basal cell carcinoma (BCC) accounts for over 85% of all non-melanoma skin cancers in Europe, followed by squamous cell carcinoma (SCC). The most important risk factor for both cancers is exposure to ultraviolet (UV) radiation, both intermittent and chronic. Sunburns significantly increase the risk, regardless of age. The pattern of exposure affects the subtype and localization of BCC: the nodular and infiltrating types occur more often on the face, whereas the superficial type predominates on the trunk. The risk of BCC also depends on skin structure (e.g., higher susceptibility of the ear epidermis), lifestyle factors (alcohol consumption increases risk, while high BMI decreases it), immunosuppression, age, and sex – men over 60 are affected more often. Risk factors for SCC are similar, with UV exposure being the most crucial. SCC usually develops on the head and face, and in transplant recipients, the risk is up to 100 times higher. Chronic skin injury, fair skin, and male sex (associated with a higher risk of metastasis) are also important.

Methods: The study began in 2023 and included patients from the Lower Silesia region. Clinical data from 25 patients with histopathologically confirmed BCC or SCC, treated in outpatient or inpatient settings, were analyzed retrospectively. Demographic data, comorbidities, and environmental-behavioral factors, including alcohol consumption and dietary patterns, were collected. The frequency of BCC and SCC, their association with comorbidities, and potential risk factors were evaluated. Statistical significance was set at $p \leq 0.05$.

Results: Data from 25 patients were analyzed—BCC was diagnosed in 92% (n = 23) and SCC in 8% (n = 2). BCC was more common in men (60%) than in women (40%), similarly to SCC (men 75%, women 25%). The most frequent comorbidity was arterial hypertension, followed by cardiovascular, thyroid, and genitourinary diseases. No statistically significant associations were found between comorbidities and the occurrence of BCC or SCC ($p > 0.05$). Most patients did not consume alcohol (BCC – 36%, SCC – 24%), and the applied dietary models (elimination, low-fat, or reduced bread consumption) showed no correlation with the incidence of skin cancer.

Conclusions: Due to the limited sample size, the obtained data do not yet allow for comprehensive statistical analysis. Therefore, data collection is ongoing to enable further evaluation of potential etiopathogenetic factors and their relationship with the risk of developing basal cell and squamous cell carcinoma of the skin.

[V-3] PRELIMINARY ANALYSIS OF SERUM CYTOKINE PROFILES IN PATIENTS RECEIVING VENOM IMMUNOTHERAPY (VIT) WITH STRATIFICATION BY SARS-COV-2 SEROSTATUS

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Introduction: Venom immunotherapy (VIT) is a model of antigen-specific immune modulation. The impact of prior SARS-CoV-2 exposure on circulating cytokine profiles during VIT remains insufficiently characterized.

Methods: We examined 28 patients (18 females, 10 males; mean age 52 ±13 years), undergoing maintenance VIT for Hymenoptera venom allergy. Serum cytokines (IL-1β, IL-4, IL-6, IL-8, IL-10, IL-12, IL-13, TNF-α) were quantified using a Luminex xMAP multi-analyte assay. Participants were stratified by SARS-CoV-2 serostatus: convalescents (S+N+), vaccine-only individuals (S+N−), and virus-naïve subjects. In the study group IgG antibodies against SARS-CoV-2 antigens (S1, S2, NP and S1-Omicron variant BA.1) and seasonal human coronaviruses (HCoV-HKU1, HCoV-229E, HCoV-OC43, HCoV-NL63) were assessed by ELISA and immunoblot. Clinical data included BMI and comorbidities (hypertension 18%, thyroid disorders 11%, asthma 7%).

Results: In the maintenance phase VIT, serum IL-10 (median: 0.34 pg/mL, Q1–Q3: 0.09–0.85) and IL-12 (median: 39.06 pg/mL, Q1–Q3: 37.43–86.90) remained within physiological values, while IL-4 and IL-13 were increased (median: 7.99 pg/mL, Q1–Q3: 5.18–26.19; 33.62 pg/mL, Q1–Q3: 28.35–67.77, respectively) and consistent with Th2 suppression and immune modulation. Patients with prior SARS-CoV-2 exposure and/or seasonal human coronaviruses vs virus-naïve participants did not show any statistically significant changes to their serum interleukin levels. The analysis of covariance (with clinical data – BMI and comorbidities) showed IL-12 difference in a group with and without IgG anti-HCoV-NL63 NP (median: 50.24 pg/mL, Q1-Q3: 37.43–197.76 and 37.43 pg/mL, Q1-Q3: 37.43–82.03, p=0.035 for negative and positive, respectively). TNFα, IL-6 and IL-8 showed lower levels in a serum of patients pre-exposed to HCoV-NL63 NP (TNFα median: 1.97 pg/mL; Q1-Q3: 1.34–3.98 and 1.73 pg/mL Q1-Q3: 1.13–3.08, p=0.043; IL-6 median: 0.885 pg/mL Q1-Q3 0.75–1.61 and 0.75 pg/mL Q1-Q3: 0.38–1.29, p=0.032; IL-8 median: 13.05 pg/mL Q1-Q3 9.27–16.31 and 7.7 pg/mL Q1-Q3 6.74–10.08, p=0.045 for negative and positive, respectively).

Conclusions: In the maintenance phase VIT, serum Th2 cytokines are increased (IL-4/IL-13), with IL-12 and IL-10 showing normal physiological range. The proportion of pro- (IL-6, IL-8, IL12, and TNFα) to anti-inflammatory (IL-10) cytokine levels indicates that prior contact with the HCoV-NL63 seasonal human coronavirus may prime and modulate the humoral immune system response against SARS-CoV-2 virus. It is possible that this phenomenon is caused by the fact that HCoV-NL63 uses ACE2 as a receptor for infection of target cells, as does the SARS-CoV-2 virus.

[V-4] DYNAMIC CHANGES IN SYSTEMIC INFLAMMATORY INDICES REFLECT TUMOR BURDEN RATHER THAN TREATMENT PARAMETERS IN CERVICAL CANCER UNDERGOING CHEMORADIOTHERAPY

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Introduction/Rationale: Systemic inflammation influences tumor progression and treatment response in cervical cancer (CC). Markers such as the neutrophil-to-lymphocyte ratio (NLR), platelet-to-lymphocyte ratio (PLR), lymphocyte-to-monocyte ratio (LMR), and systemic immune-inflammation index (SII) are increasingly recognized as prognostic indicators. However, their longitudinal dynamics during definitive chemoradiotherapy (dCRT) remain insufficiently characterized.

Methods: A prospective cohort of 106 women with FIGO stage IB–IVA CC undergoing dCRT was analyzed. Peripheral blood was collected before treatment initiation and within three days after completion of teletherapy. NLR, PLR, LMR, and SII were calculated from complete blood counts. Tumor volume was measured by MRI, and associations with clinical, treatment, and volumetric parameters were assessed using non-parametric tests, correlation analysis, and multivariable regression.

Results: Significant treatment-related hematologic shifts were observed: NLR and PLR increased, while LMR decreased after teletherapy ($p < 0.05$). SII showed a non-significant upward trend. Pre-treatment indices correlated positively with tumor volume and FIGO stage, whereas post-therapy NLR and LMR correlated with residual tumor volume but not with radiation dose or histologic grade. Multivariate regression confirmed tumor volume as the principal independent determinant of post-therapy inflammatory indices ($\beta\_NLR = +0.41$, $p = 0.002$; $\beta\_LMR = –0.33$, $p = 0.01$). Kaplan–Meier analysis revealed a tendency toward poorer progression-free survival in patients with elevated post-treatment NLR and PLR.

Conclusions/Novel aspect: Dynamic alterations in systemic inflammatory indices during dCRT primarily mirror tumor burden and immune response rather than treatment intensity or dose parameters. The consistent correlation between tumor volume and NLR, PLR, and LMR highlights their potential as accessible, noninvasive biomarkers for monitoring disease activity and therapeutic response in cervical cancer.

[V-5] THE IMPACT OF N501Y AND E484K MUTATIONS IN THE SARS-COV-2 SPIKE PROTEIN ON THE BINDING WITH AN ALPACA NANOBODY

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Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) causes the infectious disease COVID-19. The virus enters host cells through a specific interaction between its spike (S) protein and the angiotensin-converting enzyme 2 (ACE2) receptor on the cell surface. Although vaccines against COVID-19 have been developed, researchers continue to focus on approaches that can block the binding of the spike protein to ACE2, a key step in viral infection. This strategy remains central to the development of therapeutic drugs. Antibody-based therapy is one of the main methods of combating COVID-19; however, the use of conventional antibodies faces several limitations. Therefore, nanobodies are considered promising therapeutic agents for viral infections due to their small molecular size, high stability, excellent tissue penetration, and cost-effective production.

The aim of this study was to determine the impact of the key N501Y and E484K mutations in the spike protein on binding strength with the alpaca nanobody.

The inhibitory effect of the alpaca nanobody (W25) on the binding strength of the SARS-CoV-2 spike protein with N501Y and E484K mutations to the ACE2 receptor was tested using an ELISA assay (Raybiotech, Cat. No. CoV-SM3ACE2-2, CoV-SM2ACE2-2, CoV-SACE2). The results indicate that the inhibitory effect of the alpaca nanobodies on the binding of ACE2 to the SARS-CoV-2 spike protein depends on the concentration of the nanobodies. At low concentrations, the nanobodies most strongly inhibit the binding of the SARS-CoV-2 spike protein with the E484K point mutation to ACE2, while they show the weakest inhibition towards the wild-type SARS-CoV-2 spike protein.

In conclusion, the inhibitory effect of the alpaca nanobodies on the binding of ACE2 to the SARS-CoV-2 spike protein depends on both the concentration of the nanobodies and the SARS-CoV-2 variants.

[V-6] LONG-TERM OCCUPATIONAL EFFECTS OF LEAD INTOXICATION ON THE HUMAN HEARING AND BALANCE SYSTEM, OXIDATIVE STRESS AND SELECTED MINERALS

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Introduction: Lead compounds cause numerous harmful effects on the human body, particularly affecting the central and peripheral nervous systems, as well as negatively impacting the hearing and balance system. The aim of the study was to examine a group of workers long-term occupationally exposed to lead, focusing on oxidative stress markers, selected minerals, and hearing and balance functions. This paper presents an integrated summary and shared conclusions of two scientific articles developed within the international project: doi.org/10.1007/s00405-024-08675-0, doi.org/10.3390/ijms232112768).

Methods: The research group included male workers of the zinc and lead smelter (hearing study: n=280, balance study: n=268), which was divided into low blood lead concentration (L-Pb), and high blood lead concentration subgroups (H-Pb). Hearing assessment was conducted using click-evoked otoacoustic emissions (CEOAE), and the balance test was performed using a computerized posturographic examination.

Results: (1) Biochemical distinctions: ZPP (zinc protoporphyrin): elevated in H-Pb subgroups, hearing study: +68%, $p < 0.001$, balance study: significantly higher in both current and 12-year average value; CdB (blood cadmium concentration): significantly increased in the H-Pb subgroups, hearing study: +33%, $p < 0.001$, balance study: +26%, $p < 0.040$. (2) Homeostasis of essential minerals: Ca: significantly lower in H-Pb subgroups: hearing study: −2%, $p = 0.037$, balance study: -3%, $p = 0.011$; Zn: no significant difference between subgroups, but within-group correlations were positive between Zn and hearing parameters. (3) Oxidative stress markers in the H-Pb subgroups: MDA (malondialdehyde): hearing study: +4%, $p = 0.041$, balance study: +13%, $p = 0.043$; LPS (lipofuscin): hearing study +9%, $p = 0.040$. (4) Otoacoustic emission findings: in the H-Pb subgroup, the CEOAE results showed significant differences in otoemission amplitudes, and signal-to-noise ratios were observed in the right ear for all tested frequencies; negative correlations between otoemission parameters and PbB, ZPP, MDA, and duration of lead exposure. (5) Postural stability was significantly worse in the H-Pb subgroup:FCOP (sway field of the center of pressure) increased by 27-41%, significant across all test variants (eyes open/closed, 0-2 foam pads); VCOP (average velocity of the center of pressure) increased by 19-24%, significant in nearly all test variants.

Conclusions: (1) Occupational long-term exposure to lead is associated with measurable functional deficits in hearing and balance. (2) Elevated ZPP confirms ongoing lead absorption, and the presence of cadmium in the exposure mixture may intensify the ototoxic effects of lead. (3) Oxidative stress markers (MDA, LPS) increase significantly with blood lead levels, correlating negatively with cochlear function. (4) Calcium and zinc may have a protective effect on human hearing.

[V-7] DIVERGENT METABOLOMIC SIGNATURES OF RADIOTHERAPY AND INFLAMMATION DESPITE SHARED PATHWAYS

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The tissue response to radiotherapy (RT) inherently involves the induction of inflammation, raising a fundamental question: are there systemic metabolic effects equivalent? Is the metabolic fingerprint observed in a patient's blood following irradiation merely a reflection of inflammation? This presentation aims to delineate and compare the "metabolic portraits" of both conditions to address these questions.

Based on a systematic review of the literature encompassing 25 metabolomic studies, our analysis revealed that both radiotherapy and inflammatory states engage a surprisingly similar set of shared metabolic pathways. These include key domains such as energy metabolism (including the tricarboxylic acid cycle), the urea cycle, and amino acid (arginine, proline) and lipid metabolism.

However, the crux of the finding lies in the distinct outcomes observed at the level of specific metabolites. Despite activation of the same pathways, the direction of changes in the concentrations of their key molecules was often opposite. The most striking example is the ratio of lysophosphatidylcholines to phosphatidylcholines (LPC/PC), which characteristically increases in chronic inflammation, whereas it undergoes a substantial decrease in the early phase of the radiotherapy response. Similar opposing trends were observed for glutamine and sebacic acid.

These observations paint a clear picture: the metabolic portrait of radiotherapy is unique and cannot be reduced to an inflammatory effect alone. Understanding these distinct metabolic signatures is crucial for monitoring treatment response and for the development of new strategies to mitigate adverse effects.

[V-8] EVALUATION OF GASTRIC COLONIZATION OF HELICOBACTER PYLORI IN CAVIA PORCELLUS MODEL IN VIVO AFTER THE USE OF MICROPARTICLES LOADED WITH MYCOBACTERIUM BOVIS BCG

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Helicobacter pylori is a gram-negative bacterium that is responsible for gastritis and approximately 90% of gastric cancer cases. Gastric cancer remains a serious clinical problem because it is very often diagnosed at an advanced stage. Therefore, successful eradication of H. pylori is essential. However, due to the increasing antibiotic resistance of this pathogen, novel therapeutic strategies are urgently required. Recent studies suggest that probiotics may support eradication therapy through competitive inhibition of bacterial adhesion to the gastric mucosa. An innovative approach involves the use of selected chitosan microparticles loaded with Mycobacterium bovis Bacillus Calmette-Guérin (BCG) to investigate their potential impact on H. pylori-induced chronic inflammation. Chitosan is a polysaccharide polymer, is widely studied for its antimicrobial, antioxidant, and mucoadhesive properties. The immunomodulatory activity of M. bovis BCG has already been exploited in the treatment of bladder cancer and melanoma. The aim of this study was to evaluate whether supplementation with microparticles coated with chitosan containing M. bovis BCG could affect H. pylori colonization in the gastric mucosa of the Cavia porcellus model *in vivo*.

In this study, tissue from 30 Himalayan guinea pigs (Cavia porcellus) was investigated (5/group). The animals were housed in the Animal Facility at the Faculty of Biology and Environmental Protection, University of Lodz. For guinea pig inoculation, the reference H. pylori Culture Collection University of Gothenburg (CCUG) 17 874 strain, positive for the vacuolating cytotoxin (VacA) and cytotoxin-associated gene A (CagA), was used. Microparticles loaded with M. bovis BCG were administered twice to the guinea pigs.

Two different variants of carriers were used: alginate microparticles and polyvinyl alcohol (PVA) microparticles covered with chitosan. Twenty-eight days after the last inoculations with H. pylori, gastric tissues were collected postmortem. DNA was isolated from the gastric tissue using a commercial kit dedicated to animal samples. Using the real-time PCR method, the H. pylori and M. bovis BCG status was confirmed. SYBR Green-based detection targeted the following genes: ACTB and PPIA (housekeeping genes of guinea pigs), ureC and cagA (H. pylori), and the Bcg gene. After amplification, cycle threshold (Ct) values were evaluated to confirm the presence or absence of genetic material in the examined samples. Neither ureC nor cagA DNA fragments of H. pylori were detected in the gastric tissues of guinea pigs, irrespective of the microparticle variant used.

Based on the conducted research, it was demonstrated that the tested variants of microparticles loaded with M. bovis BCG did not influence the reduction of H. pylori colonization.

[V-9] THE EFFECT OF CHEMOTHERAPY ON APOLIPOPROTEIN PROFILE BASED ON ANALYSIS OF THEIR CONCENTRATIONS IN BREAST CANCER PATIENTS – A PILOT STUDY

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Introduction: Apolipoproteins are molecules involved in lipid metabolism and transport. Measuring their levels can be helpful in assessing the risk of cardiovascular disease. Both cancer cells and chemotherapy change the lipid profile. In a study, we determined apolipoprotein concentrations in breast cancer patients before and during anthracycline therapy. The aim was to analyze changes in their concentrations in the context of parameters related to the toxicity of chemotherapy in patients with breast cancer.

Materials and Methods: The study included 32 patients with triple-negative breast cancer (TNBC) diagnosed at the Maria Sklodowska-Curie National Institute of Oncology in Warsaw. The control group consisted of 10 healthy people. Serum samples from patients were collected before anthracycline administration and 6 months after starting therapy to determine the effect of treatment on apolipoprotein concentrations. Apolipoprotein concentration and CRP were determined using the Luminex X-MAP system (BIO-RAD) and the Bio-Plex Pro Human Apolipoprotein Assay Panel. The Mann-Whitney U test and Chi2 were used for statistical calculations, as well as nonparametric pairwise comparisons of adjacent AUCs using the Wilcoxon test. The diagnostic power of determined parameters was analyzed using MedCalc software. Receiver operating characteristic (ROC) analysis was used to determine cut-off points for the studied parameters.

Results: The Mann-Whitney test demonstrated that the concentrations of apolipoproteins A2, B, C3, D, E, H, and J in patients before treatment were significantly higher than in the control group ($p<0.005$). Only Apo A1 ($p=0.092$) and Apo C1 ($p=0.140$) concentrations did not differ between the patients and healthy individuals. For comparison, the concentration of CRP protein, which is an indicator of inflammation, was determined; significantly higher concentrations were found in patients before treatment than in healthy individuals ($p=0.001$). ROC curve analysis demonstrated high diagnostic sensitivity and specificity for most apolipoproteins: A2 (AUC=0.791), B (AUC=0.778), C3 (AUC=0.703), D (AUC=0.724), E (AUC=0.823), H (AUC=0.834), and J (AUC=0.798). The highest AUC was observed for CRP (AUC=0.856). Our own cut-off points for the apolipoproteins were determined based on the ROC curves. The Ch2 test showed that the percentage of patients with elevated Apo A1 levels before chemotherapy was higher (59%) than after 6 months of therapy (38%), while the percentage of patients with elevated Apo E levels before treatment (56%) was lower than after 6 months (72%).

Conclusion: Our preliminary studies confirm the association between elevated levels of Apo A2, B, C3, D, E, H, and J, and cancer. However, determining Apo A1 and Apo E concentrations may be useful in monitoring anthracycline therapy in patients with malignant tumours. However, this requires further research on larger groups of patients.

[V-10] ANALYSIS OF INFLAMMATORY MARKERS: NLR, PLR, SIRI, PIV, SII AND IL-6 CONCENTRATIONS IN BLOOD SERUM IN PATIENTS WITH CLEAR CELL RENAL CELL CARCINOMA (CCRCC) DURING IMMUNOTHERAPY

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Introduction: The inflammatory reaction accompanying cancer is an unfavourable prognostic factor. There are studies indicating poorer outcomes of treatment with immune checkpoint inhibitors in patients with significantly elevated inflammatory parameters. Monitoring the inflammatory process through the analysis of indicators based on quantitative measurements of blood cell counts, such as NLR (neutrophil to lymphocyte ratio), PLR (platelets to lymphocyte ratio), SIRI (systemic inflammation response index), PIV (pan immune inflammation value), and SII (systemic immune inflammation index), assesses the severity of the inflammatory process in relation to the patient's immune capacity. In addition, IL-6 plays a key role in the initiation and development of the acute inflammatory response and is also a good biomarker of inflammation. The aim was to evaluate the inflammatory response based on changes in inflammatory markers: NLR, PLR, SIRI, PIV, SII, and IL-6 levels before and during immunotherapy combined with ipilimumab and nivolumab in patients with clear cell renal cell carcinoma.

Materials and Methods: The study included 20 patients with clear cell renal cell carcinoma treated with ipilimumab + nivolumab at the Maria Sklodowska-Curie National Institute of Oncology in Warsaw. Serum samples were collected before immunotherapy, one and three months after starting treatment to determine the effect of therapy on inflammatory markers. Hematological markers were measured using a Mindray BC6800 Plus device, while serum IL-6 concentrations were determined using a Roche CobasPro800 device. The Mann-Whitney and Wilcoxon tests were used for statistical analysis.

Results: The median baseline values of PLR (161), PIV (419), SII (733), and IL-6 concentrations (9.3 pg/ml) were lower than the median values one month after starting immunotherapy: PLR (170), PIV (556), SII (939), and IL-6 concentrations (11.8 pg/ml). The increase in the median PLR value (216) three months after the start of immunotherapy was observed, while the median PIV and SII values remained unchanged. The median of IL-6 concentrations was slightly higher after the first month of treatment (11.8 pg/ml) than after 3 months of treatment (10.6 pg/ml). The Mann-Whitney and Wilcoxon tests show no significant differences in IL-6 concentrations ($P=0.696$) and the values of inflammatory markers ($P>0.05$) before and during immunotherapy.

Conclusions: In our study, the use of immunotherapy did not significantly affect changes in inflammatory parameters during the first three months of treatment in clear cell renal cell carcinoma patients. The study requires further research on a larger group of patients and expanding the research group to include patients receiving a combination of immunotherapy with a targeted drug.

[V-11] LIQUID BIOPSY ANALYSIS FOR PREDICTING (PSEUDO) PROGRESSION IN ADVANCED MELANOMA PATIENTS

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Despite advances in melanoma treatment, approximately 70-75% of patients with advanced melanoma will experience disease progression within five years. It is estimated that about 10% of patients treated with immunotherapy experience pseudoprogression, in which tumours may initially appear to grow due to immune cell infiltration rather than actual tumour growth. Predicting progression and distinguishing true progression from pseudoprogression remains challenging. Liquid biopsy (LB), particularly the analysis of circulating tumour DNA (ctDNA), offers a minimally invasive tool for real-time disease monitoring. One of the key methods for LB analysis is digital PCR (dPCR), which allows highly sensitive and absolute quantification of ctDNA, making it a promising biomarker for predicting disease progression.

This study evaluates the potential of ctDNA analysis to predict progression and distinguish true progression from pseudoprogression in patients undergoing immunotherapy.

A total of 60 blood samples were obtained from 50 advanced melanoma patients (stage III and IV) treated with targeted therapy or immunotherapy. Cell-free DNA (cfDNA) was isolated from plasma and quantified using a Qubit Fluorometer 4. The amount of ctDNA was measured by dPCR for the following variants: BRAF V600E, BRAF V600K, NRAS Q61K, NRAS Q61L, NRAS Q61R, and TERT C250T.

Based on ctDNA status up to 12 weeks after therapy initiation, patients were grouped by detectable or undetectable ctDNA. Patients with detectable ctDNA showed a higher progression rate (76%) compared to patients with undetectable ctDNA (20%). Kaplan-Meier survival analysis confirmed that detectable ctDNA significantly increased the risk of progression within 12 months of follow-up. Additionally, we compared the predictive value of ctDNA with lactate dehydrogenase (LDH), a widely used clinical biomarker. Kaplan-Meier survival analysis revealed that ctDNA provides a more accurate prediction of progression than LDH, which did not show a statistically significant association with progression risk during the same period. In the study group, pseudoprogression was identified by medical imaging in 11 patients treated with immunotherapy. Four patients (80%) with true progression had detectable ctDNA at the time of imaging, and four patients (67%) with pseudoprogression had undetectable ctDNA.

In conclusion, ctDNA analysis is a promising predictive biomarker and potential tool for distinguishing pseudoprogression from true progression during immunotherapy. However, these findings require validation in a larger patient cohort.

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[V-12] MOUSE MODEL OF AUTOIMMUNE MYOCARDITIS – TOWARDS OPTIMIZATION OF EXPERIMENTAL PROTOCOL

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Introduction/Rationale: Myocarditis is a complex cardiac disorder characterized by inflammation, necrosis, and fibrosis. This condition is underdiagnosed cause to sudden death and chronic dilated cardiomyopathy. Mouse models of myocarditis serve as potential tools facilitating the development of new therapies. The purpose of this work was to test the common experimental protocol for induction of autoimmune myocarditis in mice.

Methods: 6–8-week-old female BALB/c mice were subcutaneously injected with 250 µg of α-MyHC614–634 peptide (Ac-RSLKLMATLFSTYASADR-OH) emulsified with complete Freund's adjuvant on days 0 and 7. The function of the mice hearts was evaluated in vivo before the treatment and around 21 days after the first peptide injection using magnetic resonance micro-imaging at 9.4T. The vertical 89 mm bore system (Bruker BioSpin, Germany) equipped with a Bruker Micro 2.5 gradient system and a transmit/receive birdcage radiofrequency coil with an inner diameter of 30 mm was exploited. During data acquisition, the animals were anaesthetized with 2-3% sevoflurane. Body temperature, ECG signal, and respiration rate were monitored using ECG/respiratory unit (SA Instruments, Inc). The imaging protocol started from the acquisition of several fast scout scans in order to plan the geometry of self-gated CINE images (acquired using Intragate FLASH sequence) in the short and long axis orientation of the heart. Based on these images, end diastolic volumes, end systolic volumes, stroke volumes and ejection fractions were evaluated. The prospectively gated multi-slice multi-echo sequence was exploited for transverse relaxation time (marker of myocardial oedema) quantitation. After MR imaging mice were euthanized by cervical dislocation. The hearts were fixed in 4% formalin and embedded in paraffin. Conventional haematoxylin/eosin staining was used to evaluate cardiac inflammation.

Results: Histological analysis revealed very small foci of immune cell infiltration in the examined mice hearts. Consistently, magnetic resonance micro-imaging did not reveal prominent changes in heart function in response to the injection of α-MyHC614–634 peptide.

Conclusions/Novel aspect: Application of the commonly used experimental protocol was ineffective in inducing massive cardiac inflammation in mice in our study. The high variability in the severity of the disease despite an identical dose of α-MHC peptide is a frequent problem in the studies of murine models of autoimmune myocarditis. The protocol will be modified by supplementing complete Freund's adjuvant with the heat-inactivated H37Ra strain of Mycobacterium tuberculosis. This modification is expected to amplify the immune response [https://doi.org/10.2478/rjc-2025-0026].

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[V-13] BIOPSY PROTEOME-BASED CLASSIFICATION OF T CELL-MEDIATED KIDNEY ALLOGRAFT REJECTION

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Introduction: Late kidney graft loss remains a major obstacle in kidney transplantation. The mechanisms that enable a transplanted kidney to maintain long-term function are still not fully understood. T cell–mediated rejection (TCMR) continues to pose a significant clinical challenge. Based on histopathological biopsy evaluation, patients are typically classified into categories such as no rejection (NR), borderline rejection (BR; Banff category 3), and acute rejection (AR; Banff category 4). However, this classification alone may be insufficient, as some patients with borderline rejection still require clinical intervention. Therefore, a more robust classification approach based on biopsy proteome profiling may provide a valuable solution.

Methods: In this study, kidney tissue samples from patients classified as NR, BR, and AR were subjected to MS-based proteomic profiling. TCMR was diagnosed through histological evaluation according to the Banff criteria. To validate the results obtained using MS techniques, a panel of four proteins (GNB4, PDK1, AGXT, and CD73) was selected for immunohistochemical (IHC) analysis using renal allograft biopsy samples from an independent patient cohort (AR: n = 8; BL: n = 9; kidney donor group (KD): n = 8).

Results: Proteomic analysis identified 2547 proteins whose abundance profiles demonstrated strong concordance between the BR and AR groups. In a quantitative comparison between the BR and AR groups, GNB4 and AGXT emerged as significantly differentiating. Moreover, AGXT was indicated as a potential biomarker following ROC analysis. PDK1 and CD73 were found to best classify the samples in a binary analysis. IHC confirmed only upregulation of PDK1 in immune cells and GNB4 in macrophages, with no significant changes in the tubular epithelium.

Conclusions: Thus, PDK1 in immune cells and GNB4 in macrophages have been identified as a potential target for further extensive studies. If their relevance were to be confirmed in a larger patient cohort, their IHC analysis could serve as an extension of established histopathological classification in the context of kidney transplant rejection.

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[V-14] DEVELOPMENT OF DOUBLE TRACER IMAGING USING MOUSE PHANTOM WITH J-PET

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Background: Positron Emission Tomography (PET) is a powerful imaging modality that works based on the emission of positrons, then annihilation by the nearby electron emitting a 511 keV photon, almost back-to-back, and then reconstructing the annihilation point based on the arrival/emission time of gamma quanta, enabling the estimation of the tracer location or the uptake value of the tracer in a certain biological organ.

Double tracer PET imaging, which is a way of using two tracers for imaging, has several advantages: it gives detailed information and tracks multiple biological molecules and different physiological processes at a single time. The image reconstruction part of PET is a vital point since it enables us to determine the location of the tracer, in other words, the degree of malignancy of the tumor.

The modular J-PET scanner, comprising 24 compact and versatile modules, each consisting of 13 plastic strips with four SiPM detectors at the ends, represents a powerful tool for clinical applications in nuclear medical imaging. The J-PET scanner has extended applications since it enables the collection of events from the electron-positron annihilations and the de-excitations.

Aim: The aim of this study is to image two different isotopes of pure β^+ and β^+ → γ emitting isotopes that are dissolved in water using the 3D-printed small animal (mouse) phantom. The imaging technique is followed by selecting a potential candidate for electron-positron annihilations, and the electron-positron annihilation is accompanied by the prompt gamma. The image subtraction is done based on the ratio of the relative efficiencies of triples to doubles.

Methods: The phantom was modeled from the CT image acquired with the Bruker Skyscan 1278 at a resolution of 50 μm at the medical physics department. The pure beta-emitting isotope (18FDG) was transferred to the head with an activity of 1.17 MBq in 0.2 ml, while the second isotope β^+ → γ was transferred to the kidney with an activity of 2.05 MBq in 0.1ml. The 18FDG was purchased from the Voxel company in Krakow, while the 44Sc was produced in the Heavy Ion Collider in Warsaw.

Results: The castor reconstructed images show distinct images both in the head and in the kidney. In the case of double coincidences, where both isotopes are contributing, both cavities are clearly visible, and in the case of triples, while the second isotope is contributing, the cavity in the kidney is visible. The substrate image based on the relative efficiency of the two coincidences shows the recontacted image from the pure beta-emitting isotope; as a result, only the head of the mouse phantom is visible.

Conclusions: We modeled the small animal phantom and transferred the tracers to the head and kidney, then reconstructed the image. The methods for reconstructing images from double tracer data, which include doubles (2γ) and triples (2γ + γ_p).

[V-15] ASSESSMENT OF THE SELECTED MIRNAS IN PATIENTS WITH ACUTE ISCHEMIC STROKE TREATED BY COMBINED STROKE REPERFUSION THERAPY

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Introduction: MicroRNAs (miRNAs) are non-coding RNAs that play key roles in post-transcriptional gene regulation. Their involvement in neurovascular remodeling and ischemia-reperfusion injury has been studied in recent years. Circulating miRNA level changes during acute ischemic stroke (AIS) may also serve as potential biomarkers. Our preliminary findings identified three miRNAs—miR-9-3p, miR-9-5p, and miR-129-5p—with significant changes in AIS patients. This study aimed to characterize the temporal dynamics of these miRNAs in patients with AIS undergoing intravenous thrombolysis (IVT) and mechanical thrombectomy (MT) in correlation to various clinical factors.

Methods: A total of 47 patients with AIS who were treated with IVT and MT were included in the final analysis. Blood samples were collected on days 1 and 10 post-treatment. MiRNAs (miR-9-3p, miR-9-5p, and miR-129-5p) were isolated and quantified using quantitative reverse transcription polymerase chain reaction (qRT-PCR). The relative expression changes were calculated using the 2^-ΔΔCT method, normalizing the results to internal controls. Subsequently, the associations between serum miRNA expression and the patients' clinical parameters were analyzed.

Results: Serum levels of miR-9-3p at day 10 post-treatment were significantly higher in patients who received tenecteplase for IVT compared to those treated with alteplase. Additionally, changes in this miRNA were less pronounced in patients who reached successful reperfusion after MT. Patients who developed acute kidney injury during their hospital stay had lower baseline levels of miR-9-3p at the time of admission. Furthermore, patients with pre-existing atrial fibrillation consistently showed higher levels of miR-9-5p at both time points.

Conclusions: Monitoring changes in circulating miRNAs may provide valuable insights into the clinical course of AIS and potentially support the development of personalized treatment strategies.

[V-16] PROTON BEAM RANGE MONITORING IN PROTON RADIOTHERAPY WITH J-PET SYSTEM

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Introduction: Using protons in radiation therapy has several advantages over conventional methods. Finite beam range, steep dose gradient, and maximal energy deposition at the end of the path allow for reducing the dose delivered to the healthy tissue and to make the dose distribution in the tumor more conformal. To fully exploit those advantages, the beam range has to be known with high precision. Hence, the beam monitoring system is needed. This role can play the J-PET detector, which is a plastic-based PET detector that allows beam range monitoring and also possibly positronium studies.

Methods: During irradiation, protons induce nuclear reactions in the tissue. Produced radioisotopes emit positrons, which annihilate and emit gamma quanta. J-PET scanner can register those gammas and obtain the information about the annihilation points that have to be transformed to information about interaction points. Combining Bethe-Bloch theory, Moliere theory, and cross-sections for nuclear reactions, one can make an attempt to compute the rate of the radioisotope production and distribution of the signal. Those computations later can be confronted with data from the proton therapy center. Data in the proton therapy center were taken with the J-PET scanner. Cuboid PMMA phantoms were irradiated with a proton pencil beam of several energies, which resulted in different ranges of protons in the phantoms.

Results: Beam monitoring can be conducted during the irradiation (in-beam) and after (off-beam) due to different lifetimes of radioisotopes. Most abundant isotopes produced in the tissue are: 11C, 10C, 15O, 14O, 13N, 30P, and 38K. 10C and 14O have short lifetimes (≈ 20s and ≈ 120s, respectively), whereas 11C has the longest lifetime of about 20 min and thus can be useful in different stages of the monitoring process. Analysis of already collected data proved that the J-PET system is suitable for proton beam range monitoring. The PMMA phantom was irradiated with a proton pencil beam. We registered a signal during the irradiation and several minutes after the irradiation. Data was analyzed with the J-PET framework and reconstructed images with CASToR software. Results show that our system is capable of conducting both online and offline monitoring. 10C and 14O emit prompt gamma. This enables obtaining information on the positronium lifetime in the tissue. This would lead to gaining additional information about the tumor itself, such as the malignancy level and hypoxia level.

Conclusion: Our research presents a novel and important approach to proton beam therapy monitoring. Obtaining the spatial distribution of radioisotopes will help find the relation between annihilation points and the Bragg peak, which will increase the precision of the treatment. Positronium studies will have an impact on the quality of the treatment when, during the therapy, one can obtain additional information about the conditions of the tumor.

[V-17] MICROBIOLOGICAL AIR QUALITY IN A UNIVERSITY SPORTS HALL: IMPACT OF INITIAL OCCUPANCY ON FUNGAL AND BACTERIAL AEROSOLS

Juhi Mishra[1]

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Indoor air quality plays a pivotal role in occupant health, especially in frequently used enclosed spaces. This study evaluates microbiological air quality in a university table tennis hall with a focus on early-stage occupancy. Measurements were taken at two time points, early morning 7:30 (background) and 14:00 (after three groups of users) at different points of the hall, to assess the effect of initial physical activity on airborne microbial concentrations. Air sampling was conducted using an aspiration sampling method with the help of an Active impactor air sampler. Fungal aerosols were cultured on MEA (Malt Extract Agar) and Czapek-Dox (CzD) media, while mesophilic bacteria were assessed using TSA (Trypticasein Soy Lab-Agar) incubated at 37°C. Simultaneous measurements of CO_2 concentration, temperature, and relative humidity.

Results showed a notable increase in fungal spores, especially on MEA and CzD substrates, following just one group's activity. Mesophilic bacterial counts also rose significantly, indicating rapid aerosolization from human presence and movement. Concurrent increases in CO_2 concentration, along with slight temperature and humidity shifts, further supported the relationship between occupancy and deteriorating air quality. These findings underscore how usage can measurably impact indoor microbial load and associated physical and chemical conditions, stressing the importance of early-stage ventilation strategies.

[V-18] PANEL-BASED PROFILING OF SOMATIC VARIANTS IN SPORADIC MEDULLARY THYROID CARCINOMA

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Medullary thyroid carcinoma (MTC) is a rare and aggressive cancer, accounting for up to 5% of all thyroid cancer cases. MTC can occur as a hereditary disease associated with germline variants in the RET gene, but most patients have no family history of the disease (~75%). Data on the molecular landscape and clinical course of sporadic medullary thyroid carcinoma (sMTC) remain limited. The aim of this study was to characterize somatic variants and their frequencies in patients diagnosed with sMTC.

The cohort included 92 tumour samples from 86 patients diagnosed at the Maria Sklodowska-Curie National Research Institute of Oncology, Gliwice Branch, Poland. The inclusion criterion for the analysis was the absence of a germline RET mutation. RNA for the study was isolated from fresh-frozen tissue, FFPE blocks (formalin-fixed, paraffin-embedded), and core-needle or fine-needle aspiration biopsy material. We used the FusionPlex Comprehensive Thyroid and Lung Kit and FusionPlex Lung v2 Kit (Archer, USA) gene panels to evaluate somatic single-nucleotide variants (SNVs) and small insertions and deletions (indels). Next-generation sequencing (NGS) was performed on an Illumina platform. Bioinformatics analysis was conducted with Archer Analysis software (v7.2.1–7.4.3).

In the cohort, we detected 23 unique pathogenic or likely pathogenic somatic variants in the following genes: RET (n=13), HRAS (n=5), KRAS (n=3), and BRAF (n=2). The most common variants were RET c.2753T>C (p.Met918Thr) (24/92, 26%), which is associated with an adverse prognosis, and HRAS c.182A>G (p.Gln61Arg) (13/92, 14%). In this cohort, RET and RAS variants were mutually exclusive. BRAF gene variants were observed in cases with coexisting papillary thyroid carcinoma (PTC), as confirmed by histopathology. Furthermore, in 19/92 samples (21%), we did not identify any pathogenic or likely pathogenic variants; in 4 cases, it was not possible to assess the presence of somatic variants due to poor sample quality.

In ~75% of patients, we found pathogenic or likely pathogenic somatic variants in either RET or RAS – mutually exclusive at the gene level. Our findings underscore the role of these genes in the pathogenesis of sMTC. The lack of clinically relevant variants in part of the cohort indicates the need for further analysis.

[V-19] TRANSCRIPTOMIC PROFILING REVEALS DISTINCT MOLECULAR SIGNATURES IN REFRACTORY AND RELAPSED T-ALL

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Introduction: T-cell acute lymphoblastic leukemia (T-ALL) is an aggressive malignancy arising from thymocytes, the precursors of T-cells in the thymus. T-ALL represents ~ 15% of acute lymphoblastic leukemia (ALL) cases, the most common pediatric cancer. Current cure rates for pediatric T-ALL reach ~ 80%. The remaining 20% are patients who suffer from a resistant form of T-ALL (Refractory T-ALL) or leukemia recurrence (Relapsed T-ALL). Development of novel treatment strategies for refractory and relapsed T-ALL is an unmet need in contemporary pediatric oncology. In the literature, these patients are often grouped together as refractory and/or relapsed T-ALL (RR-T-ALL) due to their shared poor prognosis and similar clinical management challenges. These combined analyses may overlook important biological differences between refractory and relapsed T-ALL, masking distinct molecular and clinical features of each group. Given this common practice of combining the RR patients, we examined whether refractory and relapsed T-ALL represent a single biological entity or should be analyzed as separate subgroups.

Methods: mRNA-seq data from 88 diagnostic samples from pediatric T-ALL patients, including those with primary therapy resistance, leukemia recurrence or neither. RNA-seq reads were aligned to GRCh38 reference genome using STAR and summarized using featureCounts. Differentially expressed genes (DEGs) between individual groups, i.e. Refractory vs. nonRefractory and Relapsed vs. nonRelapsed, were identified with edgeR. The following analyses and visualizations were performed with RStudio utilizing appropriate packages. Gene set enrichment analysis (GSEA) was performed using: KEGG and MSigDB: – hallmark gene sets (H), and chosen gene sets related to stemness and chemoresistance from curated gene sets (C2) and ontology gene sets (C5).

Results: PCA based on RNA-seq data showed noticeable differences between groups, with refractory samples forming a distinct and homogeneous group, while relapsed cases being dispersed among non-relapsed ones, indicating higher transcriptional heterogeneity. We identified 2578 DEGs between Refractory vs. nonRefractory T-ALL, while only 224 DEGs between Relapsed vs. nonRelapsed groups. GSEA revealed processes potentially associated with worse prognosis of both analysed groups, showing minimal overlap and opposite directions of enrichment.

Conclusions: Our results demonstrate that refractory and relapsed T-ALL differ substantially at the transcriptional and pathway level, challenging the popular practice of combining them as one group (RR T-ALL). These findings highlight the molecular heterogeneity of pediatric T-ALL and indicate that refractory and relapsed cases may arise from distinct biological mechanisms and should be considered separately in both research and clinical contexts.

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[V-20] MIR-153-3P AS AN INDICATOR OF CIGARETTE SMOKING IN REPERFUSION-TREATED ISCHAEMIC STROKE

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Introduction: The global burden of nicotine dependence and stroke remains substantial, with smoking markedly increasing stroke risk via endothelial dysfunction, inflammation, oxidative stress, and prothrombotic pathways. MicroRNAs (miRNAs) are short, single-stranded regulators of post-transcriptional gene expression. Several miRNAs linked to smoking-related vascular injury and cerebrovascular diseases may help distinguish nicotine exposure-driven stroke biology and therapeutic responsiveness when compared to non-smokers.

Methods: Paired blood samples were obtained from 68 patients (18 smokers, 50 non-smokers) on day 1 (acute phase) and day 10 following reperfusion therapy. Functional status was assessed at both time points using the modified Rankin Scale (mRS). Serum-derived exosomal miRNAs were isolated and quantified by RT-qPCR, with expression normalized to miR-320a. Change was expressed as a fold change (day 10 vs day 1). To provide external context, we analysed two independent NCBI GEO datasets: GSE69960 (healthy smokers vs non-smokers) and GSE55937 (stroke vs non-stroke).

Results: Our findings have shown a significant difference in miR-153-3p levels (p= 0,027; Mann–Whitney U test) between the smoking and non-smoking population, with a Hodges-Lehmann median difference of -0.53, indicating lower levels in non-smokers. In silico analysis of NCBI's GSE69960 dataset shows that, while miR-153-3p is associated and slightly downregulated with smoking exposure, it does not reach statistical significance (p = 0.132). Good functional outcome (mRS 0-2) on the tenth day was more frequent in smokers than non-smokers (72.22% vs 46.30%). Our analysis has shown further differences between groups – smokers were younger (65.89 ± 11.01 years) than non-smokers (71.56 ± 13.71 years), with more frequently observed minor ischaemic changes (44.44% vs 28.00%). On the contrary, atrial fibrillation was more common in non-smokers (42.00% vs 33.33%).

Conclusions: The observed elevation of miR-153-3p in smoking stroke patients shows its potential as a discriminator of nicotine exposure. However, the higher short-term functional status recovery, age, and comorbidities differences in groups are concordant with the "smoker's paradox", underscoring the need for further multivariable analysis separating exposure effects from confounding interactions.

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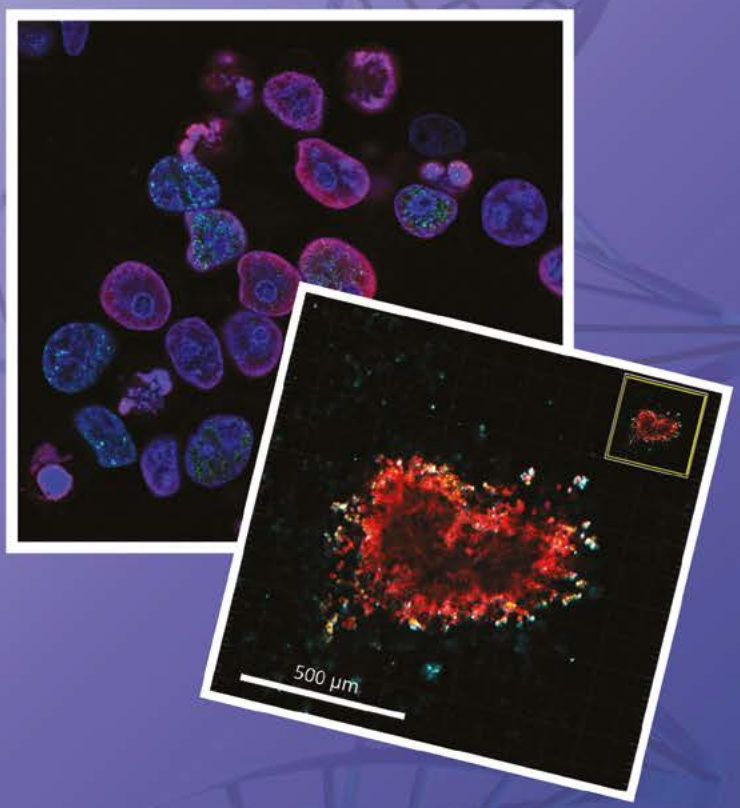

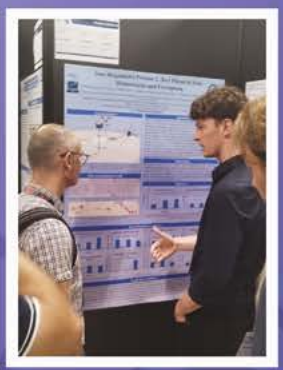

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