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POSTERS – RESEARCH

Bioenergetics

P-01-001
Infrared light neuroprotection: transcriptomic changes and mitochondrial potassium channel activity

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According to the World Health Organization, stroke is the second leading cause of death and the third leading cause of disability worldwide. Numerous research articles and few clinical trials have described protective effects of photobiomodulation (PBM) after stroke. Inconsistent effectiveness may result from variations in treatment protocols, such as differences in light wavelength, power, and timing. A complete understanding of the mechanisms underlying PBM can help optimize study designs. One proposed mechanism involves enhancing the performance of the mitochondrial enzyme cytochrome c oxidase (COX) - a main cellular chromatophore. COX has four redox active metal centers: the binuclear CuA, CuB, heme a and heme a3, all of which absorb red and near infrared (NIR) light with maxima at 620, 680, 760 and 825 nm. Additionally, neuroprotective and cardioprotective effects have been described in some studies as a result of mitochondrial potassium channels activity. Here, we demonstrate increased activity of mitochondrial large-conductance calcium-activated potassium (mitoBK$_{Ca}$) channels following NIR light illumination, using the patch-clamp technique on mitoplasts isolated from rat hippocampi. Furthermore, we compare the preconditioning effects of mitoBK$_{Ca}$ channel activators and PBM prior to an N-Methyl-D-aspartic acid (NMDA) insult on rat hippocampal slices. We also present transcriptomic changes induced by the same NIR illumination in astrocytoma cells with either naturally expressed or CRISPR-Cas9-deleted mitoBK$_{Ca}$ channels. Our results indicate that incubation of hippocampal slices with a mitoBK$_{Ca}$ channel activator provides greater neuroprotection than NIR illumination, however the latter is expected to offer a higher biosafety profile. Additionally, illumination rapidly and durably activates mitoBK$_{Ca}$ channels and alters the expression levels of thousands of genes. This research was funded by the National Science Center, grant no. 2019/34/A/NZ1/00352.

P-01-002
The redox state of cysteines in Voltage-Dependent Anion Channels (VDACs): roles in biogenesis, redox homeostasis and oligomer assembly

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The Voltage-Dependent Anion-selective Channel (VDAC) is the main pore-forming family in the outer mitochondrial membrane (OMM), where it guarantees the passage of various metabolites. In mammals, three isoforms – VDAC1, VDAC2, and VDAC3 – exhibit similarity in sequences and β-barrel structures. Despite numerous reports on the redox modifications of proteins in the mitochondrial matrix, intermembrane space (IMS), and inner membrane, less is known about the redox modifications of OMM proteins. Intriguingly, VDACs' cysteine content and redox state are highly variable among the isoforms and probably correlate with distinct biological functions. Cysteines in VDAC1 appear non-essential for the pore-forming activity, while those in VDAC2 enhance its association with OMM. In contrast, VDAC3 has been proposed as a main player in redox homeostasis, counteracting oxidative stress through cysteine residues exposed to the IMS[1]. Recently, our group determined disulfide bonds in VDAC2 using mass spectrometry[2], underscoring the necessity to clarify the biological roles of these redox modifications. Hence, we aimed to investigate: 1) The relationship between cysteine residues and the biogenesis of VDACs; 2) ROS-mediated disulfide bonds formation of VDACs. Here, we report that VDAC3's cysteines slow down its mitochondrial import kinetics and complex assembly. Secondly, we show for the first time that VDAC isoforms respond differently to oxidative stimuli *in vitro*. VDAC2 and VDAC3 form oligomers under non-reducing conditions, but, surprisingly, only VDAC2 increases oligomer formation upon H_2O_2 exposure through disulfide bonds. Together, these results suggest that variations in the oxidative state of cysteine residues among VDAC isoforms could contribute to their distinct biophysical properties, influencing VDACs' biogenesis, assembly and their role in redox homeostasis. [1] Reina S. et al. (2022) Redox Biol. 51:102264. [2] Pittalà MGG et al. (2024) J Am Soc Mass Spectrom. 35(7):1422-1433.

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P-01-003
Glutamine supplementation: a new promising treatment for LIG3-dependent chronic intestinal pseudo-obstruction (CIPO)

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Chronic intestinal pseudo-obstruction (CIPO) is a rare and severe gastrointestinal motility disorder, that alters peristalsis, mimicking a mechanical obstruction. Whole exon sequencing on 7 CIPO patients from 3 families led to the identification of a syndromic form due to biallelic mutations in *LIG3*, a DNA ligase essential for mtDNA maintenance, thus for cell energy production. As biochemical analysis on patients' fibroblasts displayed mitochondrial dysfunction, we explored the possible glutamine (L-Gln) anaplerosis, and the possibility to establish a new treatment for these patients. We obtained fibroblasts from skin biopsies to conduct *in vitro* assays. After RNA-seq analysis revealed a significant dysregulation of genes involved in cellular bioenergetics and mitochondrial metabolism in mutant cells, we confirmed a severe energy impairment and morphological and functional alterations in mitochondria by different functional studies and cellular imaging. In fact, we observed a fragmented mitochondrial network, together with an impaired mitophagy and lower Ca^{2+} levels in the mitochondrial matrix. Western blot revealed autophagy activation, a dysfunction in the mitochondrial network dynamics, and lack of integrity in mitochondria-associated membranes (MAMs). L-Gln supplementation rescued growth and bioenergetic impairments *in vitro*, in terms of ATP content, leaving unvaried the transcriptional pattern. These *in vitro* effects of L-Gln led us to parenterally administer a dipeptide containing L-Gln to 3 siblings carrying biallelic *LIG3* mutations that resulted in a reduction of the severity of gastrointestinal symptoms after 8 months. After 2 years of supplementation, patients are gradually resuming oral feeding and improving body weight. We have now identified 2 new variants of LIG3 and performed *in vitro* analyses that display similar results, supporting the use of L-Gln as a potential treatment option for the complex syndrome caused by mutations in *LIG3*. Previously published in: Diquigiovanni C et al. (2024) Gastroenterology 5085:1-16.

P-01-004
Impact of co-enzyme Q10 deficiency on mitochondrial super complex biogenesis in the neuroblastoma cell line SH-SY5Y

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Genetic mutations in the nuclear DNA or mitochondrial DNA that codes for mitochondrial proteins or proteins involved in mitochondrial activity leads to dysfunction of the oxidative phosphorylation, causing mitochondrial diseases. The nervous system heavily relies on ATP produced from oxidative phosphorylation and is steadily involved in patients with mitochondrial disorders like Leighs syndrome, Alpers syndrome, cognitive degeneration, etc. The functions carried out by the mitochondria are dependent on five intramembrane complexes and two electron carriers (cytochrome c and COQ10), which are assisted by super complexes. Usually, therapeutic intervention involves COQ10 supplementation. COQ10, also called ubiquinone, is found in all cell membranes and is synthesised in almost all mammalian cells by mitochondrial proteins, although the head and tail precursors are synthesised in the cytosol. The redox ability of benzoquinone provides COQ10 valuable functions inside the mitochondria, like the transport of electron. Additionally, COQ10 provides the electrochemical proton gradient that permits ATP synthesis and transports electrons from complexes I and II to complex III. Also, COQ10 is a structural constituent of both Complexes I and III and influences complex I stability. Hence, COQ10 deficiency can manifest in various ways. In this study the neuroblastoma cell line SH-SY5Y was cultured and COQ10 deficiency was induced using treatment with para-aminobenzoic acid (PABA) an inhibitor of COQ2 (an enzyme involved in COQ10 synthesis). Here we demonstrate that COQ10 deficiency results in impairment of mitochondrial function and Complex I deficiency without loss of cellular viability.

P-01-005
LAT1-mediated amino acid transport supports thermogenesis of human brown adipocytes

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Thermogenically active adipocytes utilize high amounts of metabolic substrates, such as glucose, fatty acids, and amino acids (AAs), which provide sufficient energy for heat generation via UCP1-mediated proton leak in the inner membrane of mitochondria. However, the mechanism of how brown adipocytes can uptake distinct types of AAs remains unclear. Our preliminary data showed that the consumption of branched-chain and other AAs as well as the expression of L-amino acid transporter (LAT) 1 (encoded by SLC7A5) and its heterodimer protein 4F2hc (encoded by SLC3A2) was upregulated during adrenergic stimulation [Previously published in: Arianti R et al. (2024) Sci Rep 14, 28272]. Therefore, we aimed to investigate the role of LAT1 in the thermogenesis of human brown adipocytes. Stromal vascular fraction was isolated from cervical adipose tissue biopsies and differentiated into brown adipocytes for 14 days. The expression of LAT1 was silenced by siRNA-mediated

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interference and then dibutyryl-cAMP was administered to mimic *in vivo* thermogenesis. We found that LAT1 deficiency in adrenergic stimulated matured adipocytes inhibited AA influx and led to reduced proton leak respiration which is associated with UCP1-dependent heat generation. Dibutyryl-cAMP-stimulated elevation of Etomoxir-resistant respiration, which correlates with glucose and amino acid utilization, was also abrogated in LAT1 knock-down adipocytes. LAT1 silencing also resulted in decreased expression of thermogenic markers, such as UCP1 and PPARGC1a during adrenergic stimulation, as well as genes which were involved in various biological pathways, such as the TGF-β, MAPK, or PI3K-Akt signaling. Our data suggest that LAT1 regulates human brown adipocyte thermogenesis by mediating the uptake of several AAs to support the high metabolic activity and by controlling the transcriptional program of thermogenesis-related genes. *The authors marked with an asterisk equally contributed to the work.

P-01-006
Lipoamide dehydrogenase (Lpd), a common E3 subunit of PDH and OGDH, links central carbon catabolism and membrane electron transfer chain in *Escherichia coli*

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Energy generation in aerobically growing *Escherichia coli* relies on glycolysis and TCA cycle connected to the membrane electron transfer chain (ETC). Pyruvate dehydrogenase (PDH) and 2-oxoglutarate dehydrogenase (OGDH) are key enzymes located at the crossroads of these pathways and controlling carbon flow. PDH and OGDH are two similar multienzyme complexes, both consisting of three subunits, E1, E2, and E3. E1 and E2 are homologous proteins, respectively, while E3 subunit (Lpd) is shared in both PDH and OGDH complexes. They not only regulate specific pathways but also influence overall energy metabolism by competing for the common coenzyme, HS-CoA [previously published in: Shimada T et al. (2021) FEBS Lett 595, 2047–2056]. In this study, we found that unlike other TCA cycle enzyme-deficient mutants, OGDH-deficient mutants display a complete growth cessation after glucose depletion in a minimal medium containing limited glucose and excess amino acids. Thus, OGDH was considered to have some unidentified role other than the well-known enzymatic activity. To explore this, based on OGDH's characteristic in generating reducing power NADH, we indirectly monitored reducing power generation in OGDH-deficient mutant by measuring the dissolved oxygen in the medium, which can index the oxygen consumption rate. The results showed that all three subunit-deficient mutants of OGDH abolished oxygen consumption after glucose depletion. Notably, the Lpd-deficient mutant exhibited a similar impaired oxygen consumption as the NADH dehydrogenases-deficient mutant, which are responsible for the electron flow to oxygen. This similarity suggests that Lpd, the shared E3 subunit of PDH and OGDH, has an uncharacterized function connecting with ETC. These findings propose that PDH and OGDH may link central carbon metabolism with the electron transfer chain, potentially serving as a critical regulator of cellular respiration beyond their traditional role in central carbon catabolism.

LB-R-01-01
Natural and synthetic iminosugars reduce colorectal cancer cell viability and alter bioenergetic metabolism via specific mechanisms

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Iminosugars are sugar mimetics where a nitrogen atom replaces the ring oxygen, conferring unique biological properties, including inhibition of cellular glycosidases and associated anti-hyperglycemic effects. Natural iminosugars are produced by plants and microorganisms as defense agent, and are found in foods like mulberries, buckwheat, rice, and fermented vegetables. Furthermore, synthetic analogues have been developed to enhance stability and target specificity. Despite the potential of glycosylation pathways as oncological drug targets, iminosugars remain underexplored in cancer research, partly due to extraction challenges and complex synthesis. In this study, we evaluated the potential antitumor activity of several natural and synthetic iminosugars, including 1-deoxynojirimycin (DNJ), N-nonyl-deoxynojirimycin (NN-DNJ), miglitol (MGLT), 2,3,4,6-tetra-O-benzyl-(+)-1-deoxynojirimycin (TBN-DNJ), fagomine (FGM), and isofagomine (IFGM), using *in vitro* screening tools requiring minimal material. Caco-2 and HCT-116 cells were used as models of early and late-stage colorectal cancer, respectively. Exposure to iminosugars always reduced cell viability, but cell responses varied depending on the compound, glucose concentration in the medium, exposure time, and culture model (2D vs 3D). In Caco-2 monolayers (but not HCT-116), NN-DNJ, FGM, and TBN-DNJ decreased lipid storage and affected intracellular glycogen distribution, while in HCT-116 spheroids, 25–50 μM iminosugar treatment significantly reduced spare mitochondrial capacity and increased lactate output. Substrate oxidation profiling indicated enhanced lipid oxidation after exposure to MGLT, TBN-DNJ, and IFGM. Our results suggest specific mechanisms of natural and synthetic iminosugars in reducing colorectal cancer cells viability. Acknowledgements: We thank the FWO-Flanders (FWO-WEAVE G000123N and FWO-SBO S001422N), Ghent University (BOF.BAS.2020.0035.01) for equipment funding.

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Biophysics

P-02-001
Role of proteins SP-B and SP-C in the formation of pulmonary surfactant interfacial 3-dimensional structures

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Pulmonary surfactant is a lipoprotein complex that facilitates breathing mechanics by coating the respiratory surface at alveoli. The special lipid and protein composition of surfactant provides the minimum surface tensions needed to stabilize open alveoli at the end of expiration. For this purpose, during compression (i.e. expiration) the interface needs to be enriched in the disaturated species DPPC, that can withstand tight packing, while unsaturated lipids need to be excluded and preserved in lipid reservoirs to be ready to refill the interface again in the subsequent expansion cycle (during inspiration). This dynamic behavior is acquired thanks to the action of the hydrophobic surfactant-associated proteins SP-B and SP-C, which are deeply embedded into surfactant lipids to supposedly create membrane–membrane contacts to ensure the connection between the surface-active interfacial film and membrane-based reservoirs. In this work, the mechanism of action of SP-B and SP-C has been further investigated using neutron reflectometry as a tool to analyze protein-dependent reservoir formation. For this purpose, an air–liquid interface was created in a Langmuir trough with a model lipid system mimicking pulmonary surfactant composition in the absence or presence of SP-B and/or SP-C, and compressed up to different stages, and the reflectivity profiles have been analyzed in order to detect the formation of surfactant reservoirs underneath the interface as a function of surface pressure. Results show the crucial role of SP-B in reservoir formation once the equilibrium surface pressure is reached. The effect of SP-B is maximized when SP-C is present, despite not being SP-C by its own to promote reservoir formation. These results highlight the need to integrate both proteins into clinical formulations useful to treat pathologies requiring surfactant replacement therapy.

P-02-002
Molecular and functional analysis of LSR: new therapeutic target in lipid homeostasis and Alzheimer's disease

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The LSR (lipolysis stimulated lipoprotein receptor) is a membrane receptor involved in peripheral and central lipid homeostasis. The *lsr* gene encodes 3 subunit types α, α′ and β resulting from alternative splicing with similar sequences due to their common mRNA [Previously published in: Stenger C. et al. (2012) Chemical Biology (Ekinci, D., ed) : pp : 267-292]. LSR deficiency leads to disturbances in lipid homeostasis associated with several diseases, such as neurodegenerative diseases, as Alzheimer's disease, making it a potential new therapeutic target, hence the interest in studying the mechanism of action and the regulation of this receptor. The LSR recognizes lipoprotein through specific interaction with its surface protein called apoprotein E (ApoE). The *ApoE* gene has three major alleles called ε2, ε3 and ε4, which respectively code for the ApoE2, ApoE3 and ApoE4 isoforms, which differ by a single amino acid involving cysteine-arginine interchanges at position 112 and 158. Particularly the e4 allele, is genetically linked to the incidence of Alzheimer's disease [Previously published in: Belloy. et al. (2019) *Neuron, 101(5), 820-838*]. Pilot studies suggest that LSR could act as a receptor to internalize ApoE and also participate in the clearance of beta amyloid (Aβ) peptide in the brain. The LSR is poorly characterized from a molecular and biochemical point of view, by virtue of LSR's novelty. Molecular characterization of the LSR/ApoE interactions has been done using interdisciplinary studies combining protein purification, protein engineering, and biophysical approaches like surface plasma resonance. In parallel, *in vitro* and *in cellulo* assays have been set up for the identification of molecules that are either recognized by the receptor or modulate its expression. The results will be presented and discussed according to what was described in the literature. *The authors marked with an asterisk equally contributed to the work.

P-02-003
Chaperones and their role in metabolostasis

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Amyloids are protein fibrils known for causing neurodegenerative diseases like Alzheimer's. The repertoire of known intracellular pathogenic fibrillations was recently expanded with the discovery of metabolite self-assemblies, which may play a role in metabolic disorders such as phenylketonuria and Gaucher's disease. These metabolite assemblies share structural and chemical similarities with protein amyloids, including their ability to cross-seed, meaning induction of protein amyloid formation. Chaperones, which prevent pathological protein aggregation, also inhibit metabolite self-assembly, suggesting a role in maintaining cellular metabolostasis. To cover a broad range of metabolite classes, my research focuses on understanding the structural properties and assembly kinetics of three metabolites – adenine, phenylalanine, and glucosylceramide – as well as their interaction with molecular chaperones. During the past months I formulated novel kinetic assembly pathways for all three metabolites, as well as identifying the first chaperone–metabolite amyloid interaction. Currently I am analyzing the distinct fibrillar structures, as well as chaperone interactions by X-ray diffraction and mass spectrometry towards mapping the interaction pathways between chaperones and metabolite amyloids, which will provide new insights for possible therapeutic approaches in metabolic disorders.

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P-02-004
DNAJB6b prevents tau aggregation both kinetically and by increasing tau solubility

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The self-assembly and fibril formation of tau is closely associated with Alzheimer's disease and other neurodegenerative diseases collectively known as tauopathies. Today there is limited treatment interventions for these maladies and no definitive treatment to cure the diseases exist. One of the body's own defence mechanisms against protein misfolding diseases is molecular chaperones. Among the molecular chaperones expressed in the central nervous system, DNAJB6b has been shown to be highly effective at slowing down the aggregation of the amyloid beta peptides, another set of aggregation prone peptides also closely associated with and involved in Alzheimer's disease. In this study, we explore the lesser know effect of DNAJ6b on the amyloidogenic core of tau. Through monomer dependent concentration kinetics, we show the remarkable nanomolar range potency of DNAJB6b and through mathematical fitting of the obtained kinetic traces we elucidate the probable mechanistic origin of the inhibition and its effect on the microscopic steps of tau fibril assembly. Futhermore, we investigate the effect of DNAJB6b on the apparent solubility of tau and find that it is increased. Our findings collectively demonstrate the promising properties of DNAJB6b and identify the key underlying molecular mechanisms of its inhibition of tau fibril formation. The delayed assembly of amyloid fibrils and the apparent increase in the solubility of tau in the presence of DNAJB6b underscore the therapeutic potential of this class of chaperones and their capacity in the prevention of misfolding diseases. By decelerating fibril formation and effectively raising the apparent solubility concentration of monomers, these chaperones could aid and grant the proteolytic machinery of the cell additional time to degrade and recycle aggregation prone proteins.

P-02-005
Enhancing β-sheet breaker peptide delivery via liposome nanocarriers

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Toxic protein fibrils, such as amyloid-beta and alpha-synuclein, play a critical role in the pathological mechanisms of Alzheimer's and Parkinson's diseases. Developing peptide-based therapeutics to inhibit these fibrils offers a promising approach for treatment, leveraging the advantages of peptides such as high specificity and ease of production. However, their limited ability to cross the blood–brain barrier (BBB), and susceptibility to enzymatic degradation present significant challenges for clinical applications. To overcome these limitations, this study aims to enhance the bioavailability and BBB permeability of β-sheet breaker peptides by encapsulating them into liposome nanocarriers. In this context, experimentally validated peptides with proven inhibitory effects on toxic protein fibrils were identified from the literature, and their binding interactions with target proteins were evaluated through molecular docking and molecular dynamics (MD) simulations. PEGylated liposomal membranes with varying cholesterol ratios and lipid compositions were prepared, and their structural and dynamic properties were analyzed in the absence and presence of the top-performing peptides based on binding affinity and stability. Cholesterol was found to increase membrane compactness and rigidity, limiting PEG chain insertion, and prolonging the lipid self-assembly process. Regardless of cholesterol concentration, peptides were observed to reside in PEG corona and primarily interact with lipid headgroups. Based on the modeling insights, the shortlisted peptide sequences are further evaluated experimentally for encapsulation efficiency in liposomes under varying conditions.

P-02-006
Probing the BRC repeats-RAD51 interaction by integrating computational and experimental biophysics

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The BRCA2/RAD51 interaction is a critical step of homologous recombination, a key mechanism for maintaining genome integrity. BRCA2 recruits and transports RAD51 to DNA damage sites through eight short BRC repeats. Nowadays, only the interaction of RAD51 with BRC4, the fourth repeat, has been structurally characterized, leaving the role of the other BRC repeats unclear. To shed light on the close relationship between these two proteins we combined experimental and computational approaches. We first used AlphaFold simulations to predict peptide binding modes and rank their affinities. To further rationalize these insights, we performed residue scanning analyses and molecular dynamics (MD) simulations. We then isolated a human monomeric RAD51 form to study the interaction of individual BRC repeat peptides using orthogonal biophysical experiments. The calculated affinities were correlated with the ability of isolated BRC repeats to interact with RAD51 WT, revealing that only the highest-affinity peptides could disassemble RAD51 fibrils. These findings guided the design of RAD51 complexes with the strongest binders, either alone or within BRCA2 truncations containing multiple peptides, which were subsequently used for structural investigations. Our results, obtained by integrating AlphaFold predictions with XL-MS, NMR and SAXS data, unveil not only the crucial role of the interaction of BRC repeats with the N-terminal domains of RAD51, but also on the dynamic and multifaceted behavior of the regions linking the first four BRC repeats. This suggests that these peptides may cooperate to disassemble RAD51 fibrils, thus allowing RAD51 translocation into the nucleus. As a future perspective, we would like to integrate information gathered through other structural techniques with Cryo-EM to provide novel insights into the interaction of these complexes with DNA.

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P-02-007
Unexpected bottlenecks in nuclear export: a study of kinetics and diffusion

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Despite the critical role of nuclear export, there is a lack of systematic studies that analyze its kinetics alongside molecular diffusion in living human cells. Here, using exogenous fluorescent probes of varying sizes and chemical structures (including mRNAs, short oligonucleotides, small dextrans, and proteins), we show that the characteristic time for nuclear export ranges from 61 to 389 minutes. Additionally, we reveal that short poly(T) oligodeoxynucleotides, which are not naturally present in the cell, exhibit a nuclear retention time more than four times longer than poly(A) strands of the same length. We demonstrate that neither hydrodynamic radius nor diffusion coefficients have a significant impact on export times. Instead, the availability of nuclear pore complexes (NPCs) and their interactions play a crucial role. We determine that fewer than 0.2% of NPCs are effectively accessible at any given moment, which accounts for the orders of magnitude slower nuclear export rates compared to those predicted from measured diffusion coefficients in the nucleus of living cells. Acknowledgement: This work was co-financed by the Polish Science Fund, Virtual Research Institute, within the grant WIB-1/2020-O11; WIB_HERO.

P-02-008
Investigating the ECM stiffness effect on talin-1 adhesion organisation and fibronectin deposition

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The stiffness of the ECM (extracellular matrix) influences numerous physiological processes, including cell migration, differentiation and proliferation. Cell-ECM adhesions, such as nascent, focal and fibrillar adhesions, facilitate the mechanosensing process. Those protein complexes link extracellular ligands to the cytoskeleton via transmembrane integrins and adapter proteins, such as talin. This study investigated how ECM stiffness influences talin-1 organization in fibroblasts using polyacrylamide substrates to mimic different tissue elasticities. We showed that the distribution of talin-1 in cells strongly depends on ECM elasticity. Additionally, we analysed the correlation between talin-1 and tensin-3 localisation. Interestingly, this correlation was independent of ECM stiffness, though we detected differences between glass and polyacrylamide substrates. Lastly, we investigated fibronectin secretion and observed enhanced colocalization between talin-1 and fibronectin for softer substrates. These findings contribute to a deeper understanding of how ECM mechanics regulate cell adhesion and extracellular matrix remodelling.

P-02-009
Determination of mechanobiological properties of *Saccharomyces cerevisiae* using optical tweezers

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Optical tweezers or traps are systems used to apply piconewton-sized forces and make precise measurements on a scale of roughly one micron. The trapping and manipulation of micrometers and submicrometer-sized particles with optical tweezers are achieved by optical forces generated by a focused laser beam using a high numerical aperture objective. Optical tweezers are used in many biological, biotechnological, nanotechnological, material science, and physical research. When used in living organisms, it offers various advantages such as non-invasive manipulation, high sensitivity, and control. This technique has been used as a powerful tool to study mechanobiological approaches, especially in yeast cells. *Saccharomyces cerevisiae (S. cerevisiae)* is preferred in genetic and molecular biology studies because it has a mechanically strong cell wall responsible for various cellular functions, proliferates rapidly, and is generally regarded as safe (GRAS). In this study, the methodology is presented to investigate the mechanobiological properties of *S. cerevisiae* using an optical tweezer system including a position sensing detector. The calibration studies of the system are carried out for yeast cells. Yeast cells are trapped and manipulated using the calibrated system. Using the obtained data, the stiffness coefficient, which is a descriptor of mechanical properties, are obtained. In addition, experimental processes are evaluated using COMSOL Multiphysics®.

P-02-010
DDfit web server: an improved processing scheme for diffusion NMR data

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Pulsed-field gradient nuclear magnetic resonance (PFG NMR) is widely used for characterizing biological macromolecules and

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their assemblies. However, when measuring samples with low concentration, poor signal-to-noise ratio and baseline distortions make accurate determination of diffusion parameters challenging. Here, we present a new data processing scheme for 1D diffusion NMR experiments which significantly improves the accuracy of the measurements. Briefly, the algorithm can be described as follows. First, the user defines the boundaries of the spectral region of interest, which are used to perform linear baseline correction. Next, the spectra are integrated to yield approximate (zero-order) signal intensities, $I_0(G_k)$. Then the model spectrum is constructed as a weighted average of all spectra in the series, with weights set to $I_0(G_k)$. Fitting the individual spectra to the model produces refined integrals, $I(G_k)$, which can be subsequently analyzed using an appropriate formula, such as Stejskal-Tanner equation. This computational scheme is rooted in optimal filter theory and maximizes the signal-to-noise ratio of the model spectrum. The algorithm has been implemented in a web server named DDfit (Diffusion Data Fit; https://ddfit.bio-nmr.spbu.ru). When using this server, the experimentalist uploads spectral data files and types in several acquisition and processing parameters. The server then finds $I(G_k)$ as described above, fits the results using a relevant formula and reports the determined diffusion coefficient. The calculations take on the order of several seconds. The DDfit server has been tested on simulated spectral data, as well as our own experimental data from hen egg-white lysozyme. The new processing scheme demonstrates improved accuracy and precision compared to standard methods implemented in TopSpin and MestreNova platforms. The work was supported by SPbU grant 127400313.

P-02-011
Amyloid aggregation of plant cysteine proteases

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Plant cysteine proteases are widely used for biotechnological applications. Structural transitions and stability of these proteins are mostly studied in the context of these applicative processes. Their structural features, including separate domains with characteristic α-helix and β-sheet fold and disulfide bonds, have remarkable effects on the overall stability and kinetical inertness of these enzymes in proteolysis and denaturation. This inspired us to use papain and ficin as model systems to test the hypothesis that all proteins can undergo amyloid aggregation upon controlled destabilization of their native state. Besides common structural characteristics of our model systems, papaya fruit and fig latex rich in ficins have different protease contents. While papain is expressed as a single isoform, ficins are a pallet of different isoforms with similar structural characteristics. This difference was exploited to analyse the propensity for ordered aggregation of two model systems with and without sequence polymorphisms. We used a single isoform of papain, as well as a mixture of ficin isoforms to screen for conditions suitable for overcoming kinetic barriers for amyloid aggregation. In this study different conditions were varied: concentrations of chaotropes, pH, temperature, ethanol concentration, and DTT presence. Aggregation was monitored by Thioflavin T fluorescence (ThT), Fourier transform infrared spectroscopy (FTIR) and atomic force microscopy (AFM). Our results show that both systems – papain and mixture of ficin isoforms tend to form fibrilar aggregates with significant ThT binding, characteristic for amyloids, and high content of aggregation-specific β-sheet after destabilization of their native folds in the presence of urea. While papain showed a uniform aggregation pattern, ficin aggregation seemed to be batch-dependent which can be in correlation with different expression patterns of ficin isoforms during fruit ripening.

P-02-012
Photoreactive properties of melanin obtained from human induced pluripotent stem cell-derived melanocytes

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Melanin serves as a natural sunscreen, protecting pigmented cells from solar radiation; however, under specific conditions, it may facilitate photoinduced oxidative damage. A critical limitation in studying this phenomenon is the availability of unmodified melanin devoid of photooxidative alterations. Melanin isolated from melanocytes derived from human induced pluripotent stem cells (hiPSC-Mel) has been proposed as a promising model with minimal photochemical degradation. Although different studies have demonstrated the feasibility of the above-mentioned technique to obtain melanin-producing cells, no thorough analysis of physicochemical properties of the pigment was performed. In this study, we systematically evaluated key physicochemical parameters, including the aerobic photoreactivity of hiPSC-Mel, and compared these properties against melanin obtained from bovine retinal pigment epithelium (bRPE) and phototype V (PT-V) hair. A wide-range of techniques including electron paramagnetic resonance (EPR) spectroscopy, dynamic light scattering (DLS), UV-Vis absorption spectroscopy, and high-performance liquid chromatography (HPLC) of melanin degradation products was used in this study. The ability of the melanin samples to generate reactive oxygen species upon photoactivation was assessed using EPR oximetry, EPR spin-trapping, and time-resolved singlet oxygen phosphorescence measurements. Our findings indicate that while hiPSC-Mel exhibits physicochemical properties characteristic of eumelanin, it also contains a fraction of pheomelanin with benzothiazine subunits. Notably, hiPSC-Mel demonstrated significantly lower photoreactivity compared to bRPE and PT-V melanin, underscoring its potential as a robust and physiologically relevant model for photoprotection studies. Founded by National Science Center of Poland: OPUS-2021/43/B/ST4/03255. *The authors marked with an asterisk equally contributed to the work.

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P-02-013
The increased ice-binding affinity of antifreeze protein is attributable to the presence of its oligomeric forms

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One of the key strategies that organisms use to survive in harsh environments is the production of antifreeze proteins, which can bind to ice (IBPs). By binding to ice crystals and thereby inhibiting their growth, IBPs assist organisms in resisting the effects of freezing. Despite the scientific community's significant interest in IBPs, the molecular basis of how these proteins recognize and bind ice remains to be fully elucidated. The present study hypothesizes that the activity of these natural proteins is equivalent to the area of the ice binding site. The object of study is a small protein of 12.5 kDA from *Rhagium mordax* (*Rm*AFP). Its crystal structure is characterised by a β-solenoid fold, which is accompanied by a broad, flat ice-binding surface. A notable structural characteristic of *Rm*AFP is that the β-layer of the ice interaction surface contains a single motif comprising Thr residues, thereby hindering the formation of the ice-like water structure necessary for binding. The data demonstrate that the oligomerisation of *Rm*AFP enhances the abundance of the Thr-rich motif, thereby facilitating the structuring of water into ice. We demonstrated by mass spectrometry the presence of oligomeric forms of *Rm*AFP in solution. Through modelling, we proposed a series of potential structures for *Rm*AFP oligomers, which possess a comparatively flat surface area deemed essential for ice binding. We also proposed a model for the binding of oligomeric forms of this protein to the ice surface. Our obtained results indicate that a molecule containing several *Rm*AFP domains in an oligomeric complex acquires an increased affinity for ice binding. The modeling of structures suggests the existence of such forms *Rm*AFP in solution, characterized by a sufficiently flat surface containing the amino acids necessary for binding to ice crystals. This work was supported by the Russian state-funded project for ICBFM SB RAS (No FWGN-2025-0020).

P-02-014
Helical conformation of a repeat expansion in a transcription factor disordered domain is key to preserving its activity

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Intrinsically disordered regions (IDRs) in proteins play a key role in regulating their functions, for example through the formation of biomolecular condensates, as is the case for transcription factors [previously published in: Basu S et al. (2023) Nat Struct Mol Biol 30, 1958-1969]. While mutations in globular (i.e. DNA binding) domains that affect transcription factor function are well characterized, increasing attention is being given to mutations within IDRs that alter their ability to engage in intermolecular interactions within transcriptional condensates – both homotypic (between the transcription factor's own IDRs) and heterotypic (between the transcription factor's IDRs and other components of the transcriptional machinery). However, the impact of disease-related mutations in neither functional regions nor motifs directly involved in condensation remains poorly understood. Here we study the effect of a repeat expansion, or tract, in the IDR of a transcription factor which causes a neuromuscular disease. The tract can adopt a helical structure, which is dependent on the repeat length and the residues flanking it at the N-terminus [previously published in: Escobedo A et al. (2022) Nat Commun 13, 7073]. Expansion of the tract above the disease threshold causes the condensates to transition into aggregates, resulting in a loss of function of the transcription factor and a gain of toxic function. To unravel the molecular basis of this phenomenon, we use a wide set of biophysical techniques (such as nuclear magnetic resonance) to characterize the structural propensity of the tract and its intrinsic aggregation propensity; as well as experiments in MN1 cells using microscopy techniques (including live-cell imaging, fluorescence recovery after photobleaching, or super resolution) and transcriptional activity assays to study the effect of tract expansion on the material properties of the condensates formed by the transcription factor and its activity.

P-02-015
Application of selected diene probes to monitor excited triplet states of synthetic melanins

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Although melanin pigments can generate reactive oxygen species upon exposure to light, the involvement of excited triplet states in these processes remains unclear. Despite the use of advanced time-resolved spectroscopic techniques, direct detection of melanin triplet states has largely been unsuccessful. This challenge may stem from the unfavorable optical properties of melanin, which strongly absorbs light across the UV-visible spectrum. However, melanin's ability to photogenerate singlet oxygen suggests an energy transfer process from its excited triplet state to molecular oxygen. In this study, two diene probes – sorbic acid and sorbic alcohol – were employed to investigate the triplet states of synthetic models of eumelanin and pheomelanin. The production of singlet oxygen by these pigments was measured using time-resolved near-infrared phosphorescence, in the absence and presence of increasing concentrations of diene probes and controlled concentration of oxygen. As efficient quenchers of triplet excited states that exhibit minimal interaction with singlet oxygen, both dienes enabled a comparative assessment of melanin triplet states. Both probes exhibited both static and dynamic quenching with sorbic alcohol demonstrating a higher efficiency in reducing melanin-generated singlet oxygen intensity, which can be attributed to the higher triplet energy of that probe. However, even at the highest concentrations of the dienes used, the singlet oxygen signal was only partially diminished, suggesting that melanin photoexcitation leads to the formation of triplet excited states with varying energies, some of which remain unquenchable. Supported by National Science Centre (grant 2021/43/B/ST4/03255).

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P-02-016
Thermal shift assay for measuring GPCR–ligand interaction

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G-protein coupled receptors (GPCR) are common human membrane proteins and extremely popular drug targets, since these receptors are involved in signaling in vast number of physiological processes. One of the most important tasks in the field of GPCR research is the search and the characterization of new ligands. Thermal shift assay (TSA) can become an easily accessible alternative for analyzing the interaction of GPCR and ligands. The speed of measurement, low consumption and accuracy of the obtained values represent the main advantages of the TSA. TSA is based on the measurement of the melting curve, which displays the dependence of the proportion of molten protein on the temperature of the solution. The amount of protein in the molten state is determined by the fluorescence of a dye reacting to protein denaturation. The dissociation constant (K_d) of the protein-ligand complex is calculated from the magnitude of the melting curve shift, which occurs when the ligand is added to the solution and the protein is stabilized by formation of the complex. In order to adapt the method for K_d measurements of GPCR-ligand complex we optimized experimental conditions for TSA method in the presence of the dye 7-diethylamino-3-(4'-maleimadylphenyl)-4-methylcoumarin (CPM). Three different mathematical models of data processing were formed and tested for the chosen approach. As a result, we obtained K_d for several orthosteric and allosteric ligands of the adenosine receptor of the second type (A2AAR) and cysteinyl leukotriene receptor 1 (CysLT1R) by the TSA method. The obtained values of constants for orthosteric ligands are close to values taken from literature and measured by radioligand assay. This fact confirms the possibility of using TSA as an alternative method and facilitate further structural and functional GPCR studies. This work was supported by the Russian Science Foundation project 24-74-10020 (https://rscf.ru/project/24-74-10020/). *The authors marked with an asterisk equally contributed to the work.

P-02-017
Structural investigation of asymptomatic microhematuria in human urine via ATR-FTIR spectroscopy

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Hematuria, defined as red blood cells in the urine, indicates urogenital tract damage and benign or malignant kidney disease. According to the American Urological Association guidelines, the presence of three or more RBCs per high power field in an adequate microscopic urinalysis is defined as asymptomatic microscopic haematuria. It has a wide range of urological and non-urological causes, both malignant and benign, which usually necessitate further investigation. This study aimed to determine the structural and contextual molecular changes specific in asymptomatic microscopic hematuria in urine using attenuated total reflectance Fourier transform infrared spectroscopy (ATR-FTIR) spectroscopy. We collected 15 samples from patients being diagnosed for asymptomatic microhematuria and 25 samples from healthy individuals and measured them with ATR-FTIR spectroscopy. Several spectral variations were observed, particularly, in the frequency range between 1800– 450 cm^{-1} region. The alteration in the ratio of band intensities of lipid to protein and contents of urea, creatinine, nucleic acid and phosphate were observed. The results demonstrate that ATR-FTIR spectroscopy has significant potential as a rapid, accurate and simple method for identifying asymptomatic microhematuria in human urine. This study is a part of uCAIR Project, which is funded by the European Union's Horizon Europe research and innovation programme under Grant Agreement no. 101135175.

P-02-018
Effect of electric fields on silk-fibroin protein self-assembly

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Bombyx mori silk fibroin (SF) protein is a biopolymer naturally synthesized and spun into a microfiber by the *B. mori* silkworms. It is composed of a $(GAGAGS)_n$ backbone, that tends to self-assemble into nanofibrillar structural organization, similarly to β-sheet rich amyloid assembly, under the action of shear forces, particularly elongational flow. Due to the intrinsic characteristics of fibroin protein in its soluble state as well as a spun fiber, such as biocompatibility, biodegradability and low cytotoxicity, and combined with its natural mechanical properties of high tensile strength and flexibility, this substrate is of particular interest for materials for tissue engineering and regeneration applications. Such usage has been registered in different forms, including films, hydrogels, scaffolds, etc. With a complex charge distribution on its monomers, electric fields can induce fibril orientation and even alter chain bonding. Therefore, in this work, we investigate the effect of electric fields (EF) in the context of being used as a tool for controlled self-assembly of SF proteins and films. SF was produced by the standard degumming method and films were made by the dropcasting method. Both alternating and direct current (AC and DC, respectively) EFs were applied using gold electrodes. For AC films, both amplitude and frequency of films were varied, as well as adsorption time. Surface topography was characterized by atomic force- and scanning electron microscopy (AFM and SEM, respectively). The secondary structure of bulk films was assessed by Fourier transform infrared spectroscopy (FTIR), while nano-FTIR was used in nanometric films. Further characterization includes Raman spectroscopy and X-ray crystallography. Preliminary results show that SF fibrils are aligned with the EF, while a longer exposure to the fields induces aggregation. Furthermore, bulk FTIR shows an increase in β-sheet content for films with higher concentration.

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P-02-019
Pancreatic cancer cells adapt to compressive mechanical stress through regulation of PI3K–MAPK interdynamics

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Pancreatic cancer (PDAC) is projected to become the second leading cause of cancer-related death by 2030. Understanding its biology is crucial for developing novel therapeutic strategies. A defining feature of PDAC is high fibrosis, which hinders treatment effectiveness. Although much is known about PDAC's molecular and morphological progression, less is understood about the impact of compressive mechanical stresses from the rigid extracellular matrix on cancer cells. We aimed to identify oncogenic pathways involved in the response to mechanical stress, to understand how PDAC cells adapt to this harsh environment. The genetically engineered KPC mouse model (LSL-KrasG12D/+; LSL-Trp53R172H/+; Pdx-1-Cre) was used to replicate mutations seen in PDAC. Advanced techniques like unbiased kinome activity analysis via PamGene were applied to explore cellular responses to mechanical compression in both *in vitro* and *in vivo* contexts. Using a tumor rigidity map from shear wave elastography, we segmented tumor subregions based on rigidity and identified kinase activity signatures. Both MAPK and PI3K pathways were prominent in rigid tumors. *In vitro*, mechanical compression caused changes in nuclear shape and the internalization of YAP, a mechanosensitive protein, into the nucleus. We also observed the activation of both MAPK and PI3K pathways, which occurred non-concomitantly. Notably, compression induced a long-term resistance to PI3K inhibitors, which could impact treatment strategies. Furthermore, several receptor tyrosine kinases were identified that respond to mechanical compression, offering potential targets to improve treatment outcomes. A better understanding of molecular responses to mechanical forces will aid in the development of new therapeutic strategies that consider the mechanical context of tumors.

LB-R-02-01
Ligand-independent dimerization of ErbB4 JM-b isoforms suggests a structural basis for isoform-specific signaling

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ErbB4, a receptor tyrosine kinase in the ErbB/EGFR family, regulates proliferation, differentiation, and survival. Alternative splicing produces JM-a (cleavable) and JM-b (non-cleavable) variants with distinct processing and signaling capacities. Depending on the isoform expressed and the cellular context, ErbB4 may act as an oncogene or tumor suppressor, a duality likely driven by differences in receptor localization, processing, and downstream pathways. How these structural differences affect dimerization and activation remains unclear. To address this, we applied Number and Brightness analysis using an ErbB4 specific DARPin conjugated with AF488 to quantify the oligomeric state of JM-b ErbB4 isoforms (CYT-1 and CYT-2) in live cells. The CYT-1 isoform can activate PI3K, whereas the CYT-2 isoform lacks the PI3K binding sequence due to alternative splicing. Our results reveal that both isoforms exhibit similar brightness values with or without ligand stimulation, consistent with a ligand-independent dimeric state. This pre-dimer formation may reflect a structural configuration unique to the JM-b variant, potentially stabilizing the receptor at the membrane and influencing its signaling capacity. The pre-dimerization of ErbB4 JM-b isoforms may partially explain its varying roles in cancer. Stable JM-b dimers could enable sustained, ligand-independent signaling through pathways like PI3K/AKT, promoting oncogenic activity. In contrast, the non-cleavable nature of JM-b may hinder tumor-suppressive functions associated with JM-a isoforms. Ongoing studies on JM-a variants aim to clarify how isoform-specific dimerization and processing affect signaling and outcomes, suggesting that ErbB4 isoform structure influences its context-dependent cancer behavior.

LB-R-02-02
Computational chemistry-driven development of DNA/RNA aptamers

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Biomolecular simulations have long been an important part of the drug discovery and development process, with techniques such as docking, virtual screening, molecular dynamics and quantum mechanics being routinely used in the study of the interaction and selection of small molecular drugs with their target proteins or enzymes. More recently, the application of these techniques in aptamer selection and aptamer engineering has algo become a reality. Such methods can help to understand aptamer-target interaction and to rationally introduce modifications in selected aptamers to modulate their affinity, specificity or ability to carry other molecules. Here, we present a computational protocol developed by us for the selection of specific aptamers for the recognition of proteins and small molecules. These protocols combine protein-DNA/RNA docking, aptamer/small molecule docking, atomistic molecular dynamics simulations and free energy calculations, including the conformation variability of the protein and aptamer in the selection process, and refined with DFT calculations. This approach is illustrated with recent examples obtained in the group [1-3]. Acknowledgements: This work received financial support from the PT national funds (FCT/MECI, Fundação para a Ciência e Tecnologia and Ministério da Educação, Ciência e Inovação) through the project UID/50006 -Laboratório Associado para a Química Verde - Tecnologias e Processos Limpos. References: [1] Vasconcelos D. et al. Biophysical Chemistry, 2024, 314, 107308. [2] Pina A. F. et al. Biochimica et Biophysica Acta, 2022, 1866, 130252. [3] Pereira A. C. et al. Molecular Systems Design and Engineering 2022, 7, 637.

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Cell biology

P-03-001
Low ambient temperatures activate mitochondrial activity in honey bees (*Apis mellifera*)

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Animals living in low ambient temperatures tend to have prolonged lifespans. Whether this extension in lifespan is related to mitochondrial activity remains unclear. In this study, newly emerged honey bees (*Apis mellifera*) from brood combs were randomly collected in cages, which were put into respective 22°C, 30°C, and 38°C incubators. Bees were fed daily with honey and fresh pollen grains mixed with honey (3:1). Bees reared at 22°C, 30°C, or 38°C were collected at 25 days. Their abdomens (excluding the digestive tract) were used to extract RNA for qRT-PCR assays. Trophocytes and oenocytes were isolated from the abdomens for mitochondrial density and mitochondrial membrane potential (Ψm) assays. These cells were also isolated, homogenized, and centrifuged to obtain the supernatant for the oxidized form of nicotinamide adenine dinucleotide (NAD^+) concentration, adenosine triphosphate (ATP) concentration, and NAD^+/reduced form of nicotinamide adenine dinucleotide (NADH) ratio assays. The results showed that honey bees reared at 22°C exhibited high mitochondrial density and elevated mRNA levels of cytochrome b (cyt b), 16S ribosomal RNA (16S rRNA), and NADH dehydrogenase 4 (ND4) compared to those reared at 30°C or 38°C. Additionally, bees reared at 22°C had higher Ψm, NAD^+ concentration, ATP concentration, and NAD^+/NADH ratio compared to those reared at 30°C or 38°C. These findings demonstrated that honey bees living in low ambient temperatures enhance mitochondrial biogenesis and activity, thereby extending their lifespans.

P-03-002
Gut region-dependent expression of NFκB p65 and its translocator protein, importin α3 in streptozotocin-induced type 1 diabetic rats

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The prevalence of type 1 diabetes is increasing worldwide, and its complications cause many deaths among diabetic patients. Long-term hyperglycaemia is usually accompanied by chronic and low-grade inflammation regulated by cytokines. Recently, we reported that expression of different inflammatory cytokines has changed along the intestinal axis of diabetic animals. These cytokines have great impact on induction of nuclear factor kappa-light-chain-enhancer of activated B cell (NFκB) pathway involved in the regulation of inflammatory and immune responses as well as neuronal fate. Therefore, our goal was to examine the effects of chronic hyperglycaemia and immediate insulin treatment on the expression of NFκB (p65 subunit)[1] and its translocator protein, importin α3 (QIP1) in myenteric ganglia of the small intestine. Ten weeks after the onset of hyperglycaemia, ultrathin sections and whole-mount preparations of control, streptozotocin-induced diabetic, and insulin-treated diabetic rats were processed for immunogold post-embedding electron microscopy and fluorescent immunohistochemistry. In the diabetic rats, the density of NFκB p65-labelling gold particles increased in the ileal ganglia, while remained unchanged in the duodenum. The ratio of both NFκB p65- and QIP1-immunoreactive myenteric neurons increased significantly in the diabetic ileum, while slightly decreased in the diabetic duodenum. Insulin treatment did not prevent all hyperglycaemia-related alterations in NFκB p65 and QIP1 protein expression. These findings reveal that expression of NFκB p65 and its translocator protein changed in the same way in myenteric ganglia. Furthermore, we observed a segment-specific NFκB p65[1] and QIP1 expression in myenteric ganglia, which may contribute to regional enteric neuropathy in type 1 diabetes. Previously published in: [1]Barta BP et al. (2024) Biomedicines. 2024;12 (10):2347.

P-03-003
Characterization and functional evaluation of dual nuclear localization signals in desmin

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Research has demonstrated that cytoskeletal proteins can be translocated into the nucleus, where they perform critical functions. Nucleocytoplasmic transport occurs with different mechanisms through the nuclear pore complex (NPC). The cytoskeletal desmin protein predominantly serves structural functions within the cytoplasm. However, *in silico* analyses have revealed that desmin harbors putative nuclear localization signals (NLSs) required for karyopherin-dependent nucleocytoplasmic transport. Furthermore, proteomic analyses and desmin's amphiphilic nature indicate an interaction with NPC components, suggesting that it may utilize both karyopherin-dependent and -independent pathways for nuclear translocation. This study aims to elucidate the mechanisms underlying desmin's nucleocytoplasmic transport. To this end, mutant desmin expression vectors lacking the predicted NLSs were transfected into myoblast cells. Confocal microscopy and co-localization analyses confirmed that the predicted NLSs are functional and essential for desmin's nuclear localization. Additionally, pharmacological inhibition of the karyopherin-dependent pathway demonstrated that desmin can enter the nucleus through this mechanism. Nuclear import assays were conducted to explore whether desmin can be translocated into the nucleus via direct interaction with NPC elements. The findings of the nuclear import assay did not substantiate a preference for a karyopherin-independent mechanism. However, hydrophobicity measurements have shown that desmin can undergo conformational changes to adapt to hydrophobic environments, such as the NPC. In conclusion, we showed that desmin can be translocated into the nucleus via a karyopherin-dependent mechanism. These findings reveal desmin's potential regulatory roles in nuclear architecture and gene expression, expanding our understanding of its transport dynamics and functions, with implications for novel therapies targeting desmin-related diseases

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P-03-004
Isolation and efficiency assessment of adipose-derived mesenchymal stem cells

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It was aimed to isolate mesenchymal stem cells (MSCs) from fat tissue lipoaspirates obtained from healthy volunteer donors, evaluate their efficiency, and highlight their potential for tissue engineering, regenerative medicine and cellular therapy applications. Adipose tissue lipoaspirates were collected under general anesthesia after obtaining signed informed consent from a healthy volunteer donor. To obtain MSCs from lipoaspirates, they were washed several times with PBS. Then, it was digested with collagenase. The collagenase effect was inhibited by the medium. It was centrifuged to collect the stromal vascular fraction (SVF) which was suspended with medium, filtered and added to flasks. The cells were maintained for 7 days. Biomarkers recommended by the International Society for Cellular Therapy (ISCT) were analyzed by flow cytometric method to confirm that MSCs were obtained from lipoaspirates: CD73+, CD90+ and CD105+ biomarkers were positive; CD34-, CD45-, CD11b-, CD79a-, HLA-DR- biomarkers were confirmed negative; CD90+ showed 97.6% positivity. With these data, it was confirmed that MSCs were obtained. The efficiency of MSC isolation was calculated as 1.052×10^5 cells/mL. MSCs can be isolated from many tissues, including bone marrow, adipose tissue, skin, and umbilical cord blood. While approximately $3.5 \times 10^5 - 1 \times 10^6$ MSCs can be obtained from 1 gram of fat tissue, only 500 to 5×10^4 bone marrow MSCs can be isolated from one gram of bone marrow tissue. [Previously published: Tsuji W et al. (2014). World J Stem Cells, 6(3), 312–321]. Adipose tissue is less invasive than others but has higher efficiency. Today, thousands of liters of lipoaspirate are disposed of every year. MSCs isolated with correct methods have the potential to be used in many areas such as immunomodulation, regenerative medicine and tissue engineering.

P-03-005
TMAO-induced endothelial mesenchymal transition and exosomal signaling leading to vascular smooth muscle cell differentiation

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Cardiovascular diseases (CVDs) are the leading global cause of death and are closely linked to vascular calcification (VC), a key predictor in atherosclerosis. Both endothelial cells (ECs) and vascular smooth muscle cells (VSMCs) maintain vascular homeostasis, while disrupted EC–VSMC interactions promote VC through inflammatory-driven phenotypic changes and calcium phosphate deposition, driven by VSMCs transforming into osteoblast-like cells. Trimethylamine-N-oxide (TMAO), a molecule derived from gut microbiota-generated TMA, exacerbates endothelial dysfunction and inflammation. The impact of TMAO-incubated EC-derived exosomes on VSMC differentiation remains to be clarified. We aim to determine whether TMAO incubation induces EndMT in ECs. Additionally, we are assessing the impact of exosomes from TMAO-incubated ECs on VSMC differentiation and migration. ECs were incubated with TMAO (1–100 μM) for 96 hours to induce EndMT. Phenotypic changes in HUVECs were assessed by gene and protein expression changes. Incubation with TMAO resulted in HUVECs transitioning to a mesenchymal phenotype, marked by decreased VE-cadherin and CD31 and increased αSMA, N-cadherin, and vimentin expression in a dose-dependent manner. Exosomes from transitioned HUVECs were isolated via ultracentrifugation and characterized by NTA, TEM, and western blotting. Incubation of VSMCs with these exosomes (5–30 μg/ml) reduced α-SMA and SM22α expression while increasing RUNX-2, OPN, and TNAP levels. High doses of these exosomes (20–30 μg/ml) exhibited a cytotoxic effect on VSMCs, while 10 μg/ml promoted a pro-migratory response, as shown by the wound healing assay. Our study establishes TMAO-induced endothelial exosomes as mediators of VSMC differentiation, emphasizing the critical impact of exosome dosage and implications on VSMC behavior. *The authors marked with an asterisk equally contributed to the work.

P-03-006
Genome-wide CRISPR/Cas9 screen identifies ubiquitin ligase TRIM25 that mediates ribosome degradation via ribophagy

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Ribophagy is a specific type of autophagy that is needed for the degradation of ribosomes. This process is necessary for the removal of defective ribosomes, the function of which can be impaired due to the action of translation inhibitors or other stress factors. At present, a small number of ribophagy receptors involved in this process are known, the key one of which is the NUFIP1 protein [Previously published in: Gregory A. Wyant et al. (2018) Science 360, 751–758]. Studying the molecular mechanism of ribophagy remains is an urgent task. To identify the genes involved in the ribophagy process, we created a fluorescent reporter (a cell line with ribosomes containing RPL29-mCherry-GFP) and performed a genome-wide CRISPR/Cas9 screening using the Brunello lentiviral library. Among the genes that were highly significant in the screening results was ubiquitin ligase TRIM25. We showed that TRIM25 specifically binds to the ribosome upon induction of ribophagy using cycloheximide (CHX). It is known from literature data that TRIM25 is involved in the antiviral protection of the cell, regulating the immune response and degradation of viral components [Previously published in: Shang Z. et al. (2024) Nat Commun 15, 4127; Choudhury N. et al. (2022) Nucleic Acids Res 50, 7097-7114]. To test whether viral infection could affect TRIM25 binding to the ribosome, we treated cells with poly(I:C), a synthetic analogue of double-stranded RNA that serves as a model of viral infection. This experiment confirmed that TRIM25 binds to the ribosome under conditions mimicking viral infection. Thus, we have shown that under stress conditions,

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such as cycloheximide treatment or viral infection, ribosomal ubiquitination occurs, which is mediated by the activity of TRIM25. Considering that ubiquitin ligases of this family are involved in the cellular antiviral response, we suggest that activation of ribophagy may be part of the cellular defense mechanism against viral infection.

P-03-007
Sox2 as a key regulator of taste bud regeneration: implications for age-related taste dysfunction

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Taste dysfunction is commonly associated with aging and is accompanied by morphological and molecular alterations in the lingual epithelium. To investigate the mechanisms underlying this phenomenon, we utilized Klotho-deficient (Klotho$^{-/-}$) mice, a model of accelerated aging. In these aging mice, the lingual epithelium was significantly thinner, exhibiting atrophic papillae, diminished muscle fibers, and increased adipose tissue deposition compared to wild-type controls. The minor salivary glands at the root of the tongue displayed acinar cell atrophy and expanded interacinar stroma. Notably, Klotho$^{-/-}$ mice showed reduced taste bud diameter and decreased expression of key taste receptors (Tas1R1, Tas1R2, Tas1R3, and Tas2R105), as well as a lower number of type II sweet-sensitive taste cells. Immunohistochemical analyses importantly revealed diminished expression of SOX2 in both taste buds and tongue epithelial cells. Overexpression of SOX2 significantly upregulated Keratin 8 mRNA, a marker of mature taste cells. Conversely, SOX2 knockdown using siRNA resulted in reduced expression of taste-related genes (Keratin 8, PLCB2, Tas1R1, Tas1R2, Tas1R3, Tas2R105) at the mRNA level and decreased KCNQ1 protein (a key ion channel in taste transduction). These findings indicate that SOX2 may contribute to taste bud homeostasis and may serve as a potential therapeutic target for age-related taste loss. This work was supported by the National Research Foundation of Korea grant funded by the Korea government (No. RS-2023-00222390).

P-03-008
The effect of visfatin on RANKL-induced osteoclastogenesis

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Visfatin is an adipokine secreted by various cell types, particularly associated with inflammatory diseases such as obesity, which is closely linked to osteoclastogenesis, a key process related to bone resorption and increased risk of osteoporosis (OP). However, the role of visfatin in osteoclastogenesis remains unclear. This study was conducted to investigate the effects of visfatin on RANKL-induced osteoclast differentiation *in vitro*. The results showed that, although visfatin exhibited a modest osteoclast-inductive effect compared to RANKL, co-stimulation of mouse bone marrow-derived macrophages (BMDMs) with visfatin and RANKL significantly enhanced osteoclast differentiation and activation compared to individual stimulation. Neutralization of visfatin activity using antibodies before differentiation markedly suppressed RANKL-induced osteoclastogenesis, as evidenced by the near-complete absence of tartrate-resistant acid phosphatase (TRAP)-positive multinucleated osteoclasts, decreased levels of nuclear factor of activated T cells cytoplasmic 1 (NFATc1) and osteoclast-specific proteins, inhibition of nuclear factor-κB (NF-κB) and mitogen-activated protein kinase (MAPK) signaling pathways, and a decrease in resorption pit formation. Therefore, this study emphasizes the critical role of visfatin in RANKL-induced osteoclastogenesis and suggests that the RANKL/visfatin signaling axis may serve as a potential therapeutic target for diseases related to destructive bone loss.

P-03-009
Role of yeast mitochondrial citrate transporters in RTG-dependent osmoadaptation

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Retrograde signaling is activated in yeast cells in response to mitochondrial damage or environmental stress to preserve cellular homeostasis. In wild-type (*WT*) yeast cells exposed to hyperosmotic stress induced by treatment with sodium chloride, the RTG-dependent retrograde signaling is activated and peroxisome–mitochondria cross-talk plays a crucial role: indeed, the citrate synthase peroxisomal isoform 2 (*CIT2*) is up-regulated, while the mitochondrial isoform 1 (*CIT1*) is down-regulated. Furthermore, in the presence of osmotic stress, intracellular citrate levels are increased compared to control cells whereas other TCA cycle intermediates are decreased, or unchanged, indicating that citrate homeostasis is required for survival under hyperosmotic conditions. To sustain cellular/mitochondrial metabolism, citrate needs to enter the mitochondria. Therefore, we investigated the involvement of mitochondrial citrate transporters in response to osmotic stress. Two mitochondrial citrate transporters, Yhm2p and Ctp1p, have been identified in *Saccharomyces cerevisiae*. We have now demonstrated through qPCR analysis that *YHM2* gene expression does not change under hyperosmotic conditions and is significantly higher than *CTP1* (5-fold). Conversely, Yhm2p is up-regulated in the presence of osmostress, suggesting a translational regulation of this carrier. Supporting the role of cytosolic citrate in mediating peroxisome-mitochondria communication, the Yhm2p-deleted strain exhibited increased sensitivity to hyperosmotic conditions. Our results show that mitochondrial citrate transporters are a key factor in regulating the intracellular citrate pool and its trafficking, which modulates the retrograde signaling in the osmoadaptive response.

P-03-010
The role of 5-methylcytosine RNA methyltransferases of NSUN family and TRDMT1 during osteosarcoma response to exogenous tRNA stimulation

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Exogenous nucleic acids can be considered as damage-associated molecular patterns (DAMPs) inducing nucleic acid sensing and

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related pro-inflammatory responses in normal and cancer cells. Different types of RNA may be released from damaged cancer cells during chemotherapeutic treatment modulating the response and fate of neighboring cells. However, little is known if exogenous tRNA may affect the transcription of tRNA associated genes and the pools of tRNA-derived small RNA fragments (tRFs), tRNA halves (tiRNAs), and microRNA, and if these effects may be regulated by 5-methylcytosine RNA methyltransferases. In the present study, four cellular models of osteosarcoma (OS) *in vitro* (U-2 OS, MG-63, SaOS-2, and 143B) were applied to investigate exogenous tRNA-based responses. Exogenous tRNA was taken up by OS cells in the form of tRNA–lipid complexes and caused an elevation in the levels of TRDMT1 methyltransferase. Gene knockout (KO) of *TRDMT1* was then considered to evaluate the role of TRDMT1 during tRNA-mediated nucleic acid sensing (MDA5, RIG-I, MAVS, cGAS, STING, TBK1, IRF3, IRF7, DDX1, DDX3, TRIF, IFN-β, NF-κB) and transcriptional changes (expression of 28 genes of tRNA transcription). *TRDMT1* KO affected tRNA-mediated transcriptional and translational responses in OS cells. The effect of exogenous tRNA and *TRDMT1* KO on the pools of tRFs, tiRNA, and microRNA was then evaluated using RNA sequencing. Changes in the distribution of tRF and tiRNA subtypes, specifically expressed tRF, tiRNA, and microRNA, and up and down-regulated tRF, tiRNA, and microRNA were revealed upon tRNA treatment and *TRDMT1* KO. Stimulation with tRNA also promoted TRDMT1 interaction with DExH-box helicase 9 (DHX9) that may affect gene regulation and RNA metabolism. In conclusion, we suggest that TRDMT1 may modulate tRNA-associated transcriptional and translational responses in OS cells. This study was supported by the National Science Centre (NCN, Poland) grant OPUS 22 no. 2021/43/B/NZ2/02210.

P-03-011
Effect of HSPA2 chaperone protein on EGFR function in human epithelial cells

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The HSPA2 protein belongs to the HSPA (HSP70) family, a group of several highly homologous molecular chaperones. In humans, HSPA2 is highly expressed in the testis, where it controls spermatogenesis, and certain extratesticular tissues, e.g., in various stratified and pseudostratified epithelia. The role of HSPA2 in epithelial cells is poorly characterized. Our previous study demonstrated its crucial role in maintaining human epidermal homeostasis. In this study, we aimed to gain deeper mechanistic insight into the involvement of HSPA2 in the epidermal homeostatic mechanism. We first searched for HSPA2 clients in immortalized keratinocyte Ker-CT line overexpressing the V5-HSPA2 fusion protein. V5-HSPA2-interacting proteins were co-immunoprecipitated with V5-Trap nanobodies. Epidermal growth factor receptor (EGFR) was among 155 proteins identified in co-immunoprecipitates by the shotgun LC-MS/MS-based proteomic approach. HSPA2 interactions with EGFR were confirmed in keratinocytes *in vitro* and human epidermis *ex vivo* by co-immunoprecipitation with anti-HSPA2 antibody and proximity ligation assay (PLA), respectively. To assess whether HSPA2 affects EGFR-dependent signaling in epithelial cells, keratinocyte (HaCaT) and bronchial epithelial (BEAS-2B) cell lines were stimulated with TGFα and EGF. We found that the loss of HSPA2 affected EGFR phosphorylation and the PI3K/Akt signaling pathway. We also examined how EGF stimulation affects the potential of HSPA2-null HaCaT and BEAS-2B cells to grow at air–liquid interface (ALI) culture to generate reconstructed human epidermis and bronchial epithelium *in vitro*. Our results suggest that HSPA2 can control the homeostasis in human multilayered epithelia via EGFR-dependent mechanisms. This study was funded by the National Science Centre, Poland, grants number 2017/25/B/NZ4/01550 and 2020/39/O/NZ4/02616 (to DS), and the MSCNRIO, Gliwice Branch, grant number SN/GW8/2023 (to DS). *The authors marked with an asterisk equally contributed to the work.

P-03-012
Regulatory roles of FACI in clathrin-mediated endocytosis and macropinocytosis

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The intestine- and liver-enriched transcription factor CREBH is a significant regulator of lipid metabolism. Fasting and CREBH-induced (FACI) gene, also known as C11ORF86, has been identified as a novel target of CREBH. Here, we report on the function of FACI in clathrin-mediated endocytosis and macropinocytosis, as well as its regulation by PKCα and its relevance to lipid metabolism and pathogen entry into host cells. FACI interacts with the adaptor protein 2 (AP2) complex to localize to clathrin-coated pits (CCPs) in the plasma membrane. Low-density lipoprotein (LDL) endocytosis is enhanced when FACI is overexpressed in AML12 cells but is compromised when FACI is depleted. Overexpression of FACI in the liver alleviates diet-induced hypercholesterolemia in mice. We also observed that FACI promotes macropinocytosis. Notably, enteric pathogens such as *Salmonella typhimurium* exploit FACI to enable their entry into host cells through macropinocytosis. FACI activity is regulated by PKCα through phosphorylation at S9 and S37, which induces its relocation to the plasma membrane, and detachment from CCPs. The functions of FACI in clathrin-mediated endocytosis and macropinocytosis are regulated by PKCα-dependent phosphorylation. Taken together, PKCα-regulated FACI is critically involved in clathrin-mediated endocytosis, macropinocytosis, and pathogen entry. Our findings have important implications for the design and development of pharmaceutical agents that regulate clathrin-mediated endocytosis and macropinocytosis.

P-03-013
Zinc deficiency inactivates lysin acetyl transferase 7 (KAT7) and promotes fatty liver

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Zinc is an indispensable micronutrient crucial for optimal physiological function. Zinc deficiency, arising from inadequate intake and impaired absorption, has been implicated in the

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pathogenesis of various diseases. However, the molecular mechanisms how zinc deficiency causes harmful effects remain poorly understood. To understand the impacts of zinc deficiency at the cellular level, we first focused on transcriptional regulation, especially histone post-translational modifications and revealed that the acetylation of histone H3 at Lys14 (H3K14ac) was decreased under zinc deficiency. As a molecular mechanism behind the reduction in H3K14ac, we found that the histone acetyltransferase KAT7 loses zinc coordination and enzymatic activity during zinc deficiency. Next, we investigate the phenotype caused by the reduction in H3K14ac. Using a mouse model of zinc deficiency by feeding a zinc-deficient diet, we revealed that zinc deficiency induced lipid accumulation in the liver. KAT7 knockdown or inhibitor treatment also increased lipid droplets in mouse primary hepatocytes, suggesting that zinc deficiency promotes fatty liver via KAT7 inactivation. Since patients with diseases associated with fatty liver, such as NAFLD and chronic liver diseases, often show decreased zinc levels, our results suggest the possibility that KAT7 inactivation during zinc deficiency alters gene expression and change the lipid metabolism, resulting in the cause of these diseases.

P-03-014
Identification of factors derived from *Mastigias papua* and their effects on neural differentiation in HeLa cells

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Jellyfish experience conspicuous metamorphosis throughout their life cycle. In *Mastigias papua*, the zygote undergoes multiple metamorphic stages, including planula, polyp, planuloid, and ephyra, before eventually developing into the medusa stage. During these drastic morphological transitions, factors that regulate programmed cell death, such as apoptosis and autophagy, and cell differentiation are believed to play critical roles in these processes. Identifying and elucidating the functions of these factors could contribute to applications in the treatment of cancer and regenerative medicine. In this study, the differentiation-inducing effects of an extract derived from the medusa stage of *M. papua* on HeLa cells were investigated, and the differentiation process was analyzed. First, the medusa of *M. papua* was homogenized, and the supernatant obtained after centrifugation was used as the extract. Subsequently, the extract was added to DMEM at a final total protein concentration of 25 ng/μl, and HeLa cells were cultured in this medium. Immunofluorescence staining was performed 6 to 8 days after the addition of the extract, and genetic markers were used to analyze cell differentiation. The results showed the expression of glial fibrillary acidic protein (GFAP), SRY-box transcription factor 2 (SOX2), and Vimentin (VIM) in the treated cells. Since these markers are all associated with neural progenitor cells or astrocytes, our findings indicate that the differentiation pathway of HeLa cells treated with the *M. papua* extract includes these cell types. This suggests the cells may have differentiated into neural progenitor cells or astrocytes, indicating that the extract contains neurogenic differentiation-promoting factors. These factors could potentially contribute to nerve cell regeneration and the recovery of damaged neural tissues.

P-03-015
Nintedanib as a treatment for muscle fibrosis in a rat soft palate cleft model

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Following cleft palate surgery, palatal muscle regeneration is often impaired by fibrosis. Nintedanib, an FDA-approved antifibrotic agent primarily used for idiopathic pulmonary fibrosis, may facilitate muscle regeneration by inhibiting fibrosis. This study evaluates the effects of Nintedanib in an *in vivo* soft palate cleft model. A 1-mm excisional wound was created in the soft palate of rats, followed by daily systemic treatment with Nintedanib for 7 days. Tissue samples were collected at 3, 7, 28, and 56 days post-surgery and analyzed using AZAN staining and immunostaining for MyHC (muscle), α-SMA (myofibroblasts), and Pax7 (satellite cells). At day 7, the area percentage of myofibroblasts was significantly lower in the Nintedanib-treated rats (21.5 ± 10.4%) compared to the wound-control group (41.3 ± 9.2%). By day 28, the collagen area percentage was also reduced in the Nintedanib group (70.3 ± 7.8%) compared to controls (80.3 ± 6.2%). Notably, a larger myofiber area was observed in the Nintedanib group (29.7 ± 7.8%) versus controls (19.7 ± 6.2%) at day 28. Similarly, the density of Pax7-positive cells was significantly higher in the Nintedanib-treated group (91.2 ± 24.2 cells/mm^2) than in controls (50.0 ± 12.6 cells/mm^2) at day 28. By day 56, however, no differences were observed between the Nintedanib-treated rats and the wounded control group. In conclusion, systemic administration of Nintedanib slightly decreases fibrosis but shows only limited improvement of muscle regeneration. These findings provide valuable insights for developing antifibrotic strategies aimed at enhancing soft palate regeneration following cleft palate surgery.

P-03-016
G-protein GNAO1 switches splice-form expression during neurodevelopment

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The gene *GNAO1* encodes an alpha subunit of the heterotrimeric G-protein signal-transducing complex (Gαo). Gαo plays a critical role in transducing signals from G-protein coupled receptors (GPCR) in brain cells. Both loss- and gain-of-function mutations in this gene lead to onset of inherited form of epilepsy and/or movement disorder. *GNAO1* is mostly expressed in two splice forms, A and B, which are translated into very similar proteins which differ only in few amino acid sequences close to C-end. Molecular functions of GNAO1 isoforms in the adult brain have been actively studied in recent years but little is known about its role in neurogenesis. To study the role of *GNAO1* in this process we made a panel of cells, including induced pluripotent stem cells (IPSC) from healthy donor and patient with G203R mutation in *GNAO1* and their differentiated derivatives (neuroepithelial cells, neurons and brain organoids). In these cells, we analyzed the expression of *GNAO1* splice-forms with RT-qPCR and nanopore sequencing of PCR-product and found out that splice form A was almost absent in IPSCs and its level of expression increased with neurogenesis, while expression of isoform B decreased. We suggested that those two

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forms of GNAO1 might have different subcellular localization due to contacts with different GPCR, including those with nuclear localization. Next, we generated a panel of lentiviral vectors expressing WT and *G203R* alleles of A and B forms of GNAO1 fused with GFP and used them to infect our panel of healthy donor cells. Live imaging of cells showed that in IPSCs, neuroepithelial cells, neurons and glial cells both forms of GNAO1 are located on the cell membrane and Golgi apparatus. Presence of *G203R* mutation did not affect localization in any cell type. Therefore, we conclude that *GNAO1* splice forms undergo a switch during neurogenesis, but their specific role is yet to be discovered. The research was supported by RSF (project No. 24-25-00360).

P-03-017
Cell death mechanism induced by *Aurelia* sp. extract in HeLa cells

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In recent years, massive jellyfish blooms have become a serious issue worldwide, causing significant damage to marine ecosystems and fisheries. In Japan, blooms of the moon jellyfish (*Aurelia* sp.) have been reported in various regions. Interestingly, *Aurelia* sp. undergoes four distinct metamorphic stages in its life cycle, suggesting that it may contain factors that regulate cell differentiation and cell death. Understanding the effects on these factors on human cells and elucidating their mechanisms could contribute to advancements of regenerative medicine and cancer therapy. Therefore, the effects of an extract from medusae of *Aurelia* sp. on HeLa cells, a human cervical cancer cell line, were investigated in this study. The bell portion of adult *Aurelia* sp. was isolated, homogenized, and centrifuged to obtain the extract. HeLa cells were cultured in DMEM supplemented with the extract for five days, and morphological changes were observed. Vacuole formation was detected in HeLa cells one day after treatment, followed by cell death within two to four days. Since vacuole formation is a characteristic feature of paraptosis, a form of cell death that selectively targets cancer cell, the same experiment was conducted on HEK293 cells, a human embryonic kidney cell line. However, no vacuole formation was observed in HEK293 cells. To determine the type of cell death, immunostaining was performed using anti-ERK and anti-Cleaved Caspase 3 antibodies. The presence of cleaved Caspase 3 in the cells at 36 hours post-treatment suggested that apoptosis was induced. Further analysis of ERK expression levels is currently ongoing. In future studies, we aim to identify the factors responsible for programmed cell death.

P-03-018
Morpho-functional changes of cardiomyocyte mitochondria in postinfarction ventricular remodeling of rat hearts

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Understanding of factors that drive left ventricular (LV) remodeling after myocardial infarction (MI) is a key challenge in heart failure treatment. In this regard, many recent studies have focused on LV alterations at the cellular and molecular levels. In particular, special attention has been paid to the structural and functional rearrangements of cardiomyocyte (CMC) mitochondria. However, most studies focus on the acute phase of the disease, while much less attention is given to the chronic stages. In this work, we investigated the ultrastructural and functional changes in different mitochondrial subpopulations of LV CMCs in rat hearts at long-term time points after experimental MI, induced by left coronary artery ligation. Morphometric analysis of transmission electron microscopy images revealed that at 2 weeks post-MI, the volume density and average area of mitochondria in all subpopulations (perinuclear, interfibrillar, and subsarcolemmal) were decreased in both the border and intact zones of the LV. At 26 weeks post-MI, we observed opposite changes in the border zone, including an increased average size and volume fraction of mitochondria [Previously published in: Fedotov et al. (2024) Cytometry A, Dec 31, doi: https://doi.org/10.1002/cyto.a.24915]. Despite these different remodeling patterns, mitochondrial functional activity, determined by potential-dependent dye staining of alive isolated CMCs, was lower than in control cells at both 2 and 26 weeks post-infarction. We suggest that the decrease in mitochondrial membrane potential observed 2 weeks after MI may be due to the reduction in mitochondrial size and volume density. In contrast, at 26 weeks post-occlusion, the decreased membrane potential may be associated with alterations in cristae structure, which appeared less tightly packed and exhibited a cell-like shape. Funding: Higher Education and Science Committee of Republic of Armenia, grant 23/2PostDoc-1F001 and the ERA Chair NAR-SAR-IPH Award from the European Union.

P-03-019
Role of cochaperone BAG3 in the stabilization of p53 under heat stress conditions

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Bcl-2 associated athanogene 3 (BAG3) is the only member of the BAG cochaperone family that is induced by stressful stimuli such as heat shock and heavy metals. In the present study, *bag3* knockout (KO) HeLa cells were generated via the CRISPR-Cas9 system, and the role of BAG3 in relation to p53 under heat stress conditions was investigated. Normally, the level of p53 is low in both wild-type (WT) and KO cells, and heat shock increases the level of nuclear p53 in both cell lines. However, the increased level of p53 was much greater in WT cells than in KO cells, suggesting that BAG3 plays a role in controlling the level of p53 under heat stress conditions. The mRNA level of p53 was not increased in either WT or KO cells during the heat stress period, suggesting that the different levels of p53 are not caused by transcriptional regulation. Compared with heat treatment, treatment with MG132, a proteasome inhibitor, largely increased the level of p53 in WT cells to a similar extent. Interestingly, both BAG3 and Hsp70 rapidly translocate to the nucleus and form a complex with p53 upon heat stress. The transcriptional activity of p53 increased up to 4.0-fold during the 1 h recovery period in WT cells, whereas it increased only 1.69-fold in KO cells. These results demonstrate that Hsp70 and BAG3 are involved in the quality control of p53 under heat stress conditions and suggest a role for BAG3 as a cochaperone protein.

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P-03-020
Discovery and characterization of a novel kinase, a regulator of the autophagy pathway

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Autophagy is a crucial cellular process responsible for degradation and recycling, ensuring cellular homeostasis and survival, particularly under stress conditions such as nutrient, energy, and amino acid deprivation. The regulatory mechanisms governing autophagy, including the role of kinases, remain an active area of research. In this study, we identified a stress-responsive kinase as a critical regulator of autophagy. Our findings demonstrate that this kinase directly interacted with ATG5, a key autophagy protein, and this interaction is increased during autophagy-inducing stress, as confirmed by SILAC-MS/MS, co-immunoprecipitation, and colocalization analyses. Functional studies revealed that knockout or knockdown of the kinase significantly reduced autophagy, underscoring its essential role in autophagy activation. Conversely, overexpression of the kinase, initiated autophagy under normal conditions and amplified autophagy under stress. We identified the kinase interactome and its phosphorylation targets using proteomics approaches. Several partners and targets were discovered in the autophagy pathway. Therefore, we introduce a novel kinase directly regulating autophagy pathways, providing new insights about this basic cellular phenomenon.

P-03-021
Is there an association between air pollution and COVID-19? The answer lies in lipid rafts

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The overlap between the geographic distribution of COVID-19 outbreaks and pollution levels suggested a strong correlation between exposure to atmospheric particulate matter and an increased risk of developing severe forms of COVID-19. Several epidemiological studies have highlighted this correlation, indicating the existence of shared molecular mechanisms. The RAS system plays a key role in the pathogenesis of inflammatory diseases induced by air pollution, but it is known that ACE2 also acts as a SARS-CoV-2 receptor. Furthermore, recent studies have highlighted the critical role of lipid rafts in facilitating viral entry as SARS-CoV-2 spike protein interacts with ganglioside GM1, which is particularly enriched in lipid rafts. Therefore, in this work, an *in vitro* model of A549 pulmonary alveolar cells was treated with a sub-toxic dose of diesel exhaust particles (DEP, 1,6 μg/ml). After treatment, a membrane-enriched fraction was prepared, from which lipid rafts were isolated. Lipid analysis of lipid rafts showed that treatment with DEP resulted in a significant increase in FAHFA (fatty acid esters of hydrofatty acid) and a decrease in ceramides. This dose did not cause significant alterations in protein levels or membrane redistribution of COX-2 and HO-1. However, it led to a significant shift of ACE2 into lipid rafts, accompanied by an increase in the immature form of ADAM17. Additionally, DEP exposure significantly increased IL-6 and IL-8 release, while no changes were observed in sACE2 release. The results suggest that living in a highly polluted environment within a "risk stratification model" could significantly increase the likelihood of developing severe forms of COVID-19. This could explain the higher virus lethality observed in areas with high pollution levels. The study of lipid rafts as a preferential door in SARS-CoV-2 infection is needed, with the aim of a mechanistic approach based on synthetic analogs of gangliosides capable of inhibiting viral entry.

P-03-022
Super-resolution microscopy reveals the submitochondrial localization of human Par17

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Peptidyl-prolyl cis/trans isomerases (PPIases) are enzymes that catalyze the cis/trans isomerization of prolyl bonds in proteins and play a crucial role in protein folding and the regulation of cellular processes. One family of PPIases are the parvulins, which also have human isoforms. These include Par14 and Par17, which are encoded by the same gene (*PIN4*) and are transcribed by alternative transcription initiation. Par14 and Par17 are structurally very similar, as both proteins have the same PPIase domain. The primary difference between them lies in the N-terminal extension of Par17 by 25 amino acids, which forms an amphiphatic helix and possibly functions as a mitochondrial targeting signal. While Par14 is predominantly localized in the nucleus, the precise subcellular localization of Par17 remains insufficiently characterized. To investigate the submitochondrial distribution of Par17, super-resolution microscopy techniques were employed. Using two-color STED nanoscopy and 3D-SIM, the localization of Par17-GFP fusion proteins was analyzed in fixed and living human cells. A specific TOM20 antibody staining revealed no co-localization with Par17, excluding its presence in the outer mitochondrial membrane. Further analysis using an aldehyde-based mitochondrial crosslinking probe indicated that Par17 is localized at or within the inner mitochondrial membrane. This localization suggests a potential involvement of Par17 in mitochondrial processes such as the electron transport chain and ATP synthesis, which occur at the inner membrane. Given the critical role of mitochondria in cellular energy metabolism, apoptosis, aging processes, and mitochondria-associated diseases, these findings suggest that Par17 may have a previously unrecognized function in mitochondrial metabolism. The precise characterization of parvulin isoforms provides new insights into their specific cellular functions and opens avenues for understanding their pathophysiological relevance.

P-03-023
Traffic communications between the organelles by RNF213

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Membrane-bound organelles, including the Golgi body, endoplasmic reticulum (ER), and lysosomes, concentrate metabolic precursors within environments for carrying out both

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anabolic and catabolic reactions. Traffic communications between organelles are an essential process in coordinating metabolic fluxes. Moyamoya disease (MMD) is caused by internal dysfunctions in the internal carotid artery and branches in the circle of Willis. *RNF213* is a causative gene for MMD, and we believe finding the substrate of the ubiquitination by RNF213 is the first step to solving the MMD mechanisms. To investigate RNF213 function, we developed RNF213 KO and KI mutant mouse embryonic fibroblast cells. RNF213 KO cells show the translocation of lysosomal TFEB transcriptional factor into the nucleus under normal conditions and lower accumulations of lysosomal proteins. Furthermore, dysregulation of growth factors from the outside is observed in RNF213 KO cells. This indicated that RNF213 plays a role in cellular trafficking communications between the Golgi, lysosome, and/or plasma membranes. On the other hand, RNF213 KO mice show suppression of glucose tolerance, and RNF213 SNPs of MMD appeared to relate to lipid droplet formation and lipid metabolisms. We will present updates on RNF213 cellular function in trafficking.

P-03-024
EGFR redistribution as a component of the multimodal mechanism of 3T3 fibroblasts electrotaxis

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Electrotaxis, the directed migration of cells in a direct current electric field (dcEF), involves an interplay of receptor redistribution and ionic mechanisms. This study investigates the role of epidermal growth factor receptor (EGFR) in the electrotaxis of 3T3 fibroblasts, focusing on its redistribution dynamics, ligand dependency, and functional activity. EGFR exhibited cathodal redistribution upon 3 V/cm dcEF exposure, reaching maximum asymmetry after 30–40 min. siRNA knockdown showed that EGFR and ErbB4, but not ErbB2, were critical for electrotaxis. However, receptor silencing primarily affected migration directionality in the first hour of stimulation, suggesting a key role in the early phase of electrotaxis. Serum-free experiments revealed that EGFR-mediated electrotaxis is largely ligand-independent, as supplementation with EGF (1–5 ng/ml) did not enhance directionality but modestly increased redistribution dynamics. Nonetheless, EGFR redistribution alone could not explain the very rapid response to dcEF reversal, which occurred within 1–2 min, significantly faster than the reversal of its asymmetry. Inhibition of inwardly rectifying potassium (Kir) channels with $BaCl_2$ suppressed the earliest responses to dcEF while maintaining receptor redistribution, indicating a distinct role for ion channels in the initial phase. Additionally, EGFR inhibition partially reduced the recovery of migration directionality, suggesting its involvement in sustained electrotaxis. These findings support a multimodal mechanism of electrotaxis in 3T3 fibroblasts, where Kir channels mediate the fastest response, while EGFR contributes to both early and sustained electrotaxis. However, even when EGF receptor family members are inhibited, partial recovery of migration directionality occurs, indicating the involvement of additional regulatory components in maintaining electrotaxis. This work was supported by a grant from the National Science Centre 2018/31/B/NZ3/01750, Poland.

P-03-025
Derivation and application of a fluorescence-based assay for testing selective p53 activation using U-2 OS cell lines

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The p53 protein is one of the main regulators of the cell cycle, detecting unfavourable changes in the cell and inhibiting the cell cycle until the repair procedure is complete. MDM2, an oncogene, inhibits p53[1], promoting the uncontrolled proliferation of defective, p53-wild-type cancer cells. Small-molecule antagonists like idasanutlin (RG7388) block MDM2, reactivate p53, and provide cell cycle arrest, thus decreasing the growth of cancer cells *in vitro* and *in vivo*[2]. This study introduces and validates a novel cellular model for assessing the proliferation of cancer cells upon p53 reactivation, via simple fluorescence measurements. The model uses a co-culture of two cell lines: one (U-2 OS G8) is sensitive to MDM2 antagonists and overexpresses eGFP, while the other (U-2 OS c5 R2.7) has acquired resistance to the treatment[2] and overexpresses eRFP. Differential viability in response to a range of concentrations of MDM2 antagonists alters ratio of these two lines in co-culture, affecting fluorescence readouts. Based on green and red fluorescence, the growth of each line can be monitored, reflecting both the specific activity and unspecific toxicity of p53-reactivating compounds. This streamlined method eliminates the need for performing separate biochemical assays for testing p53-dependent and independent growth inhibition, offering a valuable tool for developing new cancer therapies and a convenient protocol for educational purposes in academia. This research has been supported by the Sonata Bis grant 2021/42/E/NZ7/00422 from the National Science Centre, Poland. 1. Liu, W. et al. Discovery of Novel p53-MDM2 Inhibitor (RG7388)-Conjugated PlatinumIV Complexes as Potent Antitumor Agents. J Med Chem 67, 9645–9661 (2024). 2. Skalniak, L. et al. Prolonged idasanutlin (Rg7388) treatment leads to the generation of p53-mutated cells. Cancers (Basel) 10, (2018).

P-03-026
Nuclear translocation of desmin in skeletal muscle: a mechanotransduction-dependent process

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Studies have demonstrated that cytoskeletal proteins, traditionally considered cytoplasmic, can translocate into the nucleus and play essential roles in nuclear functions. Desmin, a muscle-specific intermediate filament protein primarily associated with structural integrity, has been extensively studied for its cytoplasmic functions. However, recent findings challenge this conventional understanding by revealing its nuclear localization and potential regulatory roles. In our study, we identified desmin within the nuclei of human and mouse skeletal muscle cells. To determine whether this localization is influenced by mechanotransduction, we examined its distribution under mechanical strain. Our findings indicate that nuclear desmin colocalization increases significantly in response to mechanical

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loading, following a strain-dependent pattern. This study highlights a previously unexplored aspect of desmin function, offering new insights into its role in nucleocytoplasmic communication in skeletal muscle cells. Our findings contribute to the growing understanding of how mechanical cues regulate intracellular protein dynamics and their potential implications for muscle biology. This research was supported by TUBITAK projects, 120E472 and 120Z946.

P-03-027
Smc2os, a novel lncRNA acting as modulator of sarcomere and cardiomyocyte proliferation

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Myocardial infarction (MI) is characterized by cardiomyocyte death secondary to ischemia. Regenerating the affected MI myocardium is one of the pivotal challenges of current molecular medicine. Recently, several reports demonstrated that sarcomere disassembly is required for cardiomyocyte proliferation after injury. Here, we explored the functional role of distinct *in silico* predicted lncRNAs that might interact with sarcomeric proteins – cardiac troponin, titin, myosin heavy chain and alpha cardiac actin – as a novel approach to modulate sarcomere disassembly. Seven different lncRNAs were identified and expression analysis demonstrated differential embryonic and adult heart expression. Among them, Smc2os displays a broad expression in several cardiac cell types and is subcellularly enriched in the cytoplasm in cardiomyocytes. RNA immunoprecipitation assays demonstrated that Smc2os interacts with cardiac TnI, but not with TnT, revealing a specific physical interaction with the troponin regulatory subunit. A gain-of-function assay of this lncRNA demonstrated that Smc2os overexpression increases mRNA and protein levels of cardiac troponin as well as distinctly balancing the expression of myosin heavy chain transcripts. Furthermore, Smc2os overexpression reduces cardiomyocyte proliferation, suggesting a possible role modulating cycle cell progression, by altering sarcomere structure. Moreover, RT-qPCR analysis proved that Smc2os is upregulated in infarcted hearts and hypertrophic mouse models. Finally, the human homologue – Smc2os_HSA – is capable of binding specifically to cTnI, but not cTnT, in line with the murine Smc2os lncRNA, demonstrating a conserved function between mice and humans. Taking all data together, we provide evidence of the first physical interaction between one lncRNA – Smc2os – and cTnI subunit of cardiac troponin, accompanied by the modulation of the cardiomyocyte proliferation rate, suggesting a possible role for this lncRNA altering sarcomere structure and function.

P-03-028
A comprehensive *in vitro* strategy for senescent cell identification using multiparametric analysis

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Cellular senescence plays a critical role in both pathological processes and normal tissue renewal and aging. It can be triggered by chronic stress factors or intrinsic mechanisms, including replicative exhaustion. Senescent cells undergo significant alterations in signaling pathways, chromatin organization, and gene expression. The mechanisms of senescence vary by physiological context, leading to a distinct spectrum of markers specific to different cell types and their microenvironment. In the absence of standardized detection methods, a promising approach combines multiple biomarkers, such as SA-β-Gal, with morphological assessment via confocal and scanning electron microscopy (SEM). This study presents a methodology for evaluating senescence in primary mouse skin fibroblasts. Cells were categorized into groups: fibroblasts from young and old donors, as well as those subjected to replicative exhaustion. Senescence was induced by doxorubicin, cisplatin, bleomycin, etoposide, H_2O_2 and assessed by β-galactosidase activity, organelle staining with specific fluorescent markers, SEM, and molecular marker expression analysis. Senescent fibroblasts exhibited distinct morphological changes, including increased size, and electron-dense structures near the nucleus. SA-β-Gal levels varied depending on the inducing factor. Differences in morphology and marker expression indicate phenotypic heterogeneity among senescent cells, highlighting the need for a comprehensive identification strategy. The findings indicate that aging and replicative exhaustion are associated with alter cellular architecture and increase SA-β-Gal activity. However, this marker has limited specificity, particularly across different senescence-inducing factors. Future studies should employ multiparametric approaches integrating morphological analysis, gene expression profiling, and functional characterization of senescent cells. This work was funded by the Russian Science Foundation (project No24-15-20003). *The authors marked with an asterisk equally contributed to the work.

P-03-029
RAB1A regulates the capacity of the oncoprotein GOLPH3 to form a distinct type of biomolecular condensate

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GOLPH3, a Golgi apparatus phosphoprotein, plays diverse roles in several cancers, including breast cancer, yet its regulation remains unclear. We discovered that in human cells, GOLPH3 interacts with RAB1A, and that RAB1A knockdown causes GOLPH3 relocation from the Golgi apparatus to distinct cytoplasmic structures. To determine whether these structures are biomolecular condensates formed by liquid-liquid phase separation (LLPS), we used fluorescence microscopy in fixed breast cancer cells, fluorescence recovery after photobleaching (FRAP) in live cells, and correlative light-electron microscopy (CLEM) in fixed cells after RAB1A knockdown. Additionally, we tested recombinant GOLPH3 for its capacity to undergo LLPS *in vitro*. We found that RAB1A knockdown induces the formation of GOLPH3-containing structures resembling biomolecular condensates, which transition from high to low liquidity and are distinct from stress granules and P-bodies. Moreover, CLEM analysis revealed a unique ultrastructure. Furthermore, we demonstrated that recombinant GOLPH3 can undergo LLPS *in vitro*. Our findings suggest that RAB1A regulates GOLPH3's unexpected ability to form biomolecular

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condensates, which may play a role in cancer cell phenotypic plasticity and tumor progression.

P-03-030
Profiling the proximity interactome of Ki67 in a cell cycle dependent manner

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Ki67 is a widely used proliferation marker in oncology. Recent studies have shown that Ki67 plays role in heterochromatin organization, ribosome biogenesis and maintenance of mitotic chromosomes[1,2]. The absence of Ki67 results in a coalescent chromosome phenotype during mitosis[3]. However, the molecular function of Ki67, whose expression and localization are dynamic throughout the cell cycle, has not been fully elucidated. To investigate Ki67's role in cell division, it is important to map its proximity interactome in a cell cycle dependent manner. Since Ki67 is localized on the surface of condensed chromosomes during mitosis and observed in the nucleolar region during interphase, we speculated that the interaction networks would be stage specific. Moreover, given that Ki67 is a very large protein (359 kDa), we hypothesized that its N- and C-terminal specific interaction networks may also show differences. To test this, we employed miniTurbo-based proximity labeling combined with mass spectrometry analysis. Hela Kyoto cells expressing miniTurbo-tagged Ki67 were synchronised to mitosis and interphase, followed by biotin supplementation to induce proximity labeling. Biotinylated proteins were then affinity purified using streptavidin beads, subjected to tryptic digestion, and analysed with LC-MS/MS. Proteomic analyses revealed that the interaction networks of Ki67 differ not only between N- and C-terminals, but also between mitosis and interphase stages. Identifying both common and distinct interaction partners across cell cycle stages will provide deeper insights into Ki67's role in cell division. References 1. Sobecki, M. et al. (2016) eLife 5: e13722. 2. Takagi, M. et al. (2016) Genes to Cells 21, 1113-1124. 3. Cuylen, S. et al. (2016) Nature 535, 308–312.

P-03-031
Saffron-derived exosome-like nanoparticles promote the proliferation of mouse GC-1 spermatogonia cells through the upregulation of VEGF-A

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Exosome-like nanoparticles (ELNs) are extracellular vesicles that mediate intercellular communication, influencing cell proliferation, differentiation, and tissue repair. Saffron (*Crocus sativus* L.), a medicinal plant rich in bioactive compounds, exhibits pro-proliferative and angiogenic effects in various cell types, primarily through VEGF-A upregulation. While saffron-derived bioactive molecules have been well studied, the effects of saffron-derived ELNs on spermatogonia cells remain unclear, requiring further investigation. In this study, Mouse GC-1 spermatogonia cells were cultured in DMEM with 10% FBS. ELNs were isolated from saffron extract via ultracentrifugation and characterized using scanning electron microscopy. Cell viability was assessed via the MTT assay, while migration capacity was evaluated using a wound healing assay. Flow cytometry was performed for cell cycle analysis, and VEGF-A expression was quantified via ELISA. Statistical analyses were conducted using GraphPad Prism 10. ELN treatment significantly enhanced GC-1 cell proliferation in a dose and time-dependent manner, with the highest proliferation observed at 50 ng/mL after 48 hours ($p < 0.05$). Wound healing assays showed increased migration, with a significantly faster closure rate in ELN-treated groups ($p < 0.05$), suggesting enhanced regenerative potential. Flow cytometry revealed a notable increase in the S-phase cell population, indicating accelerated cell cycle progression ($p < 0.05$). ELISA confirmed VEGF-A upregulation following ELN treatment ($p < 0.05$), reinforcing its role in ELN-induced proliferation. These findings suggest that ELNs act as biogenic modulators of germ cell proliferation, likely through VEGF-upregulation. Their ability to enhance cell proliferation and migration highlights potential applications in reproductive medicine, particularly in fertility preservation and spermatogonial stem cell-based therapies. However, further *in vivo* studies are required for clinical translation. *The authors marked with an asterisk equally contributed to the work.

P-03-032
Senescence-associated alterations in PML bodies in human fibroblasts

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Aging is one of the fundamental physiological processes with age-related changes at various levels of biological organization. One aspect of organismal aging is the acceleration of replicative aging or cellular senescence. It is known that many proteins are involved in these processes, including the promyelocytic leukemia (PML) protein, which is a core component of nuclear membraneless organelles known as PML bodies. However, the molecular mechanisms underlying the involvement of the PML protein and PML bodies in aging and senescence remain poorly understood. In this study, immunofluorescence analysis was used to investigate morphological changes in PML bodies and alterations in the localization of the PML protein during the transition of primary fibroblasts from healthy donors and donors with accelerated aging syndromes (ataxia-telangiectasia, Cockayne syndrome) to a senescent state. An increase in the size and number of PML bodies, as well as an increase in nuclear size, was observed in senescent cells, suggesting that these changes could serve as potential markers of cellular aging. Bioinformatics analysis and mass-spectrometry analysis of proteins of primary fibroblasts from different donors revealed that 30% of the proteins in the PML interactome and 45% of the proteins in the PML body proteome are directly associated with senescence and aging processes (according to the CellAge and GenAge databases). It is hypothesized that these proteins are involved in post-translational modifications and protein sequestration within PML bodies, influencing the activation/inactivation of transcription factors, the DNA damage response, and the negative regulation of apoptosis. The obtained results highlight the important role of PML bodies in cellular aging processes and open new avenues for exploring the mechanisms of senescence and age-related diseases.

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P-03-033
Mapping interactions of CCDC43 using proximity-dependent biotinylation labeling

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CCDC43 (Coiled coil domain containing protein 43) is a eukaryotic protein with alpha-helical domains consistent with the CCDC family. CCDC43 contains a putative RNA binding site in its protein structure and is widely localized in the cytoplasm. This protein, which has been studied at varying expression levels in different cancer types, has been shown to play a role in cell proliferation, invasion, metastasis, and epithelial-mesenchymal transition in gastric and colorectal cancers. To date, CCDC43 studies have predominantly been performed in cancer cells, but there is limited information on its basic function and cellular interactions. In this study, we used a proximity-based biotin identification (BioID2) screening approach to reveal protein–protein interactions to elucidate the cellular significance of CCDC43. Using mass spectrometry integrated with streptavidin pull-down, we identified protein interactors for CCDC43. Our results suggested YBX-1, a highly conserved cold shock domain and nucleic acid-binding protein with multiple essential functions in the cell, as the strongest candidate.

P-03-034
Investigating the RNA binding protein HuR in actinomycin D-induced stress response and its implications for cytosolic condensate formation

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Stressors in cancer cells can lead to the formation of cytosolic condensates that play roles in the translation, degradation or protection of mRNAs for survival. mRNAs are known to be regulated post-transcriptionally by RNA Binding Proteins (RBP), microRNAs, etc. to regulate gene expression. Some RBPs are involved in the formation of cytosolic condensates where they act as matchmakers of functionally related mRNAs and proteins. HuR is a stabilizing RBP that binds to target mRNA in the nucleus and transports it to the cytoplasm for post-transcriptional regulation. HuR was reported to be found in various cytoplasmic phase separated condensates, suggesting a possible role of the protein in stress response. Actinomycin D (Act D) inhibits transcription and is therefore considered to be a stressor. We observed that short term treatment of cancer cells with Act D led to the translocation of many RBPs to the cytoplasm including HuR, which is likely to have crucial implications on mRNA stability. A Turbo-ID assay followed by mass spectrometry indicated that upon Act D treatment, several different RBPs could interact with HuR. Many of these RBPs are known to be a part of different forms of membraneless condensates. All cytoplasmic condensates identified to date invariably contain RBPs; therefore, we evaluated if HuR forms such structures with Act D treatment. We observed that indeed HuR formed bright, punctate structures in the cytosol, resembling condensates. This study is the first to show that Act D-mediated global inhibition of transcription leads to a stress response whereby cytoplasmic aggregates are formed upon the translocation of several different RBPs, including HuR, to the cytoplasm perhaps to protect their constituent mRNAs, which can be translated when the stressors are removed. Our study therefore provides novel insights into why RBPs move to the cytosol when transcription is globally inhibited.

P-03-035
Searching the role of AKT expression on adiponectin induced cardiac cells

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Diabetic cardiomyopathy (DCM) refers to abnormalities in the structure and function of the myocardium resulting from diabetes. In the healthy heart, glucose and long-chain fatty acids are used equally as energy sources. There is a dynamic balance in their uptake and transport. Adiponectin (APN) is an adipokine synthesized and secreted by adipose tissue. APN concentration is reduced in insulin resistance and type 2 diabetes. APN prevents insulin resistance by increasing fatty acid excretion and glucose by skeletal muscle. Insulin-activated phosphatidylinositol 3′-kinase-dependent serine/threonine kinase AKT functions as a regulator of glucose transport, glycolysis, glycogen synthesis, suppression of gluconeogenesis, protein synthesis, lipogenesis, cell survival, cell size determination, and cell cycle progression. Accumulation of AKT in the plasma membrane of insulin-stimulated adipocytes results in different substrate specificity and involvement in the regulation of GLUT4 transport. Activated AKT converts to p-AKT which translocates to the nucleus to activate its substrates. p-AKT can phosphorylate a wide range of proteins. The aim of this study is to search the role of AKT expression on adiponectin-induced cardiac cells *in vitro*. The cardiac characteristics of H9c2 cells were confirmed through immunofluorescence staining followed by fluorescence microscopy imaging of muscle-specific desmin intermediate filaments, cardiac-specific myosin and perinuclear atrial natriuretic factor (ANF). A DCM model was created using the H9c2 cell line and time course experiments were made with different concentrations of adiponectin and to examine the expression change of Akt by western Blot method. An immunofluorescence study showed the cardiac behaviour of the H9c2 cell line. Time-dependent treatment of APN at different concentrations in H9c2 cells resulted in changes in AKT expression. This study aims to define new mechanisms that play a role in the pathogenesis of diabetic cardiomyopathy.

P-03-036
Intracellular movement and stress-triggered condensation of CCDC124 protein

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Inside cells, numerous biochemical reactions occur simultaneously, requiring precise spatial and temporal

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organization to maintain cellular function. Many of these processes are coordinated within membrane-less organelles, which are dynamic compartments that lack a surrounding lipid bilayer. These structures provide specialized microenvironments for essential cellular activities, such as RNA metabolism, stress responses, and signal transduction. Recent studies have shown that membrane-less organelles are often formed through liquid–liquid phase separation (LLPS), where biomolecules condense into distinct liquid-like droplets, physically separated from the surrounding cytoplasm. LLPS plays a critical role in dynamically organizing the intracellular space and facilitating efficient biochemical interactions. In our research, we focused on the localization and subcellular translocation of the Ccdc124 protein. Using advanced microscopy techniques, we observed that Ccdc124 shows dynamic localization patterns, moving between a variety of different cellular compartments including nucleoli, centrosomes, stress-granules, or mitochondria in response to various stimuli. Interestingly, under specific cellular stress conditions, Ccdc124 formed liquid-like condensates, exhibiting characteristic features of phase-separated structures, including spherical shape, fusion events, and the ability to dissolve upon environmental changes. These findings suggest that Ccdc124 may contribute to cellular organization through phase separation, potentially acting as a scaffold to cluster specific interaction partners. Further research into the molecular mechanisms driving Ccdc124's phase behavior could provide valuable insights into the role of phase separation in cellular homeostasis and adaptive responses.

P-03-037
Src and Hippo signaling pathway relationship in adipose-derived mesenchymal stem cells

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Understanding stem cell proliferation mechanisms is crucial for their more effective clinical application. While FGF-2 promotes the proliferation of mesenchymal stem cells (MSCs) via Src activation, the exact interaction with Hippo pathway and YAP needs clarification. In our study to determine Src/Hippo/YAP relationship in the proliferation of MSCs with FGF-2, cells were isolated from lipoaspirate and characterized by flow cytometry and differentiation capacity. FGF-2 concentration was determined by WST-1 assay. PP1 was used as an Src inhibitor. Key proteins (Src, pSrc, LATS1, pLATS, YAP, pYAP-Y357, pYAP-S127, TEAD, and PCNA) were determined by western blotting. Localization of pYAP-Y357, pYAP-S127, YAP and TEAD was shown by immunofluorescence. YAP-TEAD interaction in the nucleus was evaluated by co-immunoprecipitation. FGF-2 treatment increased cell viability and PCNA levels by 1.4-fold, while both decreased in the PP1 + FGF-2 group compared to the control ($p \leq 0.01$) and FGF-2 group ($p \leq 0.001$). The p-Src levels increased 1.15-fold with FGF-2 treatment ($p \leq 0.05$) but decreased significantly in the PP1 + FGF-2 group ($p \leq 0.0001$ for both comparisons). Total YAP levels showed no significant change, while pYAP-Y357 increased with FGF-2 ($p \leq 0.01$ vs control), but decreased in the PP1 + FGF-2 group ($p \leq 0.01$ vs FGF-2). The level of p-LATS protein did not change with FGF-2, but increased 2.4-fold in the PP1 + FGF-2 group. TEAD protein levels increased 1.3-fold with FGF-2 ($p \leq 0.05$) but decreased in the PP1 + FGF-2 group compared to the FGF-2 group. FGF-2 increases pYAP-Y357 via Src activation and inactivates the canonical Hippo pathway through LATS1, promoting YAP translocation to the nucleus, and enhancing proliferation. The Src inhibitor PP1 reverses these effects. In conclusion, FGF-2 regulates cell proliferation through the Src/Hippo-YAP pathway. This study is supported by Hacettepe University Scientific Research Projects Coordination Unit (Project No: TSA-2022-19706).

P-03-038
The effect of microRNAs within extracellular vesicles of mesenchymal stromal cells on myofibroblasts dedifferentiation in cellular models of fibrosis

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Multiple microRNAs (miR) are involved in the regulation of fibrosis, a pathological process that leads to the connective tissue overgrowth due to the proliferative and synthetic activity of activated stromal cells and myofibroblasts. We found that specific miRs transferred within extracellular vesicles secreted by mesenchymal stromal cells (EV-MSCs) inhibit fibrosis. To reveal the molecular mechanisms, we explored how EV-MSCs and selected miRs as their cargo regulated myofibroblast dedifferentiation during fibrogenesis. The EV-MSC fraction was obtained by ultrafiltration of MSC conditioned medium and used in different models of fibrosis. We observed reorganization and decrease of fibrotic extracellular matrix (fECM) with *ex vivo* mice precision-cut fibrosis lung slices and *in vitro* 3D fibrotic focus model. To study mechanisms of cellular behavior in more detail, we added EV-MSCs to the 2D-model of myofibroblast culture. That led to a decrease in the myofibroblast marker signs and fECM proteins expression. To study miRs (selected by RNA-seq), we performed an inhibitory analysis by transfecting EV-MSCs with antagomiRs and adding them to myofibroblasts. We demonstrated that miR-129 and -92a contributed to a decrease in the signs of myofibroblasts and remodeling of fECM. Probably, this miRs stimulate myofibroblasts dedifferentiation to activated stromal cells due to shift in cells function. The mimics of these microRNAs added separately did not exhibit this effect indicating their activity only as part of EV-MSCs. Thus, we confirmed the role of EV-MSCs in the regulation of myofibroblast dedifferentiation and fECM remodeling using different model of fibrosis. We found that miR-129 and -92a contributed to this effect. These data can be further used to consider EV-MSCs and antifibrotic miRs as promising candidates for the treatment of fibrosis. The study was supported by the Russian Science Foundation (#23-15-00198, https://rscf.ru/project/23-15-00198/).

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P-03-039
Mechanical and biochemical signatures of pancreatic stellate cell activation

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Pancreatic fibrosis, driven by the pathological activation of pancreatic stellate cells (PSCs), is a hallmark of chronic pancreatitis and pancreatic cancer. Understanding the alterations that occur during PSC activation is critical for identifying potential therapeutic targets for pancreatic diseases. This collaborative interdisciplinary study integrates atomic force microscopy (AFM) and Raman spectroscopy to characterise the mechanical and biochemical alterations during pancreatic stellate cell activation. To achieve this, we used human PSCs in four distinct phenotypes: quiescent PSCs (qPSCs), spontaneously activated PSCs due to surface stiffness (sPSCs), and PSCs undergoing early (2 days, 2dPSCs) or late (5-7 days, 5/7dPSCs) activation induced by TGF-β. AFM revealed a progressive increase in PSC stiffness from qPSCs to 7dPSCs, accompanied by cytoskeletal reorganisation and α-SMA upregulation. Activated PSCs became progressively flattened and more rigid, as confirmed by AFM-based quantitative imaging, which mapped cellular stiffness and morphological alterations. Raman spectral analysis demonstrated a decrease in nucleic acid signal intensity in activated PSCs, indicative of chromatin decondensation. Concurrently, cytochrome levels increased, suggesting heightened metabolic activity. There was also significant lipid droplet accumulation and increased lipid unsaturation, particularly in 7dPSCs. Transcriptomic analysis supported these findings, showing upregulation of genes involved in fatty acid elongation, desaturation, autophagy-mediated lipid turnover, and membrane remodelling, alongside downregulation of de novo lipogenesis pathways. These findings suggest that PSC activation involves a coordinated interplay between mechanical adaptation and metabolic remodelling, enabling cells to meet their energy demands for extracellular matrix production while maintaining their altered morphology.

P-03-040
Oncostatin M primes mesenchymal stem cells, modulating their migratory, secretory, and miRNA-regulatory behaviors in a manner that may promote fibrosis resolution

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Fibrosis arises from excessive extracellular matrix accumulation, leading to organ dysfunction. Oncostatin M (OSM), an IL-6 family cytokine, regulates inflammation, tissue remodeling, and wound healing. Mesenchymal stem cells (MSCs) also play key roles. In a bleomycin-induced lung fibrosis model OSM-primed MSCs reduced fibrosis, lowered inflammation, and improved lung function. Here, we examined the effects of OSM preconditioning on bone marrow (BM) and umbilical cord (UC) MSCs. We evaluated surface marker expression, wound healing capacity, cytokine secretion, and fibrosis-related miRNA profiles. Both MSC types were primed with 2 ng/mL OSM. Flow cytometry assessed surface markers, a scratch assay measured migration, the Human Cytokine Array C5 determined cytokine profiles, and the Human Fibrosis Focus miRNA PCR Panel evaluated fibrosis-related miRNAs. A fibrosis score was created by categorizing differentially expressed miRNAs ($\geq$1.5-fold change) as fibrotic (-1) or antifibrotic (1), then integrating their fold-change values. Flow cytometry showed elevated HLA-DR (34.55%) and CD45 (14.4%) in OSM-primed BM-MSCs. The scratch assay revealed that OSM-primed UC-MSCs migrated 29% faster, while BM-MSC migration dropped by 36%. Cytokine analysis indicated reduced BDNF secretion in OSM-primed BM-MSCs, suggesting an antifibrotic shift. IL-6 and TIMP-2 levels rose in both MSC types. Twenty-four miRNAs were differentially expressed in BM-MSCs and 18 in UC-MSCs. Their fibrosis scores were 9.16 and 10.36, respectively, pointing to an antifibrotic phenotype. Overall, OSM preconditioning modifies MSC migration, cytokine secretion, and miRNA expression, potentially promoting fibrosis resolution. Further functional studies are needed to confirm these findings.
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P-03-041
Generation of simple and convenient set of cellular models for smart cell sorting surfaces optimization and tunning

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The aging population, genetic disorders, and injuries resulting from armed conflicts are significant challenges for the modern regenerative medicine. Current approaches require massive production of therapeutic cells to restore damaged tissues and organs. A breakthrough in this field was the discovery of induced pluripotent stem cells (iPSC), which can differentiate into any cell type under appropriate control. One of the main issues is transformation iPSC to cancer cells (cancerogenesis). The key challenge remains the effective selection of therapeutic cells. The commercial sorting methods, which help identify cells, need expensive antibodies. Two of these methods – fluorescence-activated cell sorting (FACS) and magnetic-activated cell sorting (MACS) – are based on specific antigen-antibody interactions. These methods are precise but may lead to mechanic damage and/or loss of target cells. In our project, we propose an innovative cell sorting method utilizing dynamic polymer brush interfaces and polymer hydrogels. The proposed sorting process is based on osmotic mechanisms and thermally induced phase separation in a polymer-water system, enabling dynamic cell separation without the need for antibodies. The efficacy of cell sorting is validated by the use of a panel of model cell lines of different tissue origin. We generated a set of fluorescently

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labelled cell lines of epithelial, mesenchymal, neural and hematopoietic origin. Enhanced green fluorescent protein (eGFP), its derivatives blue (eBFP) and yellow (eYFP) as well as mFruite variants mCherry and mOrange were used. The separate fluorescently labelled variants can be easily and readily detected with the fluorescence microscopy as both uniform cultures or co-cultured mixes. The proposed technology simplifies the process of cell sorting optimization and tuning. This work was supported by grants no. 2023/05/Y/NZ3/00189 National Science Centre, Poland, and National Science Foundation, USA.

P-03-042
Fine localization patterns of myosin 1C in prostate epithelial cells and prostate cancer specimens

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Myosin 1C is a monomeric motor protein belonging to the class I unconventional myosins. It plays key roles in modulating membrane tension, vesicle transport, tethering and secretion, as well as regulating transcription and chromatin remodeling in the nucleus. Furthermore, myosin 1C has been implicated in tumor progression, particularly in prostate cancer. Despite its significance in various cellular processes and potential prognostic value, the precise subcellular localization of myosin 1C remains poorly characterized. Clarifying its distribution is crucial for understanding the mechanisms of myosin 1C function in both normal and tumor cells. In this study, we investigated the localization of myosin 1C using immunohistochemistry on human prostate tissue sections. Myosin 1C was also visualized by high-resolution confocal microscopy in RWPE-1 (normal prostate epithelial) and PC-3 (prostate cancer) cell lines. Histological analysis revealed that myosin 1C is present in the ductal and acinar epithelial cells of the prostate gland, exhibiting an asymmetrical distribution within the cells, with accumulation predominantly at the luminal side. Quantitative analysis of subcellular localization in RWPE-1 and PC-3 cells demonstrated that, during interphase, myosin 1C preferentially localizes to active lamellar ruffles and the substrate-free regions of the plasma membrane. During mitosis, myosin 1C remains associated with the plasma membrane, showing minimal presence in the cytoplasm and an absence on chromosomes. Additionally, we analyzed the colocalization of myosin 1C with β- and γ-isoforms of cytoplasmic actin. In conclusion, our findings not only quantitatively confirm previously published data but also describe new patterns of myosin 1C localization in human prostate tissue and cell models under normal conditions and during tumor progression.

P-03-043
Functional heterogeneity of mesenchymal stromal cells in the profibrotic microenvironment

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Fibrosis is an undesirable outcome of tissue response to injuries. Multipotent mesenchymal stromal cells (MSCs) have a dual role in the development of fibrosis: they can contribute to the myofibroblast pool promoting fibrosis progression but also have the potential to inhibit fibrosis by releasing anti-fibrotic factors and microRNAs. In fibrotic tissue, MSCs are exposed to various stimuli significantly affecting the composition of both MSC subpopulations and their secretome. To explore this potential heterogeneity, we cultured human adipose-derived MSCs for 4 days with a key profibrotic factor TGFb on fibrotic-like decellularized extracellular matrix (ECM) produced by fibroblasts. Single cell RNA-seq (scRNAseq) was performed (10× Genomics) and datasets were analyzed using Seurat, development trajectories, a graph of latent time (pseudo-time) and RNA-velocity. Subpopulations with distinct transcriptomic patterns were separated by flow sorting; their functional properties were analyzed in models of migration, invasion and contraction as well as by proteomic analysis of cell secretome. ScRNAseq-based transcriptome profiling of MSCs cultured in profibrotic and standard conditions reveals subpopulations with different responses to profibrotic stimuli: part of cells differentiate into myofibroblasts, while others exhibit a distinctive transcriptomic pattern enriched by matrix-remodeling factors. The latter subpopulation, as analyzed by bioinformatics, can be isolated by sorting based on PDGFRα expression and, according to functional analysis, possess higher invasive properties and a reduced ability to contract ECM. The secretome of PDGFRα+ subpopulation is significantly depleted of ECM proteins. Therefore, MSCs include distinct subpopulations that exhibit varying functional responses to fibrotic stimuli which could have strong impact on the fibrosis development. The study was supported by the Russian Science Foundation grant 19-75-30007, https://rscf.ru/project/19-75-30007/.

P-03-044
Moved to LB-R-03-01

P-03-045
Ciliopathy protein CCDC66 is required for spindle positioning and cytokinesis

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CCDC66 is a protein associated with ciliopathies affecting the eyes and brain and plays a key role in the proper functioning of cilia. In addition to its association with the ciliary microtubules, our lab showed that CCDC66 localizes to spindle poles, mitotic and central spindle, and the midbody. CCDC66 depletion leads to defects in spindle orientation, midzone organization, and an increase of binucleated cells. However, the molecular mechanism of CCDC66's mitotic and cytokinetic roles are not yet fully

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understood. To address this question, we used loss-of-function experiments combined with various imaging approaches. First, we examined CCDC66-depleted cells progressing through cytokinesis using live imaging, which revealed delays or inhibition of furrow ingression formation and cytokinetic abscission. Moreover, we examined CCDC66's effect on spindle positioning and central spindle via immunofluorescence of known regulators of these processes and structures. In CCDC66-depleted cells, we observed impairment in the spatial distribution of NuMa, a crucial component of the cortical force-generating machinery, and the centralspindlin complex, which is essential for central spindle formation. We also found that depletion of KIF23/MKLP1, another component of the centralspindlin complex, disrupts CCDC66 localization during division. Additionally, our data suggest that CCDC66 affects the expression of KIF20A and Aurora B during anaphase and cytokinesis. Notably, there was a significant reduction in the levels of active Aurora B levels at the midbody in CCDC66-depleted cells. Collectively, our findings show that CCDC66 regulates crucial mitotic and cytokinetic factors. By highlighting the role of ciliary proteins like CCDC66 in non-ciliary processes, our findings provide insights into ciliopathies and related diseases, offering potential avenues for novel therapeutic approaches.

P-03-046
IIIG9 and PP1 alpha ciliary proteins form a protein complex in the adherens junctions of polarized ependymal and MDCK cells

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Madin-Darby canine kidney (MDCK) and ependymal cells are both polarized epithelial cell models, which can be used for identifying new molecular mechanisms underlying epithelial polarization and neoplastic events. IIIG9 was initially proposed to be a novel protein phosphatase 1 (PP1) regulatory subunit (PPP1R32) in ependymal cells, where it is localized in cilia with a dotted pattern and in the adherens junctions. However, it is unknown if IIIG9 is necessary for the specific function of PP1 in these regions. PP1 catalytic subunits (a, b, and g) are highly conserved and ubiquitous Ser/Thr phosphatases. PP1a is localized to the cilia of *Chlamydomonas* and in the cell junctions of MDCK cells. Here, we have characterized the expression of PP1 subunits in ependymal cells by qRT-PCR coupled with laser microdissection and *in situ* hybridization. *In situ* and *in vitro* colocalization of catalytic PP1 and IIIG9 isoforms was analyzed by confocal microscopy, showing that IIIG9 and PP1a colocalized in cilia and adherens junctions. Similar analyses were done in 3D and cysts of MDCK cultures. FRET analysis was used to analyze the *in vitro* interaction between IIIG9 and PP1a in HEK cells, and IIIG9/PP1a interaction in the adherens junctions and cilia was examined by proximity ligation assays in adult brain and MDCK cells. Our data confirm IIIG9/PP1a interaction in ependymal cells and MDCK cells. This interaction may drive the subcellular action of PP1 in adherens junctions and cilia of polarized cells. Funding: Fondecyt grant N° 1240486 (KS). *The authors marked with an asterisk equally contributed to the work.

P-03-047
Dissecting molecular and functional regulation of centriolar satellites by DYRK3 kinase

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Centriolar satellites are membrane-less granules that cluster around the centrosome. They are scaffolded by pericentriolar material 1 (PCM1) and regulate a wide range of cellular processes such as primary cilium assembly, Hedgehog signaling, motility, autophagy, and proteostasis. Although phosphorylation of PCM1 by mitotic kinases is known to control centriolar satellites' role in ciliogenesis, the precise regulatory mechanisms remain poorly understood. DYRK3 (Dual-specificity tyrosine-phosphorylation-regulated kinase 3) is a multifunctional enzyme involved in key cellular processes such as cell cycle progression, mTORC1 signaling, secretory trafficking and cell proliferation. Recent studies show that DYRK3 promotes dissolution of membrane-less organelles including centriolar satellites in both interphase and mitotic cells. While proteomics studies have identified an interaction between DYRK3 and PCM1, the specific PCM1 phosphorylation sites targeted by DYRK3 and their functional significance remain unknown. In this study, we aim to identify the DYRK3-mediated phosphorylation sites on PCM1 and their role in centriolar satellite-regulated processes. We first confirmed the interaction between DYRK3 and various PCM1 deletion mutants to map the regions required for phosphorylation. Next, we performed lambda phosphatase treatment to confirm the phosphorylation of the DYRK3-interacting mutants. Additionally, *in vitro* kinase assays using purified PCM1 deletion constructs further confirmed DYRK3-dependent phosphorylation. Our results demonstrate that DYRK3 interacts with and phosphorylates PCM1 at the specific site both *in vivo* and *in vitro*. Identification of PCM1 phosphosites and generation of phospho-mutants will provide insights into DYRK3-mediated regulation of PCM1 in various processes, including cell cycle, ciliogenesis and signaling.

LB-R-03-01
CSPP1 has key regulatory function in differentiation processes of multiciliated epithelial cells

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Multiciliated cells (MCCs) across various organisms and tissues exhibit shared transcriptional profiles and differentiation program including centriole amplification mechanisms, and ciliogenesis. However, differences arise in basal body distribution and number, cilia length, and beating frequency. CSPP1 has been identified as a critical protein for proper primary cilia formation, with established expression profiles in lung MCCs and

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localization at motile cilia tips. Recent investigations have unveiled interactions between CSPP1 and proteins associated with Joubert syndrome, namely Armc9 and TOGARAM1. Utilizing mouse tracheal epithelial cells (mTECs) and *Xenopus laevis* models we implemented a focused strategy to study CSPP1 implication in MCCs. Our observations revealed various dynamic/various localization of CSPP1 in differentiating and mature MCCs. Functional approaches revealed abnormalities during differentiation of multiciliated cells upon depletion of CSPP1. Additionally, phenotypic outcomes show high degrees of similarity in both model systems. Altogether, these findings extend previous research offering deeper insights into the conserved functional role of CSPP1 during MCC differentiation and development. *The authors marked with an asterisk equally contributed to the work.

LB-R-03-02
Golgi-resident VAPA/B plays an essential role in initiating Rab9-dependent alternative autophagy

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VAMP-associated proteins A (VAPA) and B (VAPB) are primarily localized to the endoplasmic reticulum (ER) and are known to regulate the initiation of LC3-dependent conventional autophagy via interactions with the ULK1 complex. However, their role in Rab9-dependent alternative autophagy remains largely unexplored. This study investigates the role of the membrane-tethering proteins VAPA and VAPB in regulating Rab9-dependent alternative autophagy at the Golgi apparatus, which serves as the initiation site for this pathway. Immunoprecipitation assays revealed that VAPA/B interact with Rab9. Subcellular fractionation and confocal microscopy showed that VAPB, but not VAPA, translocates from the ER to the Golgi apparatus under autophagy-inducing conditions in wild-type cells. In VAPB knockout cells, VAPA was shifted to the Golgi, suggesting a compensatory mechanism. Notably, alternative autophagy activity, indicated by the phosphorylation of Rab9 at Ser179, was significantly impaired in VAPA/B double knockout cells even under autophagy-inducing conditions. Moreover, VAPA/B were found to interact with WIPI4, and their loss markedly reduced WIPI4 puncta formation upon Tat-Beclin1 treatment. These findings identify VAPA/B as key regulators of Rab9-dependent alternative autophagy, acting at the Golgi apparatus. Their dynamic localization and interaction with Rab9 and WIPI4 highlight their essential role in initiating alternative autophagy and provide new insights into its regulation.

LB-R-03-03
Tensin 1 condensation regulates integrin adhesion dynamics

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Integrin adhesion complexes (IACs) mediate cell interaction with the extracellular matrix (ECM) components and represent one of the key determinants in maintaining tissue homeostasis. While the function and mechanistic regulation of IACs are currently relatively well understood, the emerging concept of biomolecular condensation by liquid-liquid phase separation in adhesion proteins has prompted investigations into the regulatory role of phase separation in IAC function. There is increasing evidence showing importance of protein condensation in organizing and compartmentalizing cellular components and regulating cellular processes, highlighting the need for study on the role of phase separation in IACs. Here we focus on an IAC scaffold protein from the tensin family, tensin 1 (TNS1), and its role in regulating IAC dynamics in cell migration. Using confocal and correlative light-electron microscopy methods, we demonstrated that TNS1 forms biomolecular condensates in cells in a concentration-dependent manner. At endogenous protein levels, TNS1 condensation is regulated by availability of integrin ECM ligands and by actomyosin contractility. Employing proximity labelling proteomics (BioID) we identified potential components of TNS1 condensates. Subsequent validation confirmed selective recruitment of several IAC proteins into TNS1 condensates and their role in buffering local concentrations of inactive adhesion components. Using experimental approaches and molecular dynamics simulations, we further identified the regions driving TNS1 condensation and their role in regulating IAC dynamics, cell migration and invasion. Finally, we demonstrated that TNS1 phosphorylation by stress-activated kinases regulates TNS1 condensation and IAC dynamics. Together, our findings highlight TNS1 condensation as a regulatory mechanism controlling local availability of inactive adhesion proteins, with direct implications on adhesion dynamics and cell behaviour.

LB-R-03-04
Kinase activity of DYRK family members is required for regulating primary cilium length, stability and morphology

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The dual-specificity tyrosine-phosphorylation-regulated kinase (DYRK) family are multifunctional enzymes crucial for diverse cellular processes, including signaling through the primary cilium. Their dysregulation has been implicated in various cancers and developmental disorders, highlighting the need to define their interactors and cellular functions to inform targeted therapeutics. In this study, we generated the proximity interactome of DYRK3, identifying 178 proteins involved in a range of cellular processes, including primary cilium biogenesis. We then investigated the specific role of DYRK3 and its cooperation with other DYRK family members in cilium assembly and maintenance. RNAi-mediated depletion of DYRK3 and pharmacological inhibition of DYRK kinase activity using GSK-626616 (GSK) led to elongation of the cilium, particularly its distal segment. GSK treatment also induced ciliary defects, length fluctuations, and increased ectocytosis. Co-depletion and phenotypic rescue experiments revealed that DYRK2 and DYRK3 cooperate in regulating cilium length. Moreover, inhibiting or depleting known cilium length regulators, or quantifying their ciliary levels in GSK-treated cells, revealed functional relationships of DYRKs to centriolar satellites and the IFT complex. Collectively, our findings uncover regulatory roles for DYRK3 and DYRK kinase activity in the assembly and maintenance of primary cilium with proper length, stability, and morphology.

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LB-R-03-05
Identifying pre-analytical, analytical, and post-analytical risks in flow cytometry-based stem cell characterization

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Flow cytometry plays a key role in the characterization of mesenchymal stem cells (MSCs). Given the high sensitivity of flow cytometry to sample preparation and instrument settings, rigorous control at each stage is vital to ensure reproducible and meaningful results. In this work, critical technical risks across the pre-analytical, analytical, and post-analytical phases were identified based on practical observations during stem cell research workflows. At the pre-analytical stage, issues such as inaccurate selection of MSC markers (e.g., overlooking CD73, CD90, CD105 positivity, CD45 and CD34 negativity, along with optional markers like CD44, HLA-DR, CD14, and CD19), use of compromised antibodies due to improper storage, and delays between staining and analysis – leading to signal degradation – were frequently encountered. Analytical challenges included incorrect voltage adjustments, suboptimal gating setup during data acquisition, and inadequate instrument calibration. Post-analytical risks mainly involved errors in data analysis and misinterpretation of sample populations, resulting in inaccurate reporting. These insights highlight the importance of systematically addressing critical factors at each stage of flow cytometry-based stem cell characterization to improve data reliability and reproducibility, and could support the development of standardized training modules and protocols to minimize technical variability in future stem cell studies.

LB-R-03-06
Development of affinity-tagged VHH proteins for capture of GFP-tagged proteins

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Antibodies play a role in regulating immune responses in the body. Due to their selective binding properties to other molecules, they are widely used in various scientific methods such as immunostaining and blotting. Traditional antibodies consist of light and heavy chain pairs with variable and constant regions and are approximately 150 kDa in size. However, animals like camelids also have antibodies about 70 kDa in size, made solely of heavy chains. Proteins called nanobodies have been developed by utilizing the variable regions of these heavy chains. Nanobodies, exhibit superior tissue penetration due to their small size (approximately 15 kDa), and disulfide bonds in their structure provide stability and durability. These features have significantly increased the use of nanobodies in scientific research. Studies conducted in our laboratory showed that some members of the ZBTB protein family form condensed structures in cells due to phase separation mediated by their BTB domains. These ZBTB proteins are also tagged with GFP. Based on this, the individual and condensed structures of these proteins will be used to produce/test nanobodies that recognize the GFP tag. To produce nanobodies that are suitable for co-immunoprecipitation (Co-IP), commercially available GFP-binding nanobody will be cloned into the Pet22b plasmid with two separate tags, Strep-Tag II and Avi-Tag. Inside of the cell the fusion protein will bind to GFP-tagged ZBTB proteins through the nanobody, while the Strep-Tag II and Avi-Tag enable the nanobody to bind to streptavidin beads. This construct will be prepared for use in Co-IP methods. The choice of different tags for streptavidin binding is due to the weaker binding of Strep-Tag II (~100 μM) compared to the strong biotin-streptavidin interaction provided by Avi-Tag (approximately 10 fM through biotinylation). At the end of this project, the purification capacity of the nanobodies will be evaluated via mass spectrometry (MS).

LB-R-03-07
Autoactive non-glycosylated FGFR1 (FGFR1.GF) has a different effect on gene expression than wild-type FGFR1

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Fibroblast growth factors (FGFs) and fibroblast growth factor receptors (FGFRs) create a system that regulates crucial cellular processes such as cell differentiation, division, motility, or death. Consequently, FGFR1 was found to be aberrant in several cancers. Fibroblast growth factor receptor 1 (FGFR1) is a heavily N-glycosylated receptor tyrosine kinase. Besides the dominating localization of FGFR1 to the plasma membrane, it was also found inside the cells. Nevertheless, the impact of FGFR1 N-glycosylation on its function is still undetermined. Here, we prepared the glycosylation-free (GF) mutant of FGFR1 (FGFR1.GF) and showed that it localizes to the nuclear envelope and endoplasmic reticulum. We verified the ability of intracellular FGFR1.GF to bind to FGF2 and discovered a high level of ligand-independent autoactivation [previously published in: Gregorczyk and Porebska et al. (2023) Cell Commun Signal 21:177]. To examine the mechanism of nuclear transport of FGFR1.GF, we performed treatment of human osteosarcoma cell line (U2OS) overexpressing FGFR1.GF (U2OS-SBP-R1.GF) with the inhibitor of nuclear import targeting Kpnβ1-mediated transport (INI-43). We observed the localization of the FGFR1.GF to the plasma membrane, similar to wild-type FGFR1. Furthermore, INI-43 treatment of the U2OS-SBP-R1.GF cells before incubation with the ligand resulted in diminishing the autophosphorylation of FGFR1.GF. Additionally, we confirmed the change in expression of 80 genes in the cells overproducing FGFR1 and FGFR1.GF using next-generation sequencing. Our data indicate that N-glycosylation of FGFR1 plays a critical role in its kinase activity regulation and Kpnβ1-dependent translocation to nuclear membrane, which has an impact on the cellular function of the receptor.

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LB-R-03-08
Intramembranal cleavage influences the localization and turnover of DNAse three prime repair exonuclease 1 (TREX1) with relevance to disease pathogenesis

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Signal peptide peptidase (SPP) is an aspartyl intramembrane protease localized to the ER membrane. Three prime repair exonuclease 1 (TREX1) is a tail-anchored protein resident in the same compartment. There are also reports about cytoplasmic and nuclear localization of TREX1, which currently need mechanistic explanation. TREX1 exhibits DNAse activity and through this function acts as a negative regulator of the DNA-sensing cGAS/STING pathway. TREX1 loss-of-function variants cause Aicardi-Goutières syndrome (AGS), a type I interferonopathy. We have identified TREX1 as a novel substrate of SPP, as explained in Tever OK et al. (2025) Cell. Mol. Life. Sci. 82,1 107. TREX1 processing by SPP regulates the enzyme by several means. First, SPP co-expression reduces TREX1 levels. Second, subcellular fractionations conducted in an inducible SPP overexpression cell line indicate that SPP-mediated proteolysis generates a shorter TREX1 fragment that localizes to the cytosol. Generation of this fragment is blocked by pharmacological inhibition of SPP. We further demonstrate that TREX1 proteolysis by SPP depends on its transmembrane segment, which is subject to disease-associated mutations. The AGS-associated T303P mutation enhances the turnover of TREX1 by SPP, leading to a significant amount of cytosolic TREX1 even without SPP overexpression. Inhibition of SPP partially restores TREX1 levels in fibroblasts from patients homozygously carrying the T303P mutation, indicating the pathophysiological relevance of SPP-dependent TREX1 processing. Furthermore, TREX1 protein levels are regulated by proteasomal degradation. Inhibition of ubiquitin E1 ligase causes TREX1 accumulation also with the no-lysine mutant. Accumulation of TREX1 is observed in ubiquilin 1/2/4 KO cells pointing to a non-canonical form of ubiquitination. Altogether, SPP-mediated intramembrane proteolysis acts as a regulator of TREX1 influencing its localization and turnover with implications of disease pathogenesis. *The authors marked with an asterisk equally contributed to the work.

LB-R-03-09
Analysis of galectin-1 from the long-lived naked mole rat

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The naked mole-rat (*Heterocephalus glaber*) is a rodent species that has gained attention among researchers due to its longevity, numerous adaptations to hypoxia and resistance to diseases, including cancer. Galectins are proteins that play a role in variety of cellular processes such as proliferation, adhesion and apoptosis by binding to intracellular and extracellular glycans. Recently, the influence of galectins on the activation of fibroblast growth factor receptor (FGFR) has been demonstrated. Here we show the analysis of biochemical properties of previously unstudied galectin-1 from the naked mole-rat and comparison to its human counterpart. We demonstrate that naked mole-rat galectin-1 exhibits a stronger binding affinity to FGFR compared to human galectin. It also participates in the activation of FGFR signaling pathways and influences cell proliferation *in vitro*. To our knowledge, this is the first study to focus on the properties of galectin from this long-lived rodent. This research was funded by SONATA BIS grant (2019/34/E/NZ3/00014) from the National Science Centre.

LB-R-03-10
Evaluation of the relationship between ACE2 expression and SIRT1 in granulosa cells under energy stress

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Reproductive cells are highly susceptible to stress and require adaptive responses to maintain functionality. Granulosa cells, key regulators of folliculogenesis and steroidogenesis, are essential for oocyte maturation and ovulation even under stress, such as during assisted reproductive technologies (ART). Diminished ovarian reserve (DOR) reflects a decline in oocyte quantity and/or quality. Polycystic ovary syndrome (PCOS) is a prevalent endocrine metabolic disorder marked by hyperandrogenism, anovulation, and cystic ovaries. Sirtuins, particularly SIRT1, are critical regulators of metabolic homeostasis and cellular stress response. ACE2 is also expressed in granulosa cells and is involved in follicular development, steroidogenesis, and ovulation. Granulosa cells were isolated from PCOS and DOR patients undergoing IVF. Controls were from patients with male factor infertility. Cells were cultured and treated with AICAR to induce energy stress via AMPK activation. SIRT1 and ACE2 expression were assessed by immunofluorescence. SIRT1 inhibition was performed with EX527. Under energy stress, SIRT1 and ACE2 expression increased in PCOS granulosa cells, particularly with nuclear localization. SIRT1 inhibition further increased ACE2 levels in PCOS. However, in DOR cells, although basal SIRT1 expression was high, it did not increase further with stress. ACE2 expression declined following stress and further decreased with SIRT1 inhibition. These findings suggest distinct stress adaptation mechanisms in PCOS vs. DOR. Given the multifactorial nature of PCOS and DOR, further studies investigating additional metabolic and stress-related signaling pathways are crucial for developing targeted infertility treatments and improving reproductive outcomes. This research was funded by Hacettepe University (Grant No: THD-2023-20668). *The authors marked with an asterisk equally contributed to the work.

LB-R-03-11
Treacle-driven condensates promote fibrillar center assembly, rRNA transcription, and TopBP1-mediated DNA repair to maintain genome stability

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Liquid-liquid phase separation has proven instrumental in explaining the formation of molecular condensates like the

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nucleolus. The multilayered structure of the nucleolus arises from three key phase-separated components, which collectively enable the initial stages of ribosome biogenesis. While the proteins driving phase separation in the dense fibrillar (DFC) and granular components (GC) are well-studied, those responsible for organizing the fibrillar centers (FC) remain poorly characterized in the context of phase separation. In this work we demonstrate that the nucleolar protein Treacle functions as a molecular scaffold for fibrillar centers (FC) in human nucleoli, forming biomolecular condensates via liquid-liquid phase separation (LLPS). We identify structural determinants governing its phase separation behavior, enabling selective recruitment and concentration of transcription factors at ribosomal DNA (rDNA) loci while maintaining spatial segregation between FC and DFC. This compartmentalization ensures distinct localization of rRNA synthesis and subsequent processing steps. Disrupting Treacle's condensation capacity causes FC-DFC mixing, impairing rRNA synthesis/processing efficiency and mirroring phenotypes observed upon endogenous Treacle depletion. Furthermore, Treacle's phase separation capability is essential for recruiting the repair factor TopBP1 to activate rDNA damage responses. In this case, TopBP1 forms distinct phase-separated condensates on the scaffold of Treacle condensates, thereby promoting the localized recruitment of DNA repair factors to rDNA. Part of the presented work was published in: Velichko AK et al. (2025) eLife 13:RP96722. 21-64-00001-P, Russian Science Foundation, RSF.

LB-R-03-12
Microtubule-associated proteins guide Dishevelled cytoskeletal localisation

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Dishevelled (DVL) is a scaffolding protein involved in the Wnt signaling pathways, which are essential for both proper embryonic development and adult tissue homeostasis. Accumulating evidence has shown that DVL localizes to various microtubule-based structures within the cell, suggesting that its functions extend beyond the well-understood roles in the Wnt signaling. Despite progress in this field, the molecular details of the crosstalk between DVL and microtubules (MTs) remain somewhat elusive. According to our data, DVL lacks intrinsic MT-binding capacity and its recruitment to MTs depends on interaction partners that facilitate this association. Recently, we have identified several microtubule-associated proteins (MAPs) that have the ability to redirect cytoplasmic pools of DVL towards the microtubule networks. These findings open avenues for exploring diverse DVL functions in regulation of MTs organisation and dynamics, with implications for normal cellular physiology and disease.

Cell fate determination

P-04-001
Phosphorylation dynamics in the regulation of BNIP3L/NIX-mediated mitophagy

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Mitophagy is a specialized form of autophagy required for the selective removal of dysfunctional and unnecessary mitochondria. Impaired mitophagy is associated with a wide range of pathologies, including cardiovascular disorders, cancer, and various neurodegenerative diseases. While the PINK1/Parkin pathway is most extensively studied form of mitophagy, receptor-mediated mitophagy plays a fundamental role in cellular development and specialization. Studies have shown that BNIP3L/NIX mitophagy receptor is essential in the programmed removal of healthy mitochondria during the terminal differentiation of erythrocytes. Our research has demonstrated that BNIP3L/NIX-mediated mitophagy is regulated by receptor dimerization and phosphorylation of the LC3-interacting region (LIR). This regulation enhances the interaction between BNIP3L/NIX and autophagosomal LC3 proteins, facilitating the recruitment of mitochondria to the autophagosome. Despite recent advances in our understanding of the regulation of this process, there are still many questions as to how BNIP3L/NIX precisely governs mitophagy pathways within the erythroid lineage. This study aims to unravel the mechanism driving the efficient removal of healthy mitochondria, focusing on identifying kinases and phosphatases that regulate the phosphorylation status of BNIP3L/NIX. *The authors marked with an asterisk equally contributed to the work.

LB-R-04-01
FRMD6 determines the cell fate towards senescence

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Cellular senescence, a hallmark of aging, is pathogenically linked to the development of aging-related diseases. This study demonstrates that FRMD6, an upstream component of the Hippo/YAP signaling cascade, is a key regulator of senescence. Proteomic analysis revealed that FRMD6 is upregulated in senescent IMR90 fibroblasts under various senescence-inducing conditions. Silencing FRMD6 mitigated the senescence of IMR90 cells, suggesting its requirement in senescence. Conversely, the overexpression of FRMD6 alone induced senescence in cells and in lung tissue, establishing a causal link. The elevated FRMD6 levels correlated well with increased levels of the inhibitory phosphorylated YAP/TAZ. We identified cellular communication network factor 3 (CCN3), a key component of the senescence-associated secretory phenotype regulated by YAP, whose administration attenuated FRMD6-induced senescence in a dose-dependent manner. Mechanistically, FRMD6 interacted with and activated MST kinase, which led to YAP/TAZ inactivation. The expression of FRMD6 was regulated by the p53 and SMAD transcription factors in senescent cells. Accordingly, the expression of FRMD6 was

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upregulated by TGF-β treatment that activates those transcription factors. In TGF-β-treated IMR90 cells, FRMD6 mainly segregated with p21, a senescence marker, but rarely segregated with α-SMA, a myofibroblast marker, which suggests that FRMD6 has a role in directing cells towards senescence. Similarly, in TGF-β-enriched environments, such as fibroblastic foci (FF) from patients with idiopathic pulmonary fibrosis, FRMD6 co-localized with p16 in FF lining cells, while it was rarely detected in α-SMA-positive myofibroblasts that are abundant in FF. In sum, this study identifies FRMD6 as a novel regulator of senescence and elucidates the contribution of the FRMD6-Hippo/YAP-CCN3 axis to senescence.

LB-R-04-02
Thioredoxin interacting protein (Txnip) regulates the pluripotency circuitry during metabolic reprogramming and differentiation

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Thioredoxin interacting protein (Txnip) is a stress-responsive factor regulating Trx1 for redox balance and involved in diverse cellular processes including proliferation, differentiation, apoptosis, inflammation, and metabolism. However, the biological role of Txnip function in stem cell pluripotency has yet to be investigated. Here, we reveal the novel functions of Txnip in cellular reprogramming and differentiation onset by involving in glucose-mediated histone acetylation and the regulation of Oct4, which is a fundamental component of the molecular circuitry underlying pluripotency. During reprogramming or PSC differentiation process, cellular metabolic and chromatin remodeling occur in order to change its cellular fate. Txnip knockout promotes induced pluripotency but hinders initial differentiation by activating pluripotency factors and promoting glycolysis. This alteration affects that the intracellular levels of acetyl-CoA, a final product of enhanced glycolysis, resulting in sustained histone acetylation on active PSC gene regions. Moreover, Txnip directly interacts with Oct4, thereby repressing its activity and consequently deregulating Oct4 target gene transcriptions. Our work suggests that control of Txnip expression is crucial for cell fate transitions by modulating the entry and exit of pluripotency.

Development

P-05-001
Does loss of myosin VI expression affect the functional organization of mouse epididymal epithelium?

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The epididymis, a coiled tube divided into distinct regions, provides specialized environments necessary for the maturation and storage of sperm. Epididymal epithelial cells, characterized by the presence of microvilli and exocytic and endocytic activity, play a crucial role in creating an environment optimal for male gametes, depending on their location within different segments of the epididymis. Myosin VI (MYO6) is an important protein in maintaining the structural integrity and function of various specialized epithelia in mammals, such as intestinal microvilli, renal proximal tubules, and cochlear sensory epithelium. Studies have shown that in the polarized cells of these epithelia, MYO6 is mainly involved in endocytosis and serves a structural function by anchoring the plasma membrane at the base of the microvilli. Loss of MYO6 function in Snell's Waltzer mouse mutants disrupts the functional organization of specialized epithelia, leading to deafness, impaired food absorption in small intestine, and poor resorption in kidneys. In Drosophila, males with a mutation in the MYO6 gene are infertile. Our research, however, has shown that male Snell's Waltzer mice have reduced fertility by about 25%. This effect may partly be due to disturbances in sperm maturation in the epididymis. Indeed, our latest studies have, for the first time, shown that MYO6 is expressed in the epithelial cells of the epididymis, and the localization pattern of this protein, as well as selected adaptor proteins for MYO6, differs in various segments of the epididymis. Moreover, comparative analysis revealed an abnormal localization of clathrin and ultrastructural defects in the organization of microvilli in male Snell's Waltzer mice compared to wildtype mice. Our findings indicate that, similar to other specialized epithelia in mammals, MYO6 is an important protein in the functional organization of the epididymal epithelium, which potentially impacts sperm maturation and male fertility.

P-05-002
Abstract withdrawn

P-05-003
Identifying transcription factors regulating the 8-cell state in human embryos

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The 8-cell stage of human embryonic development is a critical stage characterized by zygotic genome activation (ZGA) and the establishment of transcriptional programs essential for further development. Despite its importance, the molecular mechanisms regulating this stage, particularly the role of transcription factors (TFs), remain poorly understood. Understanding these regulatory networks is crucial for elucidating early human development and addressing challenges in developmental biology and reproductive health. In this study, we aim to identify the key transcription factors that govern the transition to and maintenance of the 8-cell-like state. Utilizing a population of 8-cell-like cells (8CLCs) derived from human pluripotent stem cells, which mimic the transcriptomic and epigenomic features of the 8-cell stage. To address this, we developed TPRX1-mCherry reporter cell line, a reliable marker for 8-cell stage embryos, and conducted a transcription factor overexpression and perturbation screen to identify factors that enhance the population of mCherry-positive cells. Preliminary findings reveal a subset of transcription factors enriched in 8CLCs, suggesting their pivotal roles in the establishment of ZGA and early lineage specification. Functional perturbations of these factors have provided insights into their individual and combinatorial contributions to the 8-cell-like state. Our study also highlights the importance of chromatin accessibility and epigenetic modifications

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in coordinating transcriptional activity during this critical stage. By identifying and characterizing these regulatory elements, our work provides a deeper understanding of human embryogenesis, offering potential applications in reproductive health, infertility treatment, and regenerative medicine. This research establishes a foundation for unraveling the mechanisms governing early human development and opens new avenues for investigating transcriptional control in other developmental contexts.

P-05-004
Structural and biochemical alterations in zebrafish embryos exposed to toothpastes with different detergent contents

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The present study investigates the effects of toothpastes with different detergent contents on zebrafish (*Danio rerio*) embryonic development. Using Alcian Blue staining, the study evaluates head cartilage morphology and oxidative stress markers, superoxide dismutase (SOD), and glutathione (GSH), to assess detergent exposure's impact on oxidant-antioxidant balance. Acetylcholinesterase (AChE) activity was also determined to evaluate the cholinergic effects of the detergents. Zebrafish embryos were exposed to sodium lauryl sulfate (SLS) and cocamidopropyl betaine (CAPB)-containing toothpastes, while the TP3 group received a detergent-free toothpaste until 72 hours post-fertilization (hpf). Zebrafish embryos were stained with Alcian Blue to visualize cartilage structures. Measurements included Meckel's cartilage-to-ceratohyal (M-CH) distance, head width, and ceratohyal angle. The results indicate structural and biochemical changes due to detergent exposure. Alcian Blue staining revealed alterations in head cartilage morphology. GSH activity significantly decreased in TP1 and TP2 groups compared to controls, while TP3 showed a significant increase compared to TP2. AChE activity significantly decreased in TP1 and TP2, with no significant difference in TP3. SOD activity significantly increased in TP1 and TP2, while TP3 showed no significant difference from control. These findings underscore the impact of detergent exposure on zebrafish embryogenesis and provide crucial insights into cartilage development under different conditions. Our studies continue to elucidate the molecular mechanisms underlying the effects of toothpastes with different detergent contents on embryogenesis.

P-05-005
Curcumin potential as a key regulator of human adipose-derived stem cells adipogenesis

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Human adipose-derived stem cells (hASCs) are characterized by their capacity for self-renewal and differentiation into multiple cellular lineages, including the adipogenic lineage. Adipogenesis is defined as the process through which stem cells differentiate into mature adipocytes via the activation of signaling pathways, transcription factors, and genes that play a significant role in promoting adipocytes terminal differentiation. Several morphological and structural changes characterize the process by which preadipocytes transform into mature adipocytes. However, the excessive increase in adipose tissue (AT) volume is associated with the development of metabolic disorders. Current research is increasingly focusing on the potential regulatory role of natural compounds in AT development and obesity-associated pathways. Understanding the molecular mechanisms and actions of these bioactive molecules can provide valuable insights into novel, natural-based therapeutic strategies, addressing metabolic dysregulation and obesity-related diseases. In this context, the present study aimed to evaluate the effect of the flavonoid curcumin (C) on hASCs adipogenesis to highlight the potential function of this compound as a regulator of AT development. Cells were induced to differentiate into mature adipocytes and monitored for 21 days in the presence or absence of C. Both gene and protein expression of specific adipogenic markers were assessed by real-time PCR and immunostaining coupled with confocal microscopy, respectively. The accumulation of lipid droplets was also analyzed histologically by Oil Red O staining. Results indicated the inhibitory effect of C on hASCs adipogenic differentiation. Curcumin decreased the expression of specific adipogenic markers and reduced lipid droplet deposition. Overall, these data suggest a potential therapeutic role of C in preventing metabolic disease development by decreasing the number of preadipocytes and regulating AT volume.

P-05-006
Cellular signaling underlying the interplay between enteric neuronal progenitors and mesenchymal cells during bowel development

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Hirschsprung disease (HSCR) is a congenital disorder of the colon characterized by the absence of specific nerve cells due to incomplete colonization of the bowel by enteric neural crest cells (ENCCs). This condition leads to functional intestinal obstruction. Stem cell therapy shows promise as a potential treatment option for HSCR. However, despite significant advancements in identifying suitable cell sources and developing transplantation strategies, our understanding of the molecular mechanisms essential for forming a functional neuronal network in the bowel remains limited. This knowledge gap has significantly impeded progress toward effective cell replacement therapies for HSCR. Vinculin (VCL) is an adaptor protein crucial for focal adhesions, as it integrates various external signals and facilitates cell-cell communication, positioning it as a potential hub gene in the development of the enteric nervous system (ENS). In this study, we aimed to create a comprehensive map of the signaling pathways involved in the formation of the ENS, utilizing neural crest-specific Vcl knockout mouse mutants (Vcl cKO). Loss of Vcl severely disrupted cell-cell interactions among ENS cells, resulting in delayed gut colonization and failure in ganglionogenesis. Subsequent high-resolution transcriptomic analysis reconstructed the differentiation

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trajectory of ENCCs along the neuronal lineage development, revealing that genes associated with axonogenesis are dysregulated in ENS cells, along with compromised cellular signaling pathways. Additionally, spatial RNA sequencing indicated that ENS-mesenchyme interactions are reduced in the Vcl cKO gut, and it was found that the pleiotrophin (Ptn) pathway is significantly dysregulated during the development of the ENS in Vcl cKO. Notably, Ptn knockout (KO) mutants consistently exhibited abnormal ganglionogenesis, highlighting the complex interplay between ENS and mesenchymal cells that guides various steps of ENS network formation.

P-05-007
ANO1 regulates GABAR-Orai1-dependent Ca^{2+} signals in ventral radial glia

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Disruptions in the development of GABAergic interneurons are implicated in a variety of neuropsychiatric and developmental disorders. During early development, ventral radial glia (vRG) in the medial ganglionic eminence (MGE) play a crucial role in determining their fate, either generating intermediate progenitors (IPs) or somatostatin-expressing (SST) interneurons. However, the precise calcium regulatory mechanisms governing vRG function remain poorly understood. This study identifies that the Ano1 transcript is specifically enriched in a subpopulation of Fabp7+/Sox10+ vRGs, as determined using RNAscope technology. Our electrophysiological recordings and live calcium imaging demonstrate that this subpopulation of vRGs generates calcium-activated chloride (CaCC) currents, which are effectively blocked by the ANO1-specific inhibitor, Ani9. Notably, Ano1-deficient knockout (KO) mice exhibit reduced cellular proliferation, significantly increasing ASCL+ IPs. Mechanistically, we show that GABA stimulates Orai1-dependent store-operated calcium entry (SOCE), a process potentiated by ANO1 activity in vRGs. Notably, ANO1 is exclusively activated by calcium release from the endoplasmic reticulum (ER), rather than from extracellular calcium sources. Moreover, ANO1 exhibits constitutive activity, resulting in a significantly more hyperpolarized resting membrane potential in ANO1+ vRGs than ANO1- vRGs. These findings provide valuable insights into the calcium-regulatory mechanisms of vRGs in the MGE, offering new perspectives on how calcium signaling influences fate determination toward IPs versus SST interneurons.

P-05-008
Van-Gogh loss of function reveals the role of planar cell polarity in *Drosophila* embryonic development

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Planar cell polarity (PCP) signaling is a conserved pathway essential for proper cell alignment and coordinated morphogenesis across animal species, playing a crucial role in multiple stages of development. Despite extensive characterization of its components, the role of PCP in *Drosophila melanogaster* embryonic development remains poorly understood. Dorsal closure, which involves the alignment and zipping of two dorsal epithelial sheets, and dorsal appendage formation, which refers to the development of tubular eggshell structures on the embryo, serve as models for investigating PCP function in embryonic development. These processes involve epithelial sheet migration, cytoskeletal dynamics, and cell-cell adhesion. In our studies we utilized *Van-Gogh* (*Vang*), a core PCP component that plays a crucial role in various developmental processes, including the proper orientation of wing bristles in *D. melanogaster*. First, we examined dorsal appendage formation in $Vang^{6}$ loss-of-function mutants. $Vang^{6}$ mutant and *Canton-S* control flies were placed on agar plates within a cage. After 14 hours of egg laying, dorsal appendage morphology was assessed. Mutants exhibited various alterations, including distortions, thinning, or a complete loss of dorsal appendages. We also examined dorsal closure using immunohistochemistry. In mutants, defective zipping resulted in abnormal closure during the final phase. Additionally, amnioserosa cells, which contribute to dorsal closure through apical constriction and force transmission, lost their characteristic hexagonal shape. These findings indicate that *Vang* is essential for epithelial dynamics and cell shape regulation during dorsal closure. Future studies will explore the effects of *Vang* and other PCP components on cytoskeletal organization and cell adhesion in other embryonic developmental processes. Investigating these processes will enhance our understanding of PCP-dependent mechanisms in embryonic morphogenesis in *D. melanogaster*.

P-05-009
Hormone cocktail and non-parenchymal cell secretomes of the liver regulate the differentiation of hepatic organoids in culture

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There is an unmet need for relevant *in vitro* liver models for the safety and efficacy testing of drugs, as well as for disease modeling, regenerative and personalized medicine. Organoids are 3D tissue models with diverse cell composition and functional similarity to their organs, capable of replicating specific organ functions. iPSC-derived hepatic organoids carry out liver functions but retain fetal characteristics (e.g., AFP) alongside mature ones (e.g., Albumin, A1AT), limiting their ability to fully mimic adult liver physiology. In this study, we hypothesized that modifications involving a hormone cocktail, which influences liver maturation during the postnatal stage of development, combined with non-parenchymal cell secretomes, could further enhance organoid maturation in culture. iPSC-derived endodermal hepatic organoids (eHEPOs), for which our group established a protocol, were incubated with non-parenchymal cell secretomes and hormone cocktails, both individually and in combination, at different differentiation time points. Albumin secretion was quantified by ELISA, and ALB, A1AT, and AFP expression was evaluated via qPCR. Cell viability was assessed with CellTiter-Glo and lipid accumulation was measured using Autodot lipid dye as a maturation parameter. The combination of secretomes and the hormone cocktail significantly enhanced

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albumin secretion and ALB gene expression at later stage of differentiation. Hormone cocktail enhanced A1AT expression at later stage. AFP expression remained consistent in the early differentiation stage, regardless of culture conditions, but varied with differentiation time and culture conditions. Non-parenchymal cell secretomes reduced AFP expression at later stages. Additionally, secretomes increased lipid accumulation capacity of organoids. The findings suggest that incorporating a hormone cocktail and non-parenchymal cell secretomes into the culture medium enhance the maturation of hepatic organoids.

P-05-010
CKIIα-i1 integrates CKIIα and PP2A signaling in Notch-mediated neural fate determination in *Drosophila melanogaster*

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The nervous system in *Drosophila melanogaster* originates from pluripotent cells called neuroblasts, which are selected from an equivalent group of ventral neuroectoderm (VNE) cells through Notch-Delta signaling. While the fate of Notch-on and Notch-off cells is understood, the initial imbalance and the mechanisms governing the selection of Notch-dominant cells remain largely unexplored. It is known that Casein Kinase II alpha (*CKIIα*) and protein phosphatase 2 (*PP2A*) regulate Notch-mediated signaling in various tissues and antagonize each other; however, the precise mechanisms underlying their interaction remain unclear. Casein Kinase II alpha interactor 1 (*CKIIα-i1*) is a novel AD-type C2H2 transcription factor that interacts with CKIIα and PP2A regulatory subunit wdb, suggesting that it may act as a molecular switch in balancing antagonistic signals from these interactors. We hypothesize that CKIIα-i1 serves as a key transcription factor integrating CKIIα and Wdb signaling, thereby controlling a regulatory network that establishes Notch-dominant cells within the equivalence group. To elucidate its expression pattern, we utilized a *Gal4* line generated from the *CKIIα-i1* upstream region and *in situ* hybridization. Our findings indicate that *CKIIα-i1* is expressed in early embryonic stages, specifically in the apical VNE cells, within a narrow time frame from cellularization to neuroblast delamination. Overexpression of *CKIIα-i1* in S2 cells resulted in a decreased growth rate, suggesting a potential role in cell cycle progression, which is also regulated by CKIIα and PP2A. To further investigate the regulatory cascade involving CKIIα and PP2A, we designed a binary *in vivo* proximity labeling system, allowing spatial and temporal resolution for identifying interactors of these proteins. Additionally, to decipher the regulatory network in which these proteins function, we plan to conduct RNA-seq analyses using mutant lines and perform CUT&RUN assays targeting *CKIIα-i1*.

Epigenetics

P-06-001
DNA methylation-based regulation and neurodevelopmental trajectories of central nervous system tumors

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Central nervous system (CNS) tumors are a diverse group of malignancies that can be accurately diagnosed and subclassified by using only genome-wide DNA methylation patterns. Cell differentiation plays a crucial role in these tumors, with a lower differentiation state being linked to increased tumor aggressiveness. However, it is unknown why DNA methylation-based classification can precisely pinpoint CNS tumor subclasses and to what extent tumor-associated DNA methylation patterns are linked to differentiation states. This project leverages publicly available DNA methylation data from 1098 normal samples, representing different brain locations and neurodevelopmental stages, as well as 2682 CNS tumors, constituting more than 70 CNS tumor types. We identified a total of 1642 CpG sites exhibiting DNA methylation changes throughout the normal neural development as well as 147 transcription factors, whose binding sites are enriched within the identified CpG sites. Preliminary data revealed drastic changes in DNA methylation taking place during the normal neural development, including a gradual decrease of DNA methylation in the astrocytic lineage. Based on the DNA methylation status of 4942 neural and progenitor cell marker genes, which we clustered into 20 subgroups, we are currently comparing CNS tumors to stages of neural cell differentiation. Next, utilising gene regulatory networks, we aim to identify master regulators within each of the 20 subgroups. This project characterises neural cell differentiation trajectories based on DNA methylation. Using these results, we will investigate to which extent differentiation-related patterns are represented in the analyzed CNS tumor types. *The authors marked with an asterisk equally contributed to the work.

P-06-002
SS18L2, a novel epigenetic regulator of triple negative breast cancer proliferation

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Triple negative breast cancer (TNBC) remains a significant challenge in oncology due to its aggressive nature and limited treatment options. Epigenetic dysregulation plays a crucial role in TNBC progression, yet the precise mechanisms underlying this dysregulation remain poorly understood. In this study, we aimed to elucidate the role of novel epigenetic regulators in TNBC cell proliferation. Through a CRISPR-based functional screen using a gRNA library targeting epigenetic modifiers (EPIKOL), we identified SS18L2 as a pivotal gene essential for the survival of

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TNBC cells both *in vitro* and *in vivo*. Knockout of SS18L2 led to significant proliferative defects in TNBC cells, through the downregulation of several cell cycle related genes. To delve deeper into the mechanistic insights underlying SS18L2-mediated cell cycle regulation, we performed a proximity labelling assay, revealing close interactions between SS18L2 and numerous members of the BAF (BRG1/BRM-associated factor) chromatin remodelling complex. This finding highlights the potential involvement of SS18L2 in modulating chromatin architecture and gene expression programs crucial for TNBC cell cycle and proliferation. Our study proposes further experiments aimed at unravelling the intricate mechanisms by which SS18L2 is involved in cell cycle-related processes in TNBC. By delineating the functional significance of SS18L2 and its interactions within the BAF complex, we aim to uncover novel elements of the epigenetic mechanisms by which TNBC cells are regulated. This study was funded by the Scientific and Technological Research Council of Turkey (TUBITAK) ARDEB 1001 (Grant No. 123S680).

P-06-003
Maternal obesity impairs neurogenesis through epigenetic alterations in the embryonic brain cortex

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Maternal metabolic disturbances, such as obesity-induced hyperglycemia and dyslipidemia, create a challenging environment for embryonic development, particularly affecting the central nervous system. Neurodevelopmental disorders often arise during early embryogenesis period and are linked to disruptions in gene regulation and developmental processes. Research increasingly highlights that maternal obesity can alter neurogenesis pathways, increasing the risk of such disorders in offspring. Using a rat model of maternal obesity induced by a high-fat diet (HFD) before and during pregnancy, we investigated its impact on embryonic cortical neurogenesis. Maternal obesity was found to impair neurogenesis by reducing cell proliferation, promoting premature neuronal differentiation, and shifting cell fate toward the production of glial cells instead of neurons. These disruptions in neurogenesis were accompanied by significant changes in gene expression and developmental signaling pathways. Epigenetic changes also played a critical role in mediating the effects of maternal obesity. Altered histone modifications were observed, which are known to influence gene activity and developmental outcomes. These findings suggest that the metabolic environment shaped by maternal obesity can interfere with the normal processes of brain development by modifying gene expression and cell fate decisions. Overall, the results highlight the profound impact of maternal metabolic health on embryonic brain development by uncovering the epigenetic and molecular mechanisms that drive these effects.

P-06-004
Recruitment of truncated CTCF modifies 3D structure of HOXD locus

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Cohesin-driven extrusion of a chromatin fiber is one of the key mechanisms of genome folding in mammalian interphase cells. Extrusion terminates at convergently orientated CTCF binding sites, resulting in chromatin loop formation. This mechanism is also involved in control of local epigenetic landscape and gene expression. A number of pathologies are characterized by alterations in the 3D genome. In particular, changes in CTCF binding profile may rewrite distant regulatory contacts between enhancers and promoters, perturbing normal gene expression. Thus, targeted CTCF recruitment to pathology-associated genome loci could be an instrument for the precise engineering of the 3D genome and transcription control. Here, we fused four truncated variants of CTCF with dCas9 and GFP to construct chimeric proteins capable of extrusion termination at selected genome positions. We found that the recruitment of a chimeric protein based on the N-terminal domain and two Zn-finger domains of CTCF results in restoration of a chromatin loop, lost after deletion of an endogenous CTCF-binding site (CBS). Recruitment of this chimeric protein into internal regions of *HOXD* locus leads to the *de novo* formation of stripes and chromatin loops with surrounding cohesin/CTCF-bound regions. Interestingly, this also decreased H3K27me3 levels at the recruitment point. We also observed expression changes for several genes of the locus. Truncated CTCF variants do not bind to endogenous CBSs that makes them precise tools for manipulating chromatin loops. This work is supported by the Russian Science Foundation (grant # 24-44-00023). This work was also supported by FEBS Summer Fellowship 2020.

P-06-005
Methylation of the GAS5 gene regulates its expression and affects the GAS5/miR-137/TRAF3 triplet interaction in ovarian cancer development

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Epithelial ovarian cancer (EOC) represents a heterogeneous group of tumors characterized by late detection and poor prognosis. Current therapeutic methods show limited efficacy in achieving favorable outcomes. Therefore, epigenetic factors, including methylation of CpG islands in the promoter regions of tumor suppressor genes, which underlie malignant

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transformation, may hold the key to developing novel targeted therapy approaches for EOC. Our study revealed a significant association between epigenetic mechanisms, particularly DNA methylation of lncRNA genes such as *GAS5*, and the regulation of their expression. This study aimed to evaluate how *GAS5* lncRNA gene methylation regulates its expression and influences interactions with microRNAs and mRNAs in EOC. Using qMS-PCR and qRT-PCR (Bio-Rad), a nonparametric Mann–Whitney U test, and Spearman correlation analysis (SPSS 27), we analyzed a representative cohort of 30 paired (tumor/normal) EOC tissue samples ($p < 0.05$). Methylation analysis of a group of lncRNA genes (*MEG3, SEMA3B-AS1, ZEB1-AS1, ZNF667-AS1, GAS5, KCNK15-AS1, SNHG6*) demonstrated that hypermethylation of the GAS5 gene significantly reduces its expression level (Rs = -0.300, $p < 0.008$). Correlation analysis revealed an inverse correlation between GAS5 expression and miR-137 expression (Rs = -0.309, $p = 0.023$) and co-expression with TRAF3 mRNA (Rs = 0.443, $p = 0.021$). Furthermore, comparison of miR-137 expression levels with TRAF3 mRNA expression levels revealed an inverse correlation (Rs = -0.427, $p = 0.026$). Thus, our findings on epigenetic alterations in EOC contribute to the "molecular portrait" of this malignancy and enhance our understanding of its pathogenesis. The identified methylation features of the studied genes may have clinical applications in the development of modern approaches for prognosis, prevention, and treatment strategies for EOC. The study was supported by the state assignment No. FGFU-2025-0010 of the MS HE of the RF to FSBSI IGPP. *The authors marked with an asterisk equally contributed to the work.

P-06-006
Products of active demethylation pathway as biomarkers of multiple myeloma development

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Multiple myeloma (MM) is a cancer originating from plasma cells, differentiating from B lymphocytes. The disease is accompanied by a characteristic set of symptoms: hypercalcemia, renal failure, anemia, and osteolytic defects, and multiple myeloma cells secrete monoclonal proteins as kappa or lambda light chains. Monoclonal gammopathy of undetermined significance (MGUS) is a precancerous condition in which the appearance of monoclonal protein in urine and serum is observed without previously mentioned symptoms. Unrepaired DNA damage can give rise to mutations that underlie the development of various diseases, including cancer. Additionally, changes in DNA methylation patterns are also common in cancer pathogenesis. The level of 5-methylcytosine (5-mCyt) can change as a result of active DNA demethylation involving TET proteins, which catalyze the oxidation of 5-mCyt to 5-hydroxymethylcytosine (5-hmCyt) and this can be further oxidized to 5-formylcytosine (5-fCyt) and 5-carboxycytosine (5-caCyt). The nitrogenous bases removed in the DNA repair process are then secreted into the bloodstream, from where they are excreted with urine. We used two-dimensional ultra-performance liquid chromatography coupled with tandem mass spectrometry (2D-UPLC-MS/MS) to determine the level of epigenetic modifications in the urine of patients with MM, MGUS, and healthy subjects. Statistically significant differences were found for 5 methyl 2' deoxycytidine (5-mdC), 5 hydroxymethyl-2'-deoxycytidine (5-hmdC), and 5-hmCyt between groups.

P-06-007
Genome-wide DNA methylation profiling for identification of DNA methylation signatures in early-onset colorectal cancer

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Early-onset colorectal cancer (EOCRC) has been increasing in incidence worldwide, but its genomic pathogenesis remains largely undetermined. This study aimed to identify specific DNA methylation markers contributing to the development of EOCRC and analyze their relevance to gene expression and prognosis. We first performed DNA methylome profiling using the Illumina Infinium 850K BeadChip on a discovery cohort comprising 12 EOCRC (age < 50) and 12 late-onset colorectal cancer (LOCRC) (age > 70) paired tissue samples. We then identified differentially methylated CpG sites where changes occurred during the progression from normal tissue to cancer in both the EOCRC and LOCRC groups. Specifically, we observed 705 hypomethylated and 5666 hypermethylated CpG sites unique to EOCRC. To assess the association between DNA methylation and gene expression, we utilized RNA-seq data from the TCGA cohort. We found that the hypermethylation of 41 CpG sites was associated with decreased gene expression in EOCRC, while the hypomethylation of 236 CpG sites was linked to increased gene expression. To further understand the functional roles of these changes, we performed pathway analysis using genes associated with DNA methylation alterations. Our analysis revealed that pathways such as MET Promotes Cell Motility, MET Activates PTK2 Signaling, inflammatory response, and Epithelial-Mesenchymal Transition were enriched in EOCRC carcinogenesis. In the survival analysis, we identified 13 hypomethylated CpG sites and one hypermethylated CpG site that showed statistically significant associations with prognosis. In this study, we investigated DNA methylation changes specific to the development of EOCRC and identified CpG sites associated with prognosis. We will validate our findings in an independent cohort.

P-06-008
N6-methyl-2′-deoxyadenosine in lymphoproliferative disorders

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Aberrant epigenetic landscapes promote tumor initiation and progression. The focus of cancer research has been on the global and local aberrations of 5-methylcytosine (5-mCyt). The role of

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other methylated nucleobases in cancer remains obscure. The methylation of deoxyadenosines has been identified, and it is a well-described epigenetic feature in prokaryotes. The presence of N6-methyl-2'-deoxyadenosine (N6-mdA) in mammalian DNA raises some controversy due to potential contamination with bacterial DNA. However, few recent studies analyzed N6-mdA levels in human cancer (gastric, liver, lung, and breast cancers – decreased N6-mdA levels; esophageal squamous cell carcinoma, hepatocellular carcinoma, tongue squamous cell carcinoma, and glioblastoma - higher N6-mdA levels). In this study, we examined levels of N6-mdA in peripheral blood mononuclear cells DNA from patients with lymphoproliferative disorders and in healthy individuals. The analysis was carried out on the whole-genome level using the isotope-dilution automated online two-dimensional ultra-performance liquid chromatography with tandem mass spectrometry (2D-UPLC-MS/MS) and stable isotope-labeled internal standards. Chronic lymphocytic leukemia (CLL) patients and individuals with monoclonal B-cell lymphocytosis (MBL) were characterized by lower levels of N6-mdA than healthy individuals. N6-mdA content negatively correlated with other epigenetic DNA modifications (e.g., 5-(hydroxymethyl)-2'-deoxyuridine) in the CLL group. Processes of methylation and demethylation of adenine share catalytic mechanisms with methylation/demethylation of cytosine. We hypothesize that these modifications in cancer may undergo similar changes to those observed with 5-mCyt and its oxidation products. Therefore, N6-methyladenine may play a role in leukemia development and progression. The work was supported by the National Science Centre, Poland, grant No 2024/53/B/NZ5/02001.

P-06-009
Novel coregulated lncRNAs MAGI2-AS3, MEG3, ZEB1-AS1, and ZNF667-AS1 reveal common mRNA targets and co-involvement in some processes in ovarian cancer

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Ovarian cancer is characterized by an asymptomatic course, which increases the mortality rate from this disease. Gene co-expression analysis is used to identify genes with common functions. The work is aimed at identifying long non-coding RNAs (lncRNAs) that have common expression regulation due to aberrant methylation of their genes and common functions in ovarian cancer. RT-qPCR and methylation specific qPCR were used to assess expression and hypermethylation level of lncRNAs and miRNAs. The GSE119055 and GSE211669 datasets from NCBI GEO were used to predict miRNAs and mRNAs potentially interacting with lncRNAs studied. Significant hypermethylation ($p < 0.001$) and pairwise co-methylation ($rs > 0.3$, $p < 0.05$) of the 4 lncRNA genes MAGI2-AS3, MEG3, ZEB1-AS1 and ZNF667-AS1 were found using a representative set of 122 clinical samples. A significant negative correlation ($rs < -0.5$, $p < 0.01$) between methylation and expression levels of these lncRNA genes confirmed the functionality of hypermethylation. The association of hypermethylation of these lncRNA genes with ovarian cancer progression (stage, metastases) was also shown ($p < 0.05$). Pairwise co-expression of these 4 lncRNAs was predicted using GEPIA 2.0 data and confirmed by RT-qPCR on a set of 56 clinical samples ($rs > 0.3$, $p < 0.05$). Furthermore, negative interactions were predicted between 4 lncRNAs and some miRNAs using the GSE119055 dataset. Inhibiting interactions in triplets MAGI2-AS3/MEG3 miR-33b-5p VIM/ZEB1 were confirmed by RT-qPCR ($rs < -0.3$, $p < 0.05$). Using GSE211669 dataset and enrichment analysis, common potentially regulated mRNAs ($rs > 0.4$, $p < 10\text{-}5$) and possible joint participation in some processes significant for oncogenesis (such as epithelial-mesenchymal transition, cell adhesion, binding, etc.) were predicted. Thus, lncRNAs MAGI2-AS3, MEG3, ZEB1-AS1 and ZNF667-AS1 exhibit co-regulation and common functions in ovarian cancer and have clinical potential. *The authors marked with an asterisk equally contributed to the work.

P-06-010
Circulating non-coding RNAs as promising biomarkers for atherosclerosis

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Cardiovascular diseases (CVD) remain the leading cause of morbidity and mortality. There is a strong emphasis on identifying biomarkers for the early diagnosis of these diseases. Multiple vascular pathology (MVP) entails the presence of atherosclerotic lesions in multiple vascular beds. We hypothesized that specific epigenetic markers, such as microRNAs (miRNAs), may indicate the presence of MVP in patients with carotid artery stenosis (CAS) or peripheral artery disease (PAD). To prove this, we aimed to evaluate six miRNAs involved in CAS and PAD (miR-223-3p, miR-142-3p, miR-155-5p, miR-122-5p, miR-486-5p and miR-92a-3p) as diagnostic biomarkers for MVP. In this study, 32 CAS patients and 55 PAD patients were enrolled. Plasma levels of the six miRNAs were measured using RT-qPCR and statistical analyses were conducted to correlate results with MVP diagnosis. Significant increases in plasma levels of miR-223-3p, miR-142-3p, miR-155-5p, miR-122-5p and miR-92a-3p were observed in CAS patients with MVP compared to those without. In PAD patients, significant increases in plasma levels of miR-223-3p, miR-486-5p and miR-92a-3p were associated with MVP. In CAS patients, plasma levels of miR-92a-3p, miR-486-5p, miR-223-3p, miR-122-5p and miR-155-5p were identified as strong independent predictors of MVP following univariate ROC analysis after adjusting for age and gender. Conversely, only miR-486-5p was an independent indicator for MVP occurrence in PAD patients in the same type of analysis. Our findings indicate that the combination of miR-223-3p and miR-486-5p represents the most effective minimal significant multivariate ROC model for predicting MVP occurrence in both CAS and PAD patients. We propose miR-223-3p and miR-486-5p as promising epigenetic biomarkers for diagnosing MVP. This research was funded by the Romanian Academy and the Romanian Ministry of Research, Innovation, and Digitization under the PNRR program (CF 197-2022/PNRR-III-C9-2022-I8).

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P-06-011
Grainyhead-like 3 (Grhl3) as a key transcriptional and epigenetic regulator of the mesenchymal-to-epithelial transition (MET)

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Epithelial-to-mesenchymal transition (EMT) and mesenchymal-to-epithelial transition (MET) regulate cellular plasticity in development, tissue regeneration, stem cell reprogramming, and cancer Cdh1 expression is essential for maintaining pluripotency, and its depletion impairs pluripotency in embryonic stem cells. Reactivation of Cdh1 during induced pluripotency occurs through MET and is essential for reprogramming. In TGFβ-induced mesenchymal cells, Grhl3 and Hnf4α cooperation is essential for MET initiation. Although EMT is well characterized at different levels, MET remains less understood. We hypothesized the presence of distinct regulatory mechanisms in MET and challenged the notion that MET is merely the reverse of EMT. In this study, we explored the transcriptional and epigenetic roles of Grhl3 in MET initiation and maintenance in MEFs. Using a gain-of-function approach, we investigated Grhl3's ability to induce MET. Gene expression changes were assessed via qPCR, while ChIP and MeDIP experiments provided insight into transcriptional and epigenetic regulation. Grhl3 transfection into MEFs strongly induced Cdh1 expression, leading to significant epithelial-like morphological changes. RNAi-mediated Grhl3 silencing resulted in a loss of epithelial traits, confirming its necessity for MET maintenance. MeDIP analysis showed that Grhl3 overexpression decreased DNA methylation at epithelial gene promoters while increasing it at mesenchymal gene promoters. ChIP experiments indicated that Grhl3 enhances active histone marks (H3K4me3, H3K9ac, H3K27ac) and reduces repressive ones (H3K27me3), suggesting its role in chromatin remodeling. Furthermore, Grhl3 occupancy at DNA methyltransferase promoters explains these methylation changes mechanistically. Grhl3 surpassed Klf4 in promoting Cdh1, highlighting its key role in MET. These findings establish Grhl3 as a crucial transcriptional and epigenetic regulator of MET with implications for cancer therapy and iPSC reprogramming.

P-06-012
Hypoxia and pseudohypoxia differentially regulate TET protein activity: insights into epigenetic responses

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Hypoxia is a key regulator of cellular metabolism and epigenetics, with oxygen-sensitive enzymes modulating DNA methylation dynamics. Ten-eleven translocation (TET) proteins catalyze the stepwise oxidation of 5-methylcytosine (5-mCyt) to 5-hydroxymethylcytosine (5-hmCyt), 5-formylcytosine (5-fCyt), and 5-carboxycytosine (5-caCyt), crucial intermediates in active DNA demethylation. Hypoxia-inducible factors (HIFs) mediate TET recruitment to specific genomic loci, linking oxygen availability to epigenetic regulation. Cobalt chloride ($CoCl_2$), a widely used inducer of pseudohypoxia, stabilizes HIFs but also perturbs iron homeostasis by displacing Fe^{2+} from the TET catalytic site. To investigate differential TET responses to hypoxia and pseudohypoxia, we exposed HAP1 cells with functional TET knockouts to low oxygen or $CoCl_2$ and quantified TET activity products using 2D-UPLC-MS/MS. Hypoxia significantly increased global 5-hmCyt, 5-fCyt, and 5-caCyt levels, whereas $CoCl_2$ exposure led to their marked depletion, particularly in HAP1 TET1KO/TET3KO cells. TET2 exhibited the highest sensitivity to CoCl inhibition, suggesting differential susceptibility among TET isoforms. Furthermore, the loss of TET2 protein activity had the most significant impact on the level of 5-hmCyt, 5-fCyt, and 5-caCyt, both in control cells and cells exposed to hypoxia, which indicates the crucial role of TET2 in hypoxia response. These findings demonstrate that while both conditions impact TET activity, their mechanisms differ fundamentally. Given the distinct epigenetic effects of $CoCl_2$, its use as a hypoxia mimetic in epigenetic studies – especially those investigating hypoxia-induced DNA demethylation – should be reconsidered. Moreover, understanding interactions between hypoxia and active DNA demethylation provides insights into tumorigenesis and disease pathogenesis, where those processes play pivotal roles. This work was supported by Polish National Science grant number: 2018/29/N/NZ1/00497.

P-06-013
Epigenetic inheritance associated with offspring mental health following parental exposure to war trauma

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DNA methylation, the most biochemically stable epigenetic modification regulating gene expression, plays a crucial role in shaping an individual's phenotype and is directly influenced by environmental factors, including social interactions. Moreover, DNA methylation biomarkers can be inherited across generations, encoding phenotypic traits in offspring born long after initial exposure. This has led to a growing hypothesis that war-related events and consequent social disruptions may leave a lasting imprint on the epigenome, contributing to mental health challenges and behavioral impairments in future generations. One of the most prominent genes associated with trauma-related epigenetic inheritance is NR3C1, which epimutations have been associated with psychiatric conditions such as depression, PTSD, and anxiety, as well as neurodevelopmental disorders. To investigate the intergenerational transmission of war-trauma-related epigenetic biomarkers, an interdisciplinary study integrating biomedical and psychosocial research was conducted, funded by the Croatian Science Foundation (IP-2020-02-5967). A targeted participant recruitment protocol resulted in a sample of 76 mothers and 45 fathers who survived war trauma during the Croatian War of Independence in the 1990s, along with 80 of their children born in the post-war era. Buccal swabs were collected, DNA isolated, and DNA methylation analysis of the NR3C1 gene was performed using pyrosequencing. The results revealed comparable average DNA methylation levels of the

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NR3C1 gene in both parental and offspring generations, suggesting a strong intergenerational transmission of war-trauma-related epigenetic biomarkers. Additionally, a significant positive correlation was observed between maternal and offspring DNA methylation, indicating a more pronounced maternal inheritance of war-related epigenetic biomarkers.

LB-R-06-01
Preliminary study on the use of microRNAs as epigenetic biomarkers for testicular germ cell tumors

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Testicular cancer represents the most common type of cancer in men 15 to 45 years old. Although the survival rate is high, its increasing incidence and consequences such as infertility have led to intensified efforts to develop methods for early diagnosis and staging of the main subtype, testicular germ cell tumor (TGCT). Currently, diagnosis is based on general serological biomarkers such as AFP, as well as on scrotal ultrasounds and biopsies. Thus, this study aimed to identify specific biomarkers, in order to establish an early and non-invasive diagnosis by liquid biopsy. To this end, blood samples were collected from patients diagnosed with TGCT (n = 20), as well as from men who were healthy at the time of collection (n = 20). The candidate microRNAs miR-371, miR-373, miR-449 and miR-514 were selected, as the current literature suggests their potential in detection of TGCT and in the association with the different subtypes and stages. For the normalization of expression levels, the endogenous control U6 was used. The isolation of microRNAs and qRT-PCR were performed with standardized kits. The results were analyzed in GraphPad Prism 9.3, calculating the relative expression levels of the microRNAs, their correlation with the TGCT subtypes, and the statistical significance. The results showed statistically significant differences in the expression levels of miR-373, miR-449 and miR-514 between the patient group and the control group ($p < 0.05$). Statistically significant differences were observed between subtypes in the case of miR-373, however miR-449 and miR-514 also showed trends of under-expression/over-expression between subtypes. The results obtained support the working hypothesis and provide new insight into the use of microRNAs for TGCT. As such, microRNAs are a promising avenue for the development of a non-invasive method for diagnosing and subtyping this type of cancer which contributes to the declining fertility rate on an international level.

LB-R-06-02
The role of nucleoli in epigenetic regulation of human DUX4 gene expression

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Using the 4C approach, we found that the nucleoli form inter-chromosomal contacts with developmental genes in human HEK293T cells (Tchurikov et al., 2019, Cells 8, 111393. DOI: 10.3390/cells8111393). Among these genes, *DUX4* genes were found, which are a double homeobox genes, specifying a transcription factor whose activity is tightly regulated and is limited to the early stages of embryonic development (Alberto De Iaco et al., 2017, Nat Genet. 49(6): 941–945. DOI:10.1038/ng.3858). In the adult organism, epigenetic repression of *DUX4* genes take place, the decrease of which causes various pathologies (Mocciaro et al., 2021, Cells 10, 3322. DOI: 10.3390/cells10123322). Using a combination of FISH and immunostaining methods, we observed the co-localization of the *DUX4* gene hybridization region with the region of immunostaining with antibodies to the histone modification H3K27ac, which is a mark of active chromatin and with antibodies to the nucleolin, which is associated with active *rDNA* transcription. This indicates that *DUX4* genes are potentially active and form contacts with *rDNA* genes involved in the formation of nucleoli. Under stress (heat shock), the Pearson correlation coefficient between *rDNA* genes hybridization foci and the nucleolin-staining region decreases, but do not change between *DUX4* genes hybridization foci and the nucleolin-staining region. Under the stress, the number of contacts in this region decreases almost 4-fold, but the activity of *DUX4* genes, according to RNA-seq data, is not restored. We assume, that the contacts formation with the nucleoli promotes direct repression of *DUX4* genes and creates the precondition for the recruitment of nucleolar proteins. Apparently, nucleolar contacts that provide repression of the genes associated with development and morphogenesis are established at the earliest stages of development, before cells acquire their specificity. The study was supported by the grant of the Russian Science Foundation No. 25-24-00329, https://rscf.ru/project/25-24-00329/

LB-R-06-03
Functional evaluation of Dnmt1 deletion mutant and construction of the expression system for the recombinant Dnmt1

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DNA methylation is one of the epigenetic modifications. Abnormalities in this modification have been reported to be involved in various diseases. In order to clarify the cause of those diseases, it is necessary to analyze DNA methylation patterns. Various methods for DNA methylation analysis have been developed, among them, bisulfite sequencing (BS-seq) is the most common analysis method. However, BS-seq has a problem that it can't distinguish between methylated cytosine (mC) and hydroxymethylcytosine (hmC) which is an intermediate in demethylation. Therefore, our laboratory aims to develop a new methylation analysis method that combines PCR and maintenance-type DNA (cytosine-5) methyltransferase 1 (Dnmt1) which does not recognize hmC in human cells. However, our laboratory has found that recombinant Dnmt1 *in vivo* indicate *de novo* activity. In this study we constructed and evaluated six types of Dnmt1 deletion mutant (full length 1–1616 a.a.) in order to obtain a recombinant Dnmt1 with low *de novo* activity and high maintenance activity *in vitro*. The 384-1616 deletion mutant had the highest maintained methylation activity, 1.8-fold higher than that of the full-length. The 646-1616 deletion mutant had the lowest *de novo* activity, which was decreased 1/25 of the full-length, whereas the 755-1616 deletion mutant showed

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approximately 1.5-fold higher activity. Next, we used BS-Seq to evaluate the methylation activity of full-length and 646-1616 deletion mutant, which has maintenance methylation activity similar to that of full-length and the lowest *de novo* activity. Full-length and 646-1616 deletion mutant had all CpG sites of the hemimethylated DNA were methylated. In addition, full-length was methylated in several places in the unmethylated DNA, whereas the 646-1616 deletion mutant was not methylated at all. We plan to construct an expression system to obtain 646-1616 deletion mutant. *The authors marked with an asterisk equally contributed to the work.

Evolution

P-07-001
Allostery and evolution: a molecular journey through the structural and dynamical landscape of an enzyme super family

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Allosteric regulation is one of the most efficient mechanisms for controlling the efficiency of enzymes. Deciphering the evolutionary mechanisms by which allosteric properties have been acquired in enzymes is of fundamental importance. We use the malate (MalDH) and lactate dehydrogenases (LDH) superfamily as model to elucidate this phenomenon. By introducing a few of mutations associated to the emergence of LDHs into a non-allosteric MalDH scaffold, we have gradually shifted its enzymatic profile towards that typical of allosteric LDHs. We first investigated how to trigger homotropic activation. The crystal structures of the resulting mutants show the typical compact organization of the R-active state of LDHs, but a distorted catalytic site. Molecular dynamics simulations confirmed the capability of these mutants to sample the T-inactive state. By adding a final single mutation to fine-tune the flexibility of the catalytic site, we obtained an enzyme with both sigmoid (homotropic) and hyperbolic (heterotropic) substrate activation profiles depending on the salt concentration. Its structure shows a typical extended T-state as in LDHs, whereas its catalytic state has as a restored configuration favorable for catalysis. Free energy calculations indicate that the T and R catalytic site configurations are in an equilibrium that depends on ion binding. The long-range modulation of the catalytic site is also confirmed, suggesting a communication between the monomers as required for allosteric activation. NMR investigations are also underway. Our work links the evolution of allosteric regulation in the LDH/MDH superfamily to the ensemble model of allostery at molecular level and highlights the important role of the underlying protein dynamics. It also shows that evolutionary based approaches when combined with crystallographic and dynamical studies provide new insight into the regulatory process of catalysis via subtle local conformational and energetic reorganization. *The authors marked with an asterisk equally contributed to the work.

Genome organization and stability

P-08-001
The dark side of the histone PARylation: what structural features of damaged nucleosome regulate the activities of PARP1 and PARP2

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Poly(ADP-ribosyl)ation (PARylation) constitutes a critical post-translational modification of biomolecules. Poly(ADP-ribose) polymerase 1 and 2 (PARP1/2) are the main enzymes, that synthase PAR in a nucleus. Histone PARylation factor (HPF1) modulates PARP1 and PARP2 activity and switches the PARylation specificity to serine residues [1]. Despite extensive investigation into PARylation, numerous facets of this process remain poorly understood. It has been demonstrated that both the steric accessibility of PARylation sites and the primary structure of the target protein influence trans-PARylation; however, the precise mechanisms underlying these interactions are yet to be elucidated. Proteomic analyses reveal that histones are among the most prevalent acceptors of serine-linked ADP-ribosylation following oxidative stress [2]. The structured domains of histones provide a foundation for the assembly of stable nucleosome core particles (NCP), while the flexible histone tails play a critical role in regulating the interactions between nucleosomes and various factors. By employing a combination of classical biochemical methods, fluorescence-based techniques, and single-molecule mass photometry, we have demonstrated that the architecture of the NCP significantly influences the efficiency and pattern of histone ADP-ribosylation and the binding to histone-associated damaged DNA, with PARP2 displaying a higher impact than PARP1. Analyses grounded in existing research on the HPF1-dependent ADP-ribosylome and the structural dynamics of NCP suggest that the localization of DNA damage and the conformational flexibility of histone tails, modulated by post-translational modifications, are crucial for distinguishing the distinct roles of PARP1 and PARP2 during responses to genotoxic stress. The work was supported by grants from RSF 25-74-30006 1. Suskiewicz MJ et al. (2020) Nature 579, 598–602. 2. Buch-Larsen SC et al. (2020) Cell Rep 32, 108176. *The authors marked with an asterisk equally contributed to the work.

P-08-002
Comparison of SMC complexes from *Ureaplasma parvum* and *Bacillus subtilis*

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Structural maintenance of chromosome (SMC) complexes are large protein structures that interact with DNA and affect its spatial organization. SMC complexes play a key role in such processes as DNA compaction and segregation, reparation, and protection from foreign DNA. SMC complexes have been found in all living organisms – bacteria, archaea, and eukaryotes – and

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are even present in bacteria from the Mollicute class, whose representatives are characterized by a reduced genome and are generally close to the concept of a "minimal cell". Bacterial SMC complexes have been less studied than their eukaryotic homologues, and in reduced Mollicutes, they have been practically not studied at all. In this work, we studied the SMC complex of *Ureaplasma parvum*, a representative of Mollicutes, distinguished by a small genome (approximately 750 kb) and a clinically significant human pathogen. The properties of this complex were compared with the better studied *Bacillus subtilis* SMC complex. A wide range of techniques were used for these purposes, ranging from traditional biochemical to single-molecule methods, including laser tweezers. The results of the work indicate many important features of the *U. parvum* SMC complex that distinguish it from *B. subtilis* SMC. In particular, *U. parvum* SMC binds better to double-stranded DNA. The study was supported by the grant of the Russian Science Foundation No. 24-74-10022, https://rscf.ru/project/24-74-10022/.

Genomics and transcriptomics

P-09-001
Association between angiotensin-converting enzyme (ACE) genetic variants and hematological and inflammatory parameters in hemodialysis patients

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The angiotensin-converting enzyme (ACE) I/D polymorphism affects blood pressure regulation and immune function through the renin-angiotensin-aldosterone system (RAAS). In hemodialysis patients, the ACE I/D polymorphism may influence inflammation, with the D allele, linked to increased ACE activity, potentially raising angiotensin II levels and exacerbating immune activation and vascular inflammation, common in chronic kidney disease (CKD). This study investigated the relationship between the ACE I/D polymorphism and hematological parameters, including inflammation markers and platelet function, in hemodialysis patients. The study involved 60 hemodialysis patients from the University Clinical Center Nis, Serbia. Complete blood count (CBC) parameters were measured using standard methods, and inflammation markers C-reactive protein (CRP) and interleukin-6 (IL6) were quantified using immunoassays. The ACE I/D polymorphism was detected by PCR. The heterozygous ID genotype was the most common (50% of patients). Significant differences were observed in total white blood cell count, neutrophil count, and monocyte count, which were higher in ID genotype patients compared to the II genotype ($p < 0.05$). No significant differences were found for lymphocyte count, CRP, or IL6 levels ($p > 0.05$). Mean platelet volume (MPV) and plateletcrit were higher in the ID genotype compared to both DD and II genotypes ($p < 0.05$). After adjusting for age, sex, hypertension, diabetes, and hemodialysis duration, lymphocyte count remained significantly different between genotypes ($p < 0.05$), but MPV variation was no longer significant ($p > 0.05$). Our study suggests that the ACE I/D polymorphism is associated with changes in certain hematological parameters in hemodialysis patients. However, the influence of genotype on inflammation markers and MPV appears to be influenced by other factors, highlighting the complex interplay between genetic and clinical variables.

P-09-002
Association between Klotho G395A polymorphism and anemia parameters in hemodialysis patients

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The G395A polymorphism of the klotho gene has been associated with reduced klotho protein levels and is linked to various age-related diseases and complications, including kidney dysfunction and vascular calcification. Reduced levels of klotho in hemodialysis patients are linked to impaired phosphate and calcium balance, which can further disrupt the activity of erythropoietin and bone marrow cells. Our study aimed to investigate a possible link between the G395A polymorphism of klotho gene and the presence of anemia and iron metabolism impairment in hemodialysis patients. The study was conducted at the Nephrology Clinic of University Clinical Center Nis, Serbia, and it included a total of 220 hemodialysis patients. Basic hematology (red blood cells, hemoglobin, hematocrit) and iron metabolism parameters (iron, UIBC, TIBC, sat%) have been measured using standard methods on automatic analyzers (Nihon Koden, Tokyo, Japan; Erba XL 600, Mannheim, GmbH, Germany), and G395A polymorphism presence was determined using a modified Shimoyama et al. PCR method. The examined polymorphism was not equally distributed in the examined population. The least frequent was a recessive homozygote AA (6), then the heterozygote GA (76), and the most frequent was the wildtype homozygote GG (138). No significant differences were found considering hematological parameters of red blood cells (RBC) and iron ($p > 0.05$), while UIBC and TIBC were significantly higher in AA carriers compared to GA and GG ($p < 0.05$). This finding was more convincing in male participants ($p < 0.05$). In our patients we did not find proof of the link between klotho G395A polymorphism and hematological anemia parameters, however a significant difference in iron distribution parameters was found when different genotypes were compared. However, this study was limited by the small frequency of AA genotype.

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P-09-003
Integrated single-cell and spatial transcriptomics reveal dynamics of demyelinating lesions

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Multiple sclerosis (MS) is characterized by recurrent demyelinating episodes resulting in cumulative neurological disability. Relapsing-remitting multiple sclerosis (RRMS), the predominant form of MS, distinctly illustrates this chronic cycle of remission and relapse. To investigate mechanisms driving acute focal demyelination and subsequent remyelination, we utilized an experimental mouse model with controlled focal lesions induced by lysolecithin (LPC) above the corpus callosum. Using our model, we conducted comprehensive single-cell RNA-seq (10× Chromium) and spatial transcriptomic analyses (MERFISH and Molecular Cartography). Our findings revealed significant compositional shifts in specific glial and T-cell populations across experimental groups, accompanied by distinct transcriptional profiles highlighting altered gene expression programs associated with lesion dynamics. This integrated single-cell and spatial transcriptomic approach provides a robust analytical framework to elucidate cellular dynamics and molecular changes underlying demyelinating events, enhancing our understanding of myelin pathology and identifying potential therapeutic targets. *The authors marked with an asterisk equally contributed to the work.

P-09-004
EIF2AK2, GSK3A, PDE4D, and PASK emerge as immune response regulators in a transcriptomic study of atherosclerosis

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Chronic inflammatory diseases, including atherosclerosis, cause immune-mediated organ damage, impacting global health. These conditions stem from inadequate innate immune responses to endogenous stimuli, leading to dysfunction. Despite advances in understanding pathology, identifying early inflammatory drivers remains a challenge. This study seeks to uncover potential master regulators (MRs) of chronic inflammation to inform disease prevention. We assessed cytokine secretion pre- and post-LPS stimulation in 13 macrophage cybrid lines modelling atherosclerosis, followed by RNA-seq analysis. The reduction of cytokine secretion on the 2nd stimulation was considered immune tolerance, and the absence of secretion decrease spoke of intolerance. Differential gene expression (DGE) was calculated using DESeq2 (-logFC- >0.6, padj <0.05), and an MR search was conducted via geneXplain and TRANSFAC. TREM2 and OSMR were downregulated in cybrids exhibiting an intolerant response across all cytokines. OSMR, part of the IL6 receptor family, aligned with our prior findings on its ligand as a master regulator [Previously published in: Orekhov AN et al. (2024) Cur Med Chem]. EIF2AK2 was identified as an MR of the tolerant immune response, showing upregulation in tolerant vs intolerant cybrids across both stimulations. Conversely, GSK3A, PDE4D, and PASK emerged as MRs of intolerant response, displaying hypoexpression in intolerant vs tolerant cybrids in the first stimulation but an opposite pattern in the second. Their expression increased from the 1st to 2nd stimulation in intolerant cybrids, a trend absent in tolerant ones. These genes function as transcription factors (TFs) or interact with TFs regulating immune response. These findings highlight TREM2 and OSMR as probable MRs and suggest EIF2AK2, GSK3A, PDE4D, and PASK as key MRs, providing a foundation for future research into chronic inflammation and its therapeutic targets. The work was supported by RSF Grant #23-45-00031.

P-09-005
Impact of FADS gene polymorphisms on fatty acid profiles in erythrocytes of breast cancer patients undergoing aromatase inhibitor therapy

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Breast cancer (BC) patients on aromatase inhibitors (AIs) have altered polyunsaturated fatty acid (PUFA) metabolism. The role of fatty acid desaturases (*FADS*) polymorphism on these alterations and health outcomes is not clear. This study explores the association between 3 *FADS* polymorphisms (rs174537, rs174576, and rs174602) and the PUFA profile in erythrocytes of 37 BC patients on AIs therapy. Genotypes were determined using real-time PCR, and fatty acid profile by gas chromatography. Results indicated that carriers of the major allele (GG) for rs174537 exhibited significantly lower levels of 18:2, 20:3n-6, and 22:4n-6 ($p<0.05$) compared to minor allele carriers. Minor allele (TT) carriers showed reduced 20:5n-3, higher n-6 PUFA and n6/n3 ratio, along with lower Δ5-desaturase activity. The rs174537 GG genotype is linked to a reduced n6/n3 ratio and higher 20:5n-3 levels, suggesting a potential protective effect against inflammatory processes that may be relevant to cancer prognosis and treatment response. Furthermore, individuals with lower FADS1 activity (TT carriers) might benefit more from ω-3 supplementation. Carriers of the major allele (CC) for rs174576 showed lower levels of 18:2 and higher Δ5-desaturase activity compared to minor allele carriers (AA), while minor allele carriers (CA/AA) had higher 20:3n-6 levels and a higher n6/n3 ratio ($p<0.05$). Finally, for rs174602 genetic variant, carriers of the minor allele (CC) had lower 16:0 compared to TT/TC carriers. Among the three *FADS* polymorphisms studied, rs174537 demonstrated the most significant impact on erythrocyte fatty acid profile. These findings highlight the impact of genetic factors on PUFA metabolism and suggest that personalized nutritional strategies may enhance health outcomes in BC patients undergoing AIs therapy, helping to optimize their overall well-being and treatment response. This research was supported by the Science Fund of the Republic of Serbia, #5050 – AID.

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P-09-006
Tissue-resident T cell immunity imbalance is linked to senescent cells accumulation with age

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Cellular senescence is a hallmark of aging and age-related diseases, characterized by the accumulation of non-functional cells that escape apoptosis. Clearance of senescent cells has been shown to improve tissue homeostasis, enhance metabolic function, and extend lifespan in animal models. While innate immune mechanisms play a key role in eliminating senescent cells, their efficiency declines with age. The contribution of adaptive immunity, particularly T cells, to senescent cell clearance remains poorly understood. Here, we investigate whether age-related accumulation of senescent cells in tissues is associated with alterations in the T cell compartment. We analyzed single-cell RNA sequencing (scRNA-seq) data from skin biopsies of 14 donors aged 23 to 90 years, obtained from the GEO NCBI database. For cell type annotation and senescent cell identification, we evaluated multiple tools and selected CellTypist and SenCID for downstream analyses. Following cell annotation, we isolated clusters of senescent cells and T lymphocytes for in-depth characterization. We assessed age-related changes in the composition of $CD4^+$, $CD8^+$, and regulatory T (Treg) lymphocyte populations and their association with senescent cell burden. Our results reveal significant shifts in the tissue-resident T cell subpopulation balance with age, correlating with increased senescent cell accumulation. These findings suggest a potential link between age-associated alterations in T cell immunity and impaired senescent cell clearance, highlighting the need for further investigation into adaptive immune contributions to tissue aging. This work was supported by Russian Scientific Foundation, project No. 24-15-20003. *The authors marked with an asterisk equally contributed to the work.

P-09-007
Next-generation sequencing analysis of SCN1A and CACNA2D2 gene variations and their effects on sodium and calcium channel blocking anti-seizure drugs in Turkish epilepsy patients

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The *SCN1A* and *CACNA2D2* genes encode critical components of ion channels that regulate neuronal excitability and electrical signaling. Mutations in these genes are implicated in various epileptic disorders. Understanding the genetic mechanisms of these ion channels can inform the development of targeted antiepileptic drug therapies, potentially leading to more precise and effective treatment strategies for epilepsy. This study aims to investigate genetic variations in the *SCN1A* and *CACNA2D2* genes in Turkish epilepsy patients using next-generation sequencing (NGS) technology and to examine the impact of these variations on the response to antiepileptic treatments. Targeted next-generation sequencing (tNGS) was used for the molecular genotyping analysis of the *SCN1A* and *CACNA2D2* genes in genomic DNA from 29 epilepsy patients. Common variants; *SCN1A* gene rs2298771:C>T, rs3032638:CT>C; CTT>C; CTTT>C; CTTTT>C, and four different splice site mutations, rs11394960:G>GA, rs67636132:AG>A, rs566839:T>A and *CACNA2D2* gene rs566839:T>A, were selected and analyzed. There was no statistically significant difference between the responsive and persistent groups carrying the mutant allele between common variants ($p > 0.05$). In our study, we demonstrated two different rs11394960 splice polyrimidine track variations in the *SCN1A* gene, Chr2:166041479GA>G (SCN1A: c.2177-11del) and Chr2:166041479G>GA (SCN1A:c.2177-11dup). Two patients who were compound heterozygous for the rs11394960 variation showed GA>G and GA>G deletion and insertion changes. In terms of drug response, one of these patients is responsive and the other is drug resistant. In conclusion, our research contributes to the growing evidence that genetic factors play a role in epilepsy management. Further studies are needed to elucidate the functional implications of identified variants and to establish a more explicit link between these genetic markers and clinical outcomes.

P-09-008
Variation-based binding affinity analysis of the human CTCF transcription factor

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Eukaryotic transcription factors (TFs) typically bind to short sequence motifs, while CTCF recognizes a more extended ~12 bp core motif. Because the CTCF DNA-binding domain contains 11 zinc fingers, it can also read unique sequences around this central motif. CTCF is distinctive because it provides anchor sites for cohesin rings, which form the DNA loops essential for gene expression regulation. Earlier, our group developed the human ChIPSummitDB database, which, among other things, holds the genome-wide consensus binding sites for the CTCF TF. We selected CTCF from this database for our study due to its abundance of 131 experiments and the longer binding site. To understand the effect of the variations in the binding site on the binding affinity of the CTCF TF, we have downloaded the available human CTCF ChIP-seq experiments and developed a pipeline that automatically analyzes this raw sequencing data as well as the consensus binding sites in the reference genome and forms the catalog of the variations in the CTCF binding sites. Also, another pipeline of us analyzed all these variations and showed the allele imbalances in the binding affinities at the different heterozygous variations. Our results demonstrate that certain bases on CTCF motif are especially critical, like the 2nd and 7th positions. The reference alleles showed multiple times more frequency than their alternate counterparts, which is explained by the high binding affinity of these conserved locations. We repeated the same pipelines using mouse CTCF

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data and observed similar patterns, highlighting the evolutionary relevance of these patterns. In summary, we have developed a complete pipeline to analyze the possible effect of heterozygous variations at the CTCF binding sites, which allows for a better understanding of the effect of the given sequence on the binding sites for the affinity of the TF. This method can also be applied to analyze other TF binding sites. *The authors marked with an asterisk equally contributed to the work.

P-09-009
Next-generation sequencing approach for CCR5, CXCR4, and IFNAR1 gene variations on HIV-1 progression and CD4+ T cell dynamics of ART patients

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HIV, which causes acquired immune deficiency syndrome (AIDS), invades the host cell via the CD4 receptor and CCR5 or CXCR4 co-receptors. Interferons induced early in HIV infection induce an antiviral defense mechanism through IFNAR signaling. Our study aimed to examine the relationship between *CCR5*, *CXCR4*, and *IFNAR1* gene variants as a risk factor in HIV+ patients and their response to their clinical parameters. Targeted next-generation sequencing (tNGS) was used to perform molecular genotyping analysis of the *CCR5*, *CXCR4*, and *IFNAR1* genes in genomic DNA from 22 HIV+ patients and 25 healthy individuals as controls. We detected 13 rare mutations in the study, including 3 missense, 1 synonymous, 2 5′UTR, 4 3′UTR, and 1 frameshift variation. We also detected 6 common variants in the *IFNAR1* and *CXCR4* genes. HIV+ patients carrying the wild-type TT genotype of the *IFNAR1* rs2856973:T>A variant had higher CD4+ T cell counts compared with patients carrying the TA+AA genotypes of the rs2856973 variant in the naive and first month of the ART period ($p = 0.0011$ and $p = 0.0019$, respectively). Similarly, participants receiving ART with a TT genotype of rs2856973: T>A showed a significantly higher CD4+ T cell count in the third month ($p = 0.0008$). Patients carrying the homozygous wild-type genotype of the *CXCR4* rs2680880:A>T SNP had lower CD4+ T cell count compared with subjects carrying the AT+TT mutant genotypes of rs2680880:A>T in the naive and first-month period ($p = 0.0152$ and $p = 0.0256$, respectively). Our results demonstrate that variations in the *IFNAR1* and *CXCR4* genes can contribute to modifications in HIV progression. The present study was supported by a Grant from the Scientific Research Projects Coordination Unit of Haliç University (Project No: HBAP604-III-2).

P-09-010
Unveiling the correlation between phenotypic and genotypic variables obtained from whole exome sequencing data associated with adenomyosis through multivariate statistical approaches in a family study

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Adenomyosis is characterized by ectopic localization of endometrial-like tissue in the myometrium. It causes several symptoms including, dysmenorrhea, and abnormal uterine bleeding. This study aimed to utilize multivariate statistical methods, such as redundancy analysis (RDA), Anosim-Simper, Bray-Curtis, and the Spearman correlation test, to correlate adenomyosis-related symptoms (abiotic variables) with genetic variants (biotic variables) obtained from whole exome sequencing (WES) performed on familial cases of adenomyosis. A three-generation family of 19 women, five of which were diagnosed with adenomyosis were recruited following gynecological and ultrasonographic examinations. WES was performed on the genomic DNA of two affected and two healthy members utilizing Illumina NextSeq550. Bioinformatic analyses were conducted on the SEQ and Pairend platforms. The multivariate statistical technique, Redundancy Analysis (RDA) with Monte Carlo tests ($p < 0.05$) was applied to the biotic and abiotic variables in R. Spearman's rank correlation assessed variable relationships, while similarities of the case profiles were evaluated by Bray-Curtis clustering and Euclidean distances in Primer-v6. Abiotic variables, assessed via clinical examination, revealed dysmenorrhea in 10 (53%), menorrhagia in 6 (32%), and irregular menstruation in 7 (37%) out of 19 women. WES revealed four genetic variants in *DNAH5, MTMR8, MIB1*, and *TTLL4* genes. RDA indicated that *DNAH5* explained the largest proportion of variance, with remarkable positive correlations between other genetic variants and abiotic variables among family members. This study is the first to evaluate genotypic-phenotypic correlations of adenomyosis in a family utilizing multivariate analysis, emphasizing the potential importance of *DNAH5* in symptom manifestation among family members.

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P-09-011
The mutations burden on mitochondrial genomes and its biological significance of medieval human populations in Russia

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The study of mitochondrial genome mutations in medieval human populations is very relevant for understanding the genetic risks of diseases in modern humans. The presented study reveals important information about the evolution of the distribution of disease-related mutations, the historical prevalence of mutation patterns and genetic markers, as well as the impact of environmental and lifestyle changes on human health. The initial material was the bones of 30 men from graves of the XIth-XIIIth centuries in the territory of modern Ryazan, Kostroma, Bryansk, Yaroslavl and Leningrad regions of Russia. NGS sequencing was performed on the Miseq platform (Illumina, USA), and genomic libraries were prepared according to the Illumina protocols (Reagent Kit v2 300-cycles). The sequencing data was processed using standard bioinformatics utilities FastQC, Trimomatic, BWA, samtools, bcftools, utilities for analyzing ancient DNA – nf-core/eager, as well as proprietary original software in Python. In total, 9427 mutations were found in the mitochondrial genomes of 30 men of the Russian Middle Ages, which are unevenly located in the genomes. Most mutations are concentrated in non-coding regions of the beginning and end of the genome, the so-called D-loop region. Ribosomal genes RNR1 and RNR2 also account for many mutations. The cytochrome b gene carries a large mutational load. In the central region of the mitochondrial genome, where the gene density is higher, the mutation load is lower – there are genes that do not accumulate mutations at all. The presented study provides important data on the frequency distribution of mutations in the mitochondrial genomes of the inhabitants of Medieval Russia, which will serve in the future to assess the genetic risks of mitochondrial and related diseases, as well as for predictive modeling of the genetic risks of modern and future generations of people. This work was supported by the Russian Science Foundation (Grant # 24-65-00027).

P-09-012
Analysis genomic profiles of type 2 diabetes patients with COVID-19 by next-generation sequencing method in Turkish population

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The global impact of the COVID-19 pandemic, caused by the SARS-CoV-2 virus, significantly disrupted public health. A strong correlation was observed between increased hospitalization and mortality rates in individuals with type 2 diabetes (T2D) and severe COVID-19 cases. In present study, we aimed to analyze the candidate genes encoding proteins associated with T2D etiology and COVID-19 pathogenesis. We included 50 T2D patients with COVID-19 and 50 T2D patients without COVID-19 who were followed-up at Istanbul University Istanbul Faculty of Medicine COVID-19 services and outpatient clinics. Additionally, 50 healthy individuals, without both T2D and COVID-19, were enrolled as a control group. Genetic analyses were performed using the next-generation sequencing (NGS). A targeted gene panel, including genes considered to be associated with the pathogenesis of T2D and COVID-19, was used for sequencing. A total of 120 variants were identified in 15 candidate genes associated with T2D using the Clinical Exome Sequencing panel. We evaluated the genomic data with the clinical characteristics of adult patients over 45 years of age and detected pathogenic variants in the HNF1A and HNF1B genes, which caused the most frameshift variants, in the T2D group with COVID-19, especially in patients with other metabolic and cardiac comorbidities. In contrast, two frameshift variants were identified in HNF1A in the T2D group without COVID-19 and were also detected predictively in younger individuals in the control group. Among the candidate genes, the incidence rates of pathogenic, likely pathogenic and uncertain significance variants (VUS) in the HNF1A gene were determined to be 3.3 times higher in the T2D group with COVID-19 and 2.8 times higher in the T2D group without COVID-19 compared to the control group. These findings based on a limited number of patient data. However, we believe that our study will provide a significant contribution to population studies.

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P-09-013
Defining hybrid EMT states in carcinomas: molecular signatures and candidate molecules for EMT reversal

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The epithelial-mesenchymal transition (EMT) is a crucial process in cancer progression that contributes to metastasis, therapeutic resistance, and tumor adaptability. In this study, we analyzed EMT states across ~700 carcinoma cell lines, classifying them into epithelial (E), mesenchymal (M), and hybrid (H) states using multiple EMT scoring methods, including 76GS, KS, and MLR. Our findings revealed that most carcinoma cells exist in hybrid EMT states, emphasizing their potential role in promoting collective migration and metastatic behavior. Using hierarchical clustering (HC) and partitioning around medoids (PAM) algorithms, we identified four distinct hybrid clusters, each exhibiting unique gene expression signatures and biological functions. Further characterization, including pathway and metabolic analysis, highlighted functional differences among these clusters. Notably, we identified candidate molecules with the potential to reverse EMT in a cluster-specific manner and are currently conducting experimental validations to assess their anti-EMT effects.

Marine biology

P-10-001
Harnessing environmental DNA metabarcoding for biomonitoring mangrove-associated fish diversity and structure in select Philippine bioregions

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The Philippines, recognized as one of the world's mega biodiversity countries, hosts 30-40 mangrove species, representing at least half of the approximately 65 known mangroves worldwide. These mangroves are among the most productive and biodiverse ecosystems, playing a vital role in maintaining ecological balance in coastal regions, supporting high fish biodiversity, and providing critical ecological services. Mangrove ecosystems are critical habitats for diverse fish communities, yet their faunal biodiversity remains underexplored. Using eDNA metabarcoding, we assessed fish diversity across four bioregions in the Philippines, including Catanduanes, Batangas, Aklan and Palawan. Based on the 2053055 sequence reads generated in the study, we annotated 276 operational taxonomic units (OTUs). Taxonomic resolution revealed 57.61% of the OTUs at the species level, with notable ecological and economic significance. Family Gobiidae dominated the assemblages, with environmental factors such as salinity, water flow, and habitat type were identified as key drivers of fish community composition. Distinct biodiversity patterns emerged across sites, with Batangas exhibiting the highest richness and diversity. Two species, the brown-marbled grouper (*Epinephelus fuscoguttatus*) and the spotted seahorse (*Hippocampus kuda*), are categorized as Vulnerable based on the IUCN conservation status. The study highlights the potential of eDNA metabarcoding for biodiversity assessment and calls for expanding genetic databases, tailored conservation strategies, and long-term monitoring to protect these vital ecosystems amidst climate change. *The authors marked with an asterisk equally contributed to the work.

P-10-002
Expression of stress-related metabolic and biosynthetic genes in seagrasses incubated in a carbon dioxide-enriched climate chamber system

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Seagrasses, known as the "Lungs of the Sea" due to their high oxygen production, are key species that directly contribute to aquatic ecosystems. They are also considered blue carbon ecosystems, as they convert atmospheric carbon dioxide into organic carbon and store it in sediments. This process significantly contributes to the removal of carbon dioxide from the ocean–atmosphere system. These highly productive ecosystems serve as oxygen reservoirs and support biodiversity by providing food and shelter to numerous marine organisms. In this study, climate chamber systems were set up under field conditions to manipulate pH by altering CO_2 levels. The experimental setup simulated the RCP 8.5 climate scenario for the year 2100, and individuals of the seagrass species *Cymodocea nodosa* were incubated in climate chambers where CO_2 was the sole variable. Samples were collected at specific intervals over 36 hours, and the expression levels of genes associated with metabolic and biosynthetic processes were analyzed. These included glutathione synthetase (related to glutathione biosynthetic processes), glutathione reductase (related to glutathione metabolism), death-specific protein 5 (associated with apoptosis), sucrose synthase (related to sucrose metabolism), and ATP synthase subunit alpha (involved in ATP biosynthesis). The results showed that the expression of genes related to metabolic and biosynthetic processes was either upregulated or downregulated in correlation with each other. These findings provide important insights into the effects of increased CO_2

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levels on the physiological mechanisms of seagrasses. This study aims to contribute to future research on the adaptation strategies of seagrasses under climate change scenarios. Acknowledgement: This study was supported by a project funded by the Scientific and Technological Research Council of Türkiye (TUBITAK) ARDEB 1001 Grant No 124Y341. *The authors marked with an asterisk equally contributed to the work.

P-10-003
Approaches for the establishment of manipulative systems in seagrass meadows and the implementation of climate change scenarios

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Seagrasses are critical habitats that support primary production in coastal ecosystems, facilitate carbon sequestration, and contribute to biodiversity conservation. To understand these impacts and develop ecosystem-based conservation strategies, manipulative experimental systems are increasingly being utilized. Manipulative systems allow for controlled alterations of specific environmental variables, enabling the measurement of physiological, biochemical and ecological responses of seagrasses to stress factors. These systems, known as microcosms, simulate climate change scenarios at a laboratory scale. In our study, the effects of temperature and CO_2-dependent pH changes were monitored over three weeks in microcosm systems established in the laboratory using the seagrass species *Cymodocea nodosa*. Stable gene regions that remained unchanged under varying experimental conditions were selected as reference genes and the expression levels of genes associated with antioxidant pathways were analyzed individually using RT-qPCR, relative to the chosen reference gene. The obtained data revealed that the expression levels of three different candidate reference genes varied under different temperature and pH conditions. The gene with the most stable expression level was selected as the reference gene. Based on this, the expression levels of oxidative stress-related genes (*CAT*, *SOD*, *APX6*, *APX7*) were found to be upregulated and/or downregulated in correlation with each other. Developing these experimental systems is crucial for enhancing the accuracy of scientific data and guiding conservation policies. In the future, manipulative studies on seagrasses should be more extensively utilized in determining ecosystem restoration and adaptation strategies to mitigate the effects of climate change. Acknowledgement: This study was supported by a project funded by the Scientific and Technological Research Council of Türkiye (TUBITAK) ARDEB 1001 Grant No 124Y341. *The authors marked with an asterisk equally contributed to the work.

P-10-004
Future projections for seagrass under mucilage: the role of epiphytic flora

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Seagrasses, as marine angiosperms, are considered key indicators of balance in aquatic ecosystems. Seagrass meadows provide shelter for many vertebrates and invertebrates, while their leaves host various filamentous and unicellular species as epiphytes. In recent years, the increased occurrence of mucilage in the Sea of Marmara has raised concerns about the potential effects of epiphytic flora on seagrasses. This study examines the seasonal variations in the natural epiphytic flora on *Cymodocea nodosa* leaves and evaluates its potential impacts in terms of cellular stress and adaptation mechanisms. Specifically, the expression levels of metal tolerance protein (MTP) (associated with ion transport), Metallothionein (related to cellular metal ion homeostasis), and death-specific protein 5 (DSP5) (associated with apoptosis) were analyzed. The results indicate that while certain diatom species are consistently present in the epiphytic flora throughout the seasons, their abundance fluctuates, and some species appear only in specific seasons. Gene expression analyses reveal that epiphytic flora undergoes seasonal changes, influencing gene expression patterns. However, further investigations are needed to determine the direct relationship between these changes and variations in light penetration and leaf temperature caused by epiphytic growth. These findings provide valuable insights into the potential long-term impacts of mucilage and climate change on seagrass ecosystems. Understanding the dynamics of epiphytic flora is crucial for assessing the resilience and adaptation capacity of seagrasses in response to environmental stressors. Acknowledgement: This study was supported by a project given by the Ege University Scientific Research Projects Coordination Unit (Project number: FGA-2020-21348).

Membrane trafficking, vesicles, organelles

P-12-001
Consequences of the inactivation of the mannose-6-phosphate pathway on the growth and drug sensitivity of cancer cells

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Mutations in *GNPTAB* are associated with mucolipidosis II and III α/β, inherited lysosomal storage disorders resulting from defective GlcNAc-1-phosphotransferase, an enzyme essential for the synthesis of the mannose-6-phosphate (Man-6-P) lysosomal sorting signal. These mutations impair lysosomal acid hydrolases trafficking, leading to the accumulation of undigested material in lysosomes. Knowing that the dysfunction of lysosomes can affect various cellular processes, including those involved in cancer progression and drug resistance, we decided to investigate the putative consequences of *GNPTAB* knockout (KO) in HeLa cancer cells. Interestingly, we found that the loss of Man-6-P synthesis in those cells enhances anchorage-independent growth, induces the activation of some metalloproteinases, and confers resistance to several chemotherapy agents. Mechanistic analyses revealed two distinct resistance pathways: drug sequestration in the expanded lysosomal population and hyperactivation of an anti-apoptotic signaling pathway. These findings support that acid hydrolase missorting may contribute to cancer cell progression and resistance to chemotherapy.

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P-12-002
In situ extracellular labeling for exosome cargo protein identification

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The exosome, an extracellular vesicle, mediates intercellular communication by transporting macromolecules such as proteins and ribonucleic acids (RNAs). Determining cargo contents with high accuracy will help decipher the biological processes that exosomes mediate in various contexts. Existing methods for probing exosome cargo molecules rely on a prior exosome isolation procedure. Here we report an *in situ* labelling approach for exosome cargo identification, which bypasses the exosome isolation steps. In this methodology, a variant of the engineered ascorbate peroxidase APEX, fused to an exosome cargo protein such as CD63, is expressed specifically in exosome generating vesicles in live cells or in secreted exosomes in the conditioned medium, to induce biotinylation of the proteins in the vicinity of the APEX variant for a short period of time. Mass spectrometry analysis of the proteins biotinylated by this approach in exosomes secreted by kidney proximal tubule derived cells reveals that oxidative stress can cause ribosomal proteins to accumulate in an exosome subpopulation that contains the CD63 fused APEX variant.

P-12-003
Mesenchymal stem cell derived exosomes isolated by tangential flow filtration exerted preserved nano-quality characteristics

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Exosomes are functional vesicles that deliver molecular cargoes from a cell of origin to target cells, modulating the cellular behaviour of the target cells. Exosomes derived from mesenchymal stem cells (MSCs) are touted as potential key mediators of cancer pathogenesis, and are thus a promising approach for cancer therapy. However, a standard exosome isolation and purification method remains elusive due to the complex organization of extracellular vesicles. Hence, this study aims to develop a less time-consuming isolation method with a high yield and while retaining the original nano-quality characteristics of exosomes. Firstly, MSCs were cultured in serum-free media and incubated for 24 and 48 hours to optimise the exosome yield. Isolation of the exosomes from MSCs was performed using tangential flow filtration (TFF) cartridges with different sizes of polyethersulfone hollow fibres (150 nm and 50 nm). The TFF cartridges provided precise filtration of vesicles larger and smaller than the exosome size range. Next, purified exosomes were prepared for validation of nano-quality features via scanning electron microscopy (SEM) and transmission electron microscopy (TEM). Fixation and dehydration were performed for SEM while negative staining for TEM to preserve exosome nanostructure. Both results of SEM and TEM revealed spherical-shaped exosomes with their nanoscale size ranging between 50 nm and 150 nm in diameter. In conclusion, TFF method presented minimally invasive and less time-consuming approach for exosome isolation, while preserving the nano-quality features of the exosome. *The authors marked with an asterisk equally contributed to the work.

P-12-004
Effective uptake of a small helical protein by extracellular vesicles in cell culture experiments

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Extracellular vesicles (EVs) are considered promising platforms for drug delivery into cells. However, there are a number of unresolved issues limiting their use, including the lack of a universal and effective approach to drug loading. In our cell culture experiments, we found that small soluble protein LCB1 (56 residues, designed *de novo* as a blocking ligand of the SARS-CoV-2 spike and reported in Cao L et al. (2020) Science 370, 426–431) becomes rapidly absorbed from the cultural medium into EVs. The effect was observed in a cell line RPMI 2650 (human nasal epithelial cells) using LCB1 tagged with the fluorescent dye Atto 647. This remarkable behavior was reproduced using LCB1 tagged with another fluorescent dye, Cy5, and also in isolated EVs outside the cell culture. In contrast, only a minimal amount of EV accumulation was observed for two control proteins, the B1 domain of the streptococcal protein G (GB1) and ubiquitin. We hypothesize that high uptake efficiency shown by LCB1 is due to its distinctive fold, three-helix bundle, which is different from GB1 and ubiquitin, featuring a β-grasp fold. To test this hypothesis, we repeated our experiments using a sample of anti-HER2 affibody Zher2, which is also a three-helix protein. Indeed, we found that Zher2 is absorbed into EVs with the same high efficiency as LCB1. The mechanism of LCB1 capture by extracellular vesicles in relation to the properties of vesicular membranes awaits further investigation. In our RPMI 2650 cell line experiments, we observed that isolated EVs loaded with LCB1 are endocytosed by cancer cells. These results hint at potential new strategies for loading vesicles with protein cargo and delivering it to cells for research or therapeutic purposes. This work was supported by SPbU grant 127400313.

P-12-005
Survival tactics of nutrient deprived cancer cells

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Cancer cells need to rewire their metabolism and activate both catabolic and anabolic pathways to cope with the requirements of rapid proliferation. The core of a tumor can be limiting in both nutrients and oxygen, especially if the vasculature is poorly developed, and many cells will undergo necrosis. Some cells

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survive, by activating pathways such as autophagy to recycle macromolecules or degrade damaged organelles in the lysosome. We observed that colorectal cancer cell lines incubated in a nutrient depletion (ND) medium containing 10% of the nutrients found in complete medium (Nutrient rich, NR) activated canonical nutrient sensors mTOR and AMPK as well as autophagy. The surviving cells were arrested, but were highly viable, formed tumors *in vivo*, relied on fatty acid oxidation for energy, and were resistant to several drugs. We discovered that the resistance was mediated by the trapping of drugs in lysosomes that were highly acidic and localized in the perinuclear region, thereby preventing their entry into the nucleus. Alkalinization of the lysosomes with Bafilomycin A1 (Baf) resulted in a remarkable change in cell shape from cobblestone to elongated; these cells also displayed markers for partial EMT (p-EMT) and motility, most likely to escape the limiting environment. Overall, our data suggests that lysosomal pH is a key factor in determining cell fate under nutrient deficiency, deciding whether the cells should activate signaling and metabolic reprograming to die, survive or move to a distant site.

P-12-006
EVs effect on systemic herpes infection in mice

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Despite extensive research on extracellular vesicles, their effect on numerous viral diseases remains unstudied. EVs have the potential to stimulate tissue regeneration, induce anti-inflammatory effects, and accelerate post-infection recovery. We have conducted pilot studies on the impact of EVs, secreted by human Wharton's jelly mesenchymal stem cells (MSCs), on systemic damage in mice of the BALB/c line by herpes simplex virus, type 1 (HSV-1). EVs were characterized by TEM, NTA, presence of tetraspanins CD9, CD63 and CD81 was confirmed by western blot (MISEV2023). Experimental mice were infected by HSV-1. The next day, EVs were administered to the experimental animals in doses equivalent to the number of MSCs ranging from 50 000 to 600 000 per animal. The controls were: 1) mice infected with the HSV-1, without administration of EV; 2) intact animals; 3) intact mice, injected with the maximum dose of EVs simultaneously with the experimental ones. As the disease progressed, the brain and internal organs of sick, dead, and recovered animals were studied. As a result, an inverse relationship was found between the administered EV dose and the mortality/survival of the experimental animals. The highest EVs doses led to a mortality increase (compared to the control). Mortality decreased as the EV doses decreased. Low doses showed an almost complete cure effect. Virological studies have shown that the highest dose of EVs enhances the reproduction of the HSV-1 and increases the viral load compared to the control 1 and to animals treated with lower doses of EVs. Histological studies have shown that systemic infection of an organism with HSV-1 leads to damage to all internal organs. The administered EVs modify the degree and nature of damage, especially in the brain, lungs, liver, and spleen. EVs administration for viral infection treatment causes dose-dependent effects that differ from those in non-infectious pathologies. The relevant mechanisms require detailed study.

LB-R-12-01
Extracellular vesicles from tumor-associated macrophages (TAMs) upon FKBP51s silencing act as a new carrier in human macrophage polarization

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The tumor microenvironment (TME) hosts a variety of cells including tumor-associated macrophages (TAMs) that play a key role in regulating the immune response through the secretion of cytokines and extracellular vesicles (EVs) that act as carriers in cellular communication. We have identified a novel splicing isoform of FKBP51 that is involved in macrophage polarization towards the pro-tumor M2 phenotype. In this study, we aim to evaluate whether the alternative splicing isoform FKBP51s can change the cellular secretome and can be a target to reprogram the M2 phenotype towards the antitumor M1 phenotype. PBMC-derived from healthy donors were isolated and polarized to M1 and M2 macrophages and were silenced for FKBP51s. The release of cytokines was studied by flow cytometry. M1 macrophages released high levels of pro-inflammatory cytokines (IL-6 and TNFα), while M2 macrophages released high levels of IL-10. Subsequently, extracellular vesicles (EVs) secreted by macrophages were isolated from culture media using a series of centrifugations to remove cells, debris, and apoptotic bodies and collected through ultracentrifuge (UC). Their characterization showed a range size of 290.6 ± 136.8 nm, confirmed by transmission electron microscopy (TEM). The expression of membrane proteins specific to the endosomal pathway and the biogenesis of EVs was detected. In addition, cytokine analysis in EVs allowed us to observe a cellular cargo that mirrors that of the donor cell. This study supports the hypothesis that FKBP51s promotes macrophage polarization towards the pro-tumor phenotype M2 by supporting intercellular communication in TME through cytokine secretion and EVs release, that was reversed by the silencing. These results, which deserve further investigation, will allow in the future to define the alternative splicing isoform of FKBP51 as a target capable of modulating the cellular secretome as well as TAMs-derived EVs cargo with the aim of improving cancer therapy.

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LB-R-12-02
Tracking spatiotemporal distribution of organelle contacts *in vivo* with SPLICS (Split-GFP-based Contact Site Sensors) reporters

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Organelle contact sites are crucial for cellular function, enabling the exchange of lipids, ions, and other molecules between different organelles. The ability to track these contact sites *in vivo* has been significantly advanced by the development of SPLICS (Split-GFP-based Contact Site Sensors) reporters, which have provided unprecedented insights into the intricate network of organelle communication. This innovative and powerful tool allows the real-time visualization of different organelle interactions in living cells and *in vivo* thus unraveling the complexity of their dynamic in the context of cellular homeostasis. Recent studies highlighted the dynamic nature of organelle contact sites either in terms of tethering/untethering and of movement of the contact itself in time and space; whether unique temporal behaviors and contact site-specific dynamics of different organelle interactions exist is currently unknown. In this study, we investigated the spatiotemporal distribution of various organelle contact sites using time-lapse imaging *in vitro* and *in vivo* and discovered an evolutionarily conserved dynamic pattern among different contact sites, influenced by the specific partner organelles involved. These findings highlight the importance of spatial and temporal regulation at organelle contact sites, which may underlie their diverse physiological functions. The discovery of contact site-specific dynamics opens new avenues for understanding the regulation of organelle interactions in health and disease, with potential implications for developing targeted therapeutic strategies.

Plant biology

P-13-001
Gastrointestinal digestion of algal phycobiliproteins releases chromopeptides with high propensity to bind Fe(III) ions

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Phycobiliproteins (PBPs) are algal photosynthetic proteins whose primary role is capturing light during photosynthesis. The presence of covalently bound tetrapyrrole chromophores gives PBPs significant biological activities. One of the promising aspects of PBPs' bioactivity is their chromophores' ability to bind redox-active metal ions. This study examines the interactions of Fe^{3+} ions with chromopeptides formed by enzymatic digestion of phycobiliproteins (PBP) from three algae: *Arthrospira platensis*, *Porphyra haitanensis* and *Porphyridium purpureum*. Chromopeptides were obtained using gastrointestinal proteases: trypsin, pepsin and pancreatin. Each enzyme was chosen for its specificity, allowing the generation of different chromopeptide profiles. A key method used to monitor these interactions was fluorescence spectroscopy, given that tetrapyrrole chromophores exhibit strong fluorescence, which can be altered by metal ion binding. Preliminary screening in microtiter plates determined the most promising samples, which were subjected to binding characterization by spectrofluorimetric titration. Titration with a $FeCl_3$ solution revealed that the pancreatin digest of microalagae *P. purpureum* exhibited the strongest binding with a K_d in the sub-micromolar range. Chromopeptides from the digest were further purified by reverse-phase HPLC. Two chromopeptide fractions were obtained and the chromopeptide sequences were determined by tandem mass spectrometry. The binding of Fe^{3+} ions to the purified chromopeptide fractions was validated and high afinity binding was confirmed by spectrofluorimetry. The results obtained suggest the PBPs-derived chromopeptides could be a promising source of more bioavailable iron.

P-13-002
Biochemical response of tomato plants treated with compost and arbuscular mycorrhizal fungi to heat stress

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Heat stress is one of the most severe abiotic factors affecting the growth and metabolism of tomatoes. In an environmentally friendly approach, this study aimed to evaluate the potential effects of biostimulants, including arbuscular mycorrhizal fungi (AMF), compost, and their combination, on enhancing tomato tolerance to heat stress. A greenhouse experiment was conducted with Campbell33 variety, where tomato seeds were treated with an AMF consortium and compost, under a control (25°C) and high (35°C) temperatures. The study focused on key biochemical parameters such as sugar, protein, chlorophyll (a and b), hydrogen peroxide (H_2O_2), and malondialdehyde (MDA) content, and the activity of antioxidant enzymes like catalase (CAT), peroxidase (POX), ascorbate peroxidase (APX), and polyphenol oxidase (PPO). The results revealed that all treatments (compost alone (C), mycorrhizae alone (M), and their combination (MC)) had positive effects on sugar content in leaves, roots, and fruits. For proteins, only C and M showed a significant effect at the leaf level. At the root level, M and MC had a positive impact, while at the fruit level, C and MC were the most effective. Regarding chlorophyll a and b, treatments C and M showed significant effects, whereas the application of M influenced carotenoid levels positively. All treatments reduced oxidative stress by decreasing H_2O_2 and MDA levels in leaves and roots, with MC treatment performing best in reducing MDA levels. Treatment M also significantly affected CAT activity, while all three treatments (C, M, and MC) improved the activity of the other antioxidant enzymes, including PPO, POX, and APX. This study provides valuable information on sustainable agricultural practices, promoting the combined use of compost and AMF to enhance tomato performance under challenging environmental conditions of thermal stress.

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P-13-003
Redistribution of phytaspase activity in *Nicotiana benthamiana* leaves under methyl viologen-induced stress

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Subtilisin-like proteases (subtilases) are a diverse family of serine proteases. They play a crucial role in the plant life cycle and biotic and abiotic stress responses, such as salt stress, agrobacterial and viral infection, and herbivorous insect attack [previously published in: Figueiredo et al. (2018) Mol Plant Pathol 19(4), 1017-1028]. Phytaspase is one of the subtilases; it has an extracellular localization under normal conditions, and its remarkable feature is returning to the cell interior during stress-mediated cell death with clathrin-mediated endocytosis (CME) [previously published in: Trusova et al. (2019) Front Plant Sci 10, 873]. We aimed to investigate *Nicotiana benthamiana* phytaspase behavior under oxidative stress conditions. The endogenous *N. benthamiana* phytaspase was previously shown to change its localization under the action of antimycin A, the inducer of oxidative stress [previously published in Trusova et al. (2019) Front Plant Sci 10, 873]. Here, we checked the *N. benthamiana* phytaspase capacity for relocalization during oxidative stress caused by another stress-inducing agent, methyl viologen (MV), which is used as a photosynthesis-inhibiting herbicide. To elucidate the mechanism, we used cycloheximide to prevent new phytaspase molecule biosynthesis in combination with the CME inhibitor Dynasore. The results demonstrated that phytaspase changes its localization from apoplastic to intracellular during MV treatment, consistent with the effects of antimycin A. Dynasore prevents this transfer, thereby confirming the clathrin-mediated mechanism of phytaspase internalization. These observations demonstrate that the relocation of phytaspase is not limited to a specific chemical but reflects a broader response in the development of oxidative stress in *N. benthamiana* leaf tissues, together with additional evidence for a clathrin-dependent mechanism. The study was funded by the Shenzhen Municipal Government and Shenzhen MSU-BIT University. *The authors marked with an asterisk equally contributed to the work.

P-13-004
Towards herbicides that target lysine biosynthesis

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Although several commercial herbicides inhibit amino acid production in plants, lysine biosynthesis is yet to be commercially exploited as a herbicide target. We have developed the first herbicidal inhibitors of lysine biosynthesis, which target the first enzyme in the pathway, DHDPS. We have shown that these compounds exhibit potent *in vitro* and *in planta* activity using enzyme kinetic and herbicidal activity assays, whilst lacking off-target effects as demonstrated by cytotoxicity and antibacterial assays. Using X-ray crystallography, we have revealed that these inhibitors bind in a novel allosteric site in DHDPS, which is highly conserved across plant species. Interestingly, we noted that this class of inhibitors has an additional mode of action through inhibition of the second enzyme in the lysine biosynthesis pathway, DHDPR, using enzyme kinetic assays and *in silico* docking. This inhibitor class represents the first example of dual-target herbicidal compounds, which may have a reduced susceptibility to the generation of resistance. Additionally, this is the first time that an inhibitor with allosteric and orthosteric activity at two different enzymes has been reported. This work has the potential to contribute novel herbicide modes of action and strategies to mitigate resistance to help combat the rise in herbicide resistant weeds globally.

P-13-005
Constitutive defense in barley seeds: screening and identifying cadmium-bound compounds in seed exudates

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The results of our previous study[1] indicate that at least a quarter of grain protein content is influenced by the environment. Seed constitutive defense before germination involves the spermosphere and covering layers, both impacted by environmental factors. Exposure to 20 mg/L cadmium (Cd) has been shown to enhance barley seed germination, suggesting the presence of Cd-resistant compounds in seed exudates or covering layers. Our study aims to identify Cd-chelating compounds in barley seed exudates. We hypothesize that exudates contain chelators like glutathione (GSH), the protein M0WBF8, and other metabolites. Omics techniques were used to analyze proteins and metabolites linked to Cd stress and determine the duration of constitutive defense. Exudates from stressed seeds were applied to control seeds to assess vitality and screen Cd-bound compounds. Our findings will advance understanding of barley seed germination under metal stress and offer a novel approach to mitigating Cd toxicity through seed priming. Preliminary results identified 790 proteins in exudates, including 128 extracellular ones, with GSH, M0WBF8, and other metabolites potentially binding Cd. Identifying Cd-resistant proteins and metabolites enhances seed adaptability and provides a foundation for biological seed priming, supporting sustainable agriculture and environmental protection. Reference: 1) Hana Dufková et al. (2023) Journal of Experimental Botany 74, 1609–1628.

P-13-006
Equipping potato with resistance against pathogens by the use of CRISPR/Cas genome editing

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Potato is a staple crop worldwide, but its cultivation is threatened by diseases like bacterial wilt caused by *Ralstonia solanacearum* and late blight as result of *Phytophthora infestans* infection. The outcome of these infections may depend on the presence or absence of plant resistance factors (physical barriers,

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recognition receptors, stress hormones, production of antimicrobial compounds and pathogenesis related proteins, etc.) and susceptibility factors (interaction partners of plant-injected bacterial proteins, plant genes feeding the pathogen like sugar transporters, and plant negative immunoregulators). Here, we targeted various potato genes for CRISPR/Cas genome editing in order to improve the metabolic and immunological response of potato plants, thereby giving them an edge over these highly effective pathogens. The knockout of polyphenol oxidase (PPO) genes caused widespread metabolic and hormonal changes (e.g., increased scopoletin, taxifolin, salicylic acid, and jasmonic acid production) but still compromised the resistance against *Ralstonia solanacearum*, underlining the importance of PPO activity as a resistance mechanism. Meanwhile the knockout of the salicylic acid-degrading downy mildew resistance 6 enzyme increased potato resistance against bacterial wilt. We also attempted to boost the immune system by knocking out the microRNA gene miR396. The miR396 knock-out plants showed a delay in bacterial wilt symptoms, but they were more sensitive to late blight. Our studies show successful application of the CRISPR/Cas system for gene function studies. Strong plant resistance may be achieved by the combination of successful single-gene strategies.

P-13-007
Impact of biochar and compost on soil fertility and plant response to salt stress

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Saline stress negatively impacts plant growth, crop yield, and soil fertility. To address this challenge, organic amendments have gained significant attention due to their potential benefits. Recent studies have demonstrated that amendments like compost and biochar improve plant nutrition and crop yield, induce differential protein expression related to abiotic stress response, and alter soil microbiology [previously published in: Ghouili et al (2023) Biology 12(4):546, Ghouili et al (2023) Plants 12(3):526; Helaoui et al. (2023) Plant Stress 9:100182]. In this context, this study aims to evaluate the effects of compost and biochar on barley growth and response, as well as on the soil microbial community under salt stress. Barley plants, treated or not with compost and biochar, applied separately or in combination, were cultivated under controlled conditions, and at the tillering stage, irrigated with a saline solution (200 mM). Leaf samples were collected for agronomic, physiological, biochemical, and molecular analyses. Simultaneously, soil samples were taken for in-depth microbiological analyses using high-throughput sequencing (MiSeq). The results showed that the application of organic amendments improved plant growth and enhanced their antioxidant responses to saline stress. On a molecular level, compost and biochar notably enhance the expression of stress-responsive genes (including *HvDREB, HvHSP, HvP5CS*, and *HvPIP*), whereas their combined effect moderates gene expression, suggesting a harmonious and efficient regulation of stress response pathways. Moreover, MiSeq analysis revealed that the addition of these amendments affected the relative abundance of bacteria and fungi, promoting the biological fertility of the soil. These findings suggest that the co-application of biochar and compost offers better protection against saline stress. As far as we know, this is the first extensive and multifaceted study exploring the synergistic effects of biochar and compost on both plants and soil.

P-13-008
Biochemical changes in leaves of two different strawberry varieties influenced by clove extract

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Plant extracts are under investigation for their positive effect on plants as protection against disease or growth promoters. However, plant extracts consisting of strong antioxidant compounds may induce stress in plants when applied externally. Therefore, we aimed to investigate the content of biochemical compounds in strawberry leaves after spraying with *Syzygium aromaticum* clove plant extract. For comparison, two different strawberry varieties 'Rumba' and 'Malwina' were chosen. Experiments were carried out at LAMMC Institute of Horticulture greenhouse. Randomized block design with three replicates of five plants was applied. *S. aromaticum* extract was homogenized with distilled water before application (2 mL/L v/v). Leave samples were collected before spraying and after 3 and 7 days. ABTS and DPPH antioxidant activity was determined from lyophilized leaves samples. Additionally, concentration of total phenolic compounds (TPC) and chlorophylls was analyzed. It was found that DPPH antioxidant activity in 'Rumba' decreased after 3 days (543.25 mM TE/g) and increased after 7 days (704.65 mM TE/g), compared to value before spraying with plant extract (629.58 mM TE/g). The same tendency was found in non-treated strawberries. In opposite, 'Malwina' DPPH activity decreased. No significant changes in ABTS antioxidant activity were observed after spraying in 'Rumba', activity varied 1687.59-1741.07 mM TE/g. Meanwhile, 'Malwina' strawberries were more sensitive to extract application and ABTS increased more than 2 times. A minor increase in TPC in both strawberry cultivars was determined after spraying with plant extract and were equal to 62.06 mg GAE/g in 'Malwina' and 79.84 mg GAE/g in 'Rumba' after 7 days. Concentration of chlorophyl a and b increased slightly after 3 days in both strawberry cultivars, however, decreased after 7 days. To conclude, *S. aromaticum* extract induces changes in strawberry accumulated compounds, which are partially dependent on the variety.

P-13-009
Nitroalkylation mediated by nitro-fatty acids plays a crucial role in regulating antioxidant activity during abiotic stress in plants

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Nitro-fatty acids (NO_2-FAs) are novel molecules resulting from the interaction of unsaturated fatty acids and nitric oxide (NO).

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Recently, NO_2-FAs have been found to enhance antioxidant defence in plants under stress. Furthermore, they exert their signalling function mainly through a reversible post-translational modification called nitroalkylation [1]. In this study, the modulation of different antioxidant systems by nitroalkylation of NO_2-FAs, including catalase (CAT), has been characterised. Additionally, changes in the levels of nitroalkylated proteins in response to the assayed abiotic stress conditions were analysed by mass spectrometry. The oxidative stress observed during the different abiotic stress situations tested in *Arabidopsis thaliana* such as salinity, heavy metals, low temperature, continuous light and darkness, resulted in a decrease in the levels of biosynthetic and stress-responsive nitroalkylated proteins, due to the oxidation of Michael adducts. On the other hand, nitroalkylation of key catalytic residues with NO_2-FAs compromised the functional capacity of key antioxidant proteins, which was restored during the establishment of stress situations after the cleavage of the nitroalkylated adduct. In this regard, nitro-linolenic acid (NO_2-Ln) reduced CAT activity by nitroalkylation of two key histidine residues (His 156 and His 248), which are crucial for its structural integrity and function [2]. However, oxidative stress induced by salt stress led to the removal of nitroalkylation through the cleavage of NO_2-Ln from CAT, thereby restoring its catalytic activity. These findings suggest that nitroalkylation is a dynamic regulatory mechanism influenced by the cell's redox state, acting as a crucial modulator of CAT function and a key control mechanism in plant antioxidant responses to abiotic stress. [1] Aranda Caño L et al. (2019) Plants (Basel) 8 (4). [2] Previously published in: Chaki M et al. (2025) Protein Sci 34(3): e70076.

P-13-010
Combined effect of cold-plasma seed treatment, aeroponics, and plasma-activated water on *Stevia rebaudiana* Bertoni sweetness

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Stevia rebaudiana Bertoni is an economically valuable plant due to its secondary metabolites, steviol glycosides (SGs), which are widely used as health-beneficial sweeteners. Seed treatment with cold plasma (CP) is a green technology that stimulates seed germination, plant morphometric parameters, biomass production, and disease resistance by inducing changes in plant biochemical phenotype. We have previously demonstrated that pre-sowing seed treatment with CP can be a powerful technique for stimulating SGs biosynthesis and/or accumulation in stevia leaves when plants are grown in soil [previously published in: Judickaite A et al. (2025) Plants 14, 271]. In aeroponics, SG concentrations are higher compared to soil, but the stimulating CP effect is diminished. This study aimed to investigate the effect of three combined treatment/cultivation techniques: seed treatment with two types of CP, plant cultivation in aeroponics, and leaf spraying with plasma-activated water (PAW). We have demonstrated that a short (5 min) seed treatment with two types of CP (dielectric barrier discharge (DBD) and capacitively coupled (CC) CP) didn't stimulate the most abundant SGs rebaudioside A (RebA) and stevioside (Stev) SGs biosynthesis, DBD treatment even decreased RebA and Stev concentrations by 20%. However, DBD didn't change RebA/Stev ratio (the indicator of taste quality of SGs), which was 1.0 as in control, but CC increased it up to 1.7. PAW treatment (leaf spraying for 12 days) increased RebA concentration in control and DBD groups by 71% and 67%, respectively. Consequently, RebA/Stev ratio increased from 1.0 to 3.5 and 2.4 in the control and DBD groups, respectively. PAW treatment did not affect SGs of the CP group. From this pilot study, it can be concluded that seed treatment with CC alone and plant treatment with PAW may be used to ameliorate the taste quality of natural sweeteners in stevia growing in aeroponics. This work was supported by the RCL (S-MIP-23-8).

P-13-011
UPLC-ESI-Orbitrap-MS/MS phytochemical analysis and characterization of different *Achyllea clypeolata* extracts

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The genus *Achillea* L. (*Asteraceae*) contains over 120 species found across Europe, Asia, America, and Australia. In Bulgaria, 22 species have been identified, including *Achillea clypeolata*. Despite its therapeutic potential, research in Bulgaria has focused on the taxonomy, morphology, and biological activities of its essential oils. Data on the non-volatile components of the plant growing in the Plovdiv region are missing. This study aims to analyze the bioactive phytochemicals in ethanolic, methanolic, and aqueous extracts using ultra-performance liquid chromatography-electrospray ionization-orbitrap-tandem mass spectrometry (UPLC-ESI-Orbitrap-MS/MS). For the analysis, 1 μL of extracts were separated on a Hypersil Gold column (100 x 2.1 mm, 1.9 μm particle size) at 35°C. The mobile phase consisted of 0.1% formic acid in water (A) and 100% acetonitrile (B), respectively. A gradient of 5% to 100% of B for 30 min at a flow rate of 0.3 mL/min was used. The eluent was electrosprayed at 3000 V and 2000 V at positive and negative mode, respectively, into an Exploris 240 mass spectrometer (Thermo Fisher). Mass spectra were acquired in the range of 70 to 1000 m/z, with a resolution of 120 000. For MS/MS, collision energy was set to 25, 50, and 75 N with a resolution of 17 500. The 15 most intense precursor ions per scan were selected for MS/MS fragmentation. Data were processed using Compound Discoverer 3.3, with identification based on accurate mass, MS/MS fragmentation patterns,

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MzCloud and ChemSpider online libraries. A MzCloud match factor threshold of 70% was set for each compound. A total of 81 compounds were identified (51 in positive and 30 in negative mode). Predominant classes included polyphenols, flavonoids, terpenes, alkaloids, organic acids, amino acids, and their derivatives, sugars, and fatty acids. These findings provide valuable insights into the phytochemical composition of *Achillea clypeolata*, contributing to its pharmacological and medicinal application.

P-13-012
In vitro antioxidant and antibacterial activity of water, methanol and ethanol extracts of the Balkan endemic species *Achillea clypeolata* Sibth. & Sm. (Asteraceae)

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Comprised of 120 wild species, mostly native to Europe, the genus *Achillea* is a significant representative of the *Asteraceae* family. *Achillea clypeolata* is an endemic species common to the eastern half of the Balkan Peninsula. The present study aimed to evaluate the antioxidant and antibacterial activities of water, methanol and ethanol extracts of the plant, growing in the region of Plovdiv, Bulgaria, for which no literature data exist. The antioxidant activity was assessed through several colorimetric assays – ferric reducing antioxidant power, cupric ion reducing antioxidant capacity and DPPH radical scavenging assay. The total flavonoid (TF) and polyphenol (TP) content was also measured. According to the results from the antiradical assays, the methanol extract had the most pronounced antioxidant effect, followed by the ethanol one ($p < 0.001$), while the water extract showed the lowest antiradical activity ($p \leq 0.001$). Similar results were obtained for the TF and TP. The antibacterial activity was evaluated with two strains of Gram(-) (*Escherichia coli* and *Pseudomonas aeruginosa*) and Gram(+) bacteria (*Staphylococcus aureus* and *Streptococcus mutans*) for which minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) of the extracts were determined. No effect of the extracts on *E. coli* was observed. The results showed that the methanol extract had the lowest MIC in the Gram(+) bacteria ($p \leq 0.001$ vs. water), while the ethanol extract inhibited the growth of *P. aeruginosa* at a lower concentration than the other two extracts ($p \leq 0.001$). Furthermore, the ethanol extract showed a bactericidal effect at the lowest concentration in *S. aureus* ($p \leq 0.001$), while in the rest of the studied bacterial strains both the ethanol and methanol extracts had equal MBC. The determined antioxidant and antibacterial activities of the plant extracts will serve as a basis for future *in vivo* analyses aiming towards elucidation of the therapeutic effects of *A. clypeolata*.

P-13-013
The extracellular life of a cytoplasmic protein: investigating profilin secretion in pollen

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Profilin, a renown panallergen, regulates actin polymerisation by binding G-actin and inhibiting its spontaneous nucleation. Profilin-actin pools are employed for the fast polymerization needed by the pollen tube growth, and profilin-dependant actin polymerisation is necessary for the directional transport of vesicles in the pollen tube. Our previous studies have demonstrated that profilin and actin are present in pollensomes, despite not having a known function in the extracellular space; which leads to question their role in pollen-pistil interactions. In this study, hazel (*Corylus avellana*) and apple (*Malus domestica*) pollen was either hydrated in phosphate buffer saline, or germinated in liquid medium. Both treatments were performed at optimal temperatures or under heat stress, which is known to disrupt the cytoskeletal organisation of pollen tubes. Actin and profilin distribution in the pollen tubes was assessed by immunofluorescence. Pollen grains (PGs), extracellular vesicles (EVs), and vesicle-free medium (EVF) were separated by filtration and differential ultracentrifugation. Pollensomes in EVs were quantified by nanoparticle tracking analysis, and stained with FM 4-64 to confirm their integrity. Total proteins were then isolated from the three fractions, quantified, resolved by SDS-PAGE, and then immunoblotted to probed for the presence of: (I) pollensomes markers (ALIX-homologs, clathrin), confirming their presence in EVs; (II) markers of other cell compartments, excluding contamination from cell debris in EV and EVF; (III) profilin and actin. Finally, the presence of actin-bound profilin in EVs or EVFs was assessed by co-immunoprecipitation. Profilin secretion within pollensomes suggests a role in long-distance signalling, possibly leading to cytoskeletal remodelling of the pistil cells. Pollensomes and profilin secretion was enhanced by heat stress, but it remains unclear whether this is an active response.

P-13-014
The role and characteristics of liquid-liquid phase separation in physiological activity of phytochrome B

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Phytochrome B (PhyB) is a photoreceptor and thermosensor in plants. It plays a key role in photosynthesis, photomorphogenesis, skotomorphogenesis, seed germination, etc. Upon absorbing red light, PhyB transitions into its active form, translocates to the cell nucleus, and forms photobodies (PBs). PBs are multifunctional membranelles nuclear organelles in plants, characterized as dynamic liquid-droplet structures. They participate in crucial intracellular processes, including biological

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activity regulation, gene transcription, and protein degradation. PB formation is based on liquid–liquid phase separation (LLPS), though the nature of interactions between their components remains unclear. This bioinformatics study aimed to investigate the role of LLPS in PhyB function and the nature of interactions within PBs. Identification of proteins that physically interact with PhyB, along with PB's proteome analysis, revealed that 30% of PhyB-interacting proteins may localize within PBs. GO analysis showed that these proteins participate in red/far-red light signaling, interact with histones and DNA, regulate transcription, and influence plant processes like photomorphogenesis, post-embryonic development, and metabolism. Within PBs, PhyB mainly interacts with proteins involved in light signaling. Analysis of intrinsic disorder and LLPS propensity indicated that over half of PB's proteins are intrinsically disordered and LLPS-prone, including PIF (phytochrome-interacting factor) proteins interacting with PhyB. A study demonstrated that PhyB-PIF interaction lowers PhyB's critical concentration for phase separation, leading to membranelles organelle formation. It suggests PhyB–PIFs interactions may initiate LLPS, incorporating other proteins into condensates. Charge analysis showed 77% of PB's proteins are negatively charged, with 34% highly so, proposing a need for charge compensation to maintain structure, possibly via polyamines. It suggests that weak electrostatic interactions play key role in PB formation.

P-13-015
Adaptation of high-throughput screening technology in 96-well plate format for suspension cultures of monocots

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Reducing the iteration time is a critical parameter affecting the creation of transgenic crops with improved properties. Currently, there are no fast, high-throughput methods for testing a large number of genetic constructs or libraries in monocotyledonous plant cells. It is necessary to either grow whole plants and conduct agroinfiltrations to test the constructs, or isolate protoplasts from plant cells, which requires enzymes and special equipment. Therefore, the aim of this work was to adapt the technology of high-throughput screening in the format of 96-well plates for suspension cultures of monocotyledonous plants. The technology was tested on the *Asparagus virgatus* culture, which was transformed with a plasmid with the transcriptional unit p35S–GFP–tOCS. The asparagus cell suspension was precipitated by centrifugation in 96-well plates. The obtained plant cell packs (PCP) were transformed with the *Agrobacterium* strain LBA4404. After 2 days, the fluorescence emitted by the asparagus suspension cells as a result of the expression of the reporter protein GFP was analysed. The effect of the sedimentation rate of the cell suspension on the expression of the reporter gene GFP was studied. It was shown that the highest fluorescence values are achieved at an acceleration of 500 *g*, which indicates optimal conditions for maintaining cellular activity. When the acceleration is increased to 1000 *g*, the cells are damaged due to increased centrifugal action. The developed technologies for transient expression of plasmids with reporter genes opens up wide possibilities for their application in practical research and agriculture. This research was supported by the grant of the Ministry of Science and Higher Education of the Russian Federation 075-15-2024-653 "Systematic characterization of promoters and terminators for gene expression in monocots".

P-13-016
Extensive mechanical damage causes an increase in phytaspase activity in *Nicotiana benthamiana* leaves

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Molecular mechanisms of stress development in response to adverse conditions in plants are a subject of intense interest to researchers. Phytaspase is a particular plant subtilisin-like protease involved in the realization of stress-induced programmed cell death (PCD) [previously published in: Trusova SV et al. (2019) Front. Plant Sci. 10, 873]. Being constitutively active, phytaspase is thought to be involved in cellular processes other than PCD, and some of its actions are already described and can be defined as protective functions. One example is the processing of the hormone systemin in tomato plants, which activates protective mechanisms preventing insect consumption [previously published in: Beloshistov RE et al. (2018) New Phytologist 218(3), 1167-1178], and another is phytaspase's involvement in the processes of flower abscission under drought conditions [previously published in: Reichardt S et al. (2020) Science. 367(6485), 1482-1485]. Here, we are interested in the phytaspase's participation in response to mechanical stress, which can be considered a simulation of consumption by insects or herbivores. We have shown that moderate mechanical disturbances do not affect phytaspase activity in 3-4-week-old *Nicotiana benthamiana* plants, while extensive leaf removal causes a systemic increase in phytaspase activity in remaining leaves and in newly formed leaves. We investigated the effect of damage intensity and the time course of the effect. To elucidate the underlying causes of the phytaspase activity increase phenomenon, we further compared the expression levels of the phytaspase gene under normal conditions and under stress conditions caused by mechanical damage. The results we obtained provide a better understanding of the role of phytaspase in plant defense responses, particularly in response to mechanical damage, which is likely adaptive rather than PCD-inducing. The study was supported by the Grant of the Education Bureau of Guangdong Province "Innovation Team" 2022KCXTD034. *The authors marked with an asterisk equally contributed to the work.

P-13-017
Curcumin analogues elicit anti-platelet and anti-thrombotic activities

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Curcumin is a bioactive molecule extracted from the rhizome of Turmeric (*Curcuma longa*), known for its anti-inflammatory, antioxidant, cardioprotective, and neuroprotective properties.

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However, its therapeutic potential is limited by poor water solubility, low bioavailability, poor pharmacokinetic profile and can interact non-specifically with a wide range of biological targets. To address these issues, several structural variants of curcumin have been developed. Curcumin derivatives have been developed in the laboratory of Professor Michela Rosini in collaboration with Professor Cristina Lanni, and tested in this study for their anti-platelet and anti-thrombotic activities. Platelet aggregation was analyzed in light transmission aggregometry, platelet adhesion was analyzed in immunofluorescence microscopy, and platelet cytoskeleton reorganization and signaling pathways were evaluated in SDS-PAGE and immunoblotting with specific antibodies. Treatment with curcumin analogues (1 and 5 μM) resulted in a 50% reduction in thrombin-induced platelet aggregation compared to curcumin (25 μM). Thrombin-induced cytoskeleton reorganization and platelet adhesion and spreading on fibrinogen were impaired by curcumin derivatives. Previous studies have shown that curcumin enhances the phosphorylation of VASP through protein kinase A activation via adenosine A2A receptor signaling [previously published in: Rukoyatkina et al. (2022) Biochem. Biophys. Res. Commun 586, 20–26]. In line with this, $VASP^{Ser157}$ and $VASP^{Ser239}$ phosphorylation was increased in the presence of curcumin derivatives. These results suggest that curcumin analogues analyzed in this study seem to be promising candidates for further investigation.

P-13-018
Soil microbiome from Hg-contaminated and uncontaminated *Tussilago farfara* habitats

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Tussilago farfara (coltsfoot), an important medicinal plant, was found to be the dominant species in a mercury-contaminated area, where it exhibited a statistically significant increase in size compared to coltsfoot growing in uncontaminated sites. Plants from the polluted area showed no signs of disease, and there were no significant differences in the maximum quantum efficiency of photosystem II (F_v/F_m ~0.8) between plants from different habitats. To analyze the soil microbiome associated with coltsfoot, the V5-V7 amplicon was used, considering four sites: the mercury-contaminated area (G) and three control sites – a nearby uncontaminated area (N), an urban area (U), and a mountain area (M). Microbiome diversity followed the order: M > N > U > G. The Hg-contaminated soil had the lowest coverage in the *Silva* database, suggesting that many species in this area remain undiscovered. Despite this, each site exhibited a distinct metagenomic profile, with only 153 common amplicon sequence variants (ASVs) shared across all four groups. The contaminated soil microbiome had the highest similarity to the nearby uncontaminated area (N) and the least similarity to the mountain area (M). Notably, three bacterial genera – *Nocardioides*, *Nakamurella*, and *Sphingomonas* – were significantly more abundant in Hg-contaminated soil than in the other groups. These taxa may serve as biomarkers of mercury-contaminated environments and could play a crucial role in plant survival, including that of coltsfoot. These findings provide valuable insights into plant-microbiota interactions in mercury-contaminated habitats and identify unique bacterial strains with potential applications in phytobioremediation.

P-13-019
TATOOINE: a new hope for plant drought resistance

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Climate change and the more severe droughts associated with it result in reduced plant growth and, consequently, lower crop yields. Crops are an important part of the human and animal diet, making it crucial to understand drought resistance mechanisms for food security. Our research focuses on TATOOINE (AT4G25290), a protein from *Arabidopsis thaliana* (L.) Heynh., whose overexpression enhances drought tolerance while increasing biomass. TATOOINE belongs to a novel plant-specific protein group with an N-terminal photolyase domain and a C-terminal hydrolase domain. These proteins remain uncharacterized in the literature. Our findings show that TATOOINE plays a role in drought response. Under water deficit conditions, *A. thaliana* plants overexpressing TATOOINE (OE) flower earlier than wild-type (WT) and *tatooine* knock-out mutants, accelerating seed production and improving survival. Unlike WT and mutants, OE plants recover after severe drought. To explore its molecular role, we analyzed the expression of abscisic acid (ABA)-dependent signaling genes, as ABA is a key hormone involved in plant responses to stress. TATOOINE is a promising genetic target for engineering drought-resistant crops. Our findings provide new insights into plant drought adaptation strategies, laying the foundation for future studies and potential agricultural applications.

LB-R-13-01
Transcriptional regulation of thioredoxin, thioredoxin reductase, and Calvin-Benson cycle genes under light and CO_2 conditions in *Arabidopsis thaliana*

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Plant growth and development are profoundly affected by light and carbon dioxide (CO_2). Variations in light intensity and CO_2 concentrations lead to significant regulation of plant metabolism and redox states. In our study, we explored these effects in *Arabidopsis thaliana* by focusing on the expression profiles of the thioredoxin machinery, including the typical and atypical thioredoxins (*TRXs*), thioredoxin reductases (*TRs*), as well as genes involved in the photosynthesis process, specifically those associated with the Calvin-Benson cycle (*CBC*). An *in-silico* analysis of gene expression was conducted to assess the response of *TRXs*, *TRs*, and *CBC* genes under different light and CO_2 conditions. Promoter analysis was also performed to identify light-responsive elements (LREs) within the gene promoters. The expression levels of *TRXs*, *TRs*, and *CBC* genes were found to be finely tuned under varying light and CO_2 conditions. *In-silico* promoter analysis identified 27 different light-responsive elements (LREs), with two specific LREs, CAG-motif and 4cl-CMA2b,

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found exclusively in the promoters of GAPA and GAPB, respectively. Additionally, variations in CO_2 concentration were observed among six *Arabidopsis* ecotypes (*Te-0*, *Yeg-1*, *Col-0*, *Sf-1*, *Fei-1*, *Bur-1*), highlighting ecotypic differences in response to CO_2 concentration. This research elucidates the genetic and molecular mechanisms utilized by plants to adapt to fluctuating light and CO_2 conditions. The findings suggest that plants can adjust their metabolic pathways through specific changes in gene expression, aiding in their survival under varied environmental stresses.

LB-R-13-02
Functional analysis of the bifunctional FMN/FHy enzyme reveals a central role in flavin homeostasis

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Riboflavin (vitamin B2) is the biosynthetic precursor of flavocoenzymes, which are essential for the cell. As the central component of FMN and FAD cofactors, riboflavin controls the function of flavoproteins, being indispensable for growth and cellular metabolism. These cofactors sustain photosynthesis, mitochondrial electron transport, fatty acid oxidation, photoreception and secondary metabolism. While animals depend on exogenous resources, riboflavin is mostly biosynthesized in plants and microorganisms. In the model species *Arabidopsis thaliana*, the key enzyme AtFMN/FHy has both riboflavin kinase and FMN hydrolase activities. Phylogenetic analysis suggests that AtFMN/FHy proteins occur in algae and land plants. AtFMN/FHy 3D structure displays two distinct domains representing the different enzymatic properties. Here, we report the characterization of the FMN/FHy mutant, which displays abnormal plant growth and leaf chlorosis. Due to the evident phenotype, the mutant was called melinoe (melin) after the Greek goddess, who had a pale skin illuminated in the moonlight. A nucleotide transition at an intron-exon junction of the FMN/FHy gene of melin homozygous plants results in alternative 3' splicing, skipping twelve amino acids within the FMN phosphatase domain. Leaf variegation was apparent during the entire life cycle of melin homozygous plant that showed a specific gradient pattern of chlorosis beginning from the center of the leaves and extending to the entire leaf lamina. The growth of melin homozygous plants ceased upon transition from the reproductive to the vegetative stage. Biochemical approaches revealed that melin mutant seedlings accumulated riboflavin to exceptionally increased levels. Further, the knockout mutant of FMN/FHy is lethal, underlining the pivotal role of the enzyme in flavin metabolism. Overall, the results shed light on the regulatory role of FMN/FHy in intracellular flavins biosynthesis.

Redox biochemistry

P-14-001
Oxidative stress levels and antioxidant enzyme activities in hypoxia-induced zebrafish embryos

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Insufficient oxygen delivery to tissues, a condition known as hypoxia, adversely affects embryonic development and leads to the onset of various neurobiological diseases. Hypoxia-induced molecular and biochemical changes may interfere with neuronal differentiation and synaptic organization, which may subsequently manifest cognitive and behavioral impairments. Once hypoxia occurs, glutamate excitotoxicity can be triggered, resulting in axonal and neuronal degeneration. Here, we aimed to investigate the effect of hypoxia in developing zebrafish embryos. We exposed zebrafish embryos to a hypoxic E3 medium induced chemically by sodium sulfite for 175 min and 250 min and examined their embryonic development for changes in morphological, glutamate levels, and oxidative stress parameters. Lipid peroxidation (LPO) levels and glutathione S-transferase (GST), superoxide dismutase (SOD), and catalase (CAT) enzyme activities of hypoxia-induced zebrafish embryos were analyzed using spectrophotometers. Glutamate levels were measured by HPLC. LPO levels decreased in the hypoxia groups compared to the control group. GST activities increased in 250 min hypoxia group compared to the controls and 175 min hypoxia group. SOD activities decreased in the hypoxia groups compared to the control group. CAT activities increased in 175 min hypoxia group compared to the controls and 250 min hypoxia group. Glutamate levels were altered in the hypoxia groups compared to the control group. In conclusion, we demonstrated that hypoxia may induce alterations in the biochemistry of the redox system in developing zebrafish embryos. Our research will continue to uncover the molecular mechanisms behind these biochemical changes and examine the effects of hypoxia in zebrafish embryos. This study is supported by Istanbul University-Cerrahpaşa Scientific Research Projects Coordination Unit. Project No: TDK-2024-37879.

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P-14-002
Development of a biosensing system in *Rhodococcus fascians* for the detection of oxidative stress-inducing natural compounds

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Redox homeostasis is essential for survival. To achieve this, organisms employ diverse strategies, including various proteins and molecules, to regulate homeostasis and prevent oxidative stress burden, also known as distress, which causes cellular damage and can ultimately lead to cell death. Building on this concept, inducing oxidative stress has emerged as an alternative and effective strategy for combating bacteria using molecules and chemical compounds beyond conventional antibiotics. Furthermore, there is an urgent need to discover new sustainable and environmentally friendly molecules in accordance with the 2030 Agenda's goal of reducing pesticide usage. However, to meet these criteria, developing a sensor capable of detecting potentially oxidative stress-inducing compounds would be advantageous. Mycoredoxins (Mrxs) are a class of disulfide oxidoreductase enzymes known to play a key role in maintaining redox homeostasis across many species in the Actinomycetota phylum. Additionally, reduction-oxidation-sensitive GFPs (roGFPs) have been engineered by modifying specific surface-exposed residues of green fluorescent protein (GFP), allowing them to function as biosensors for oxidative stress. Building on these insights, this study focuses on developing a biosensing system in *Rhodococcus fascians* D188, an actinobacterial phytopathogen. Using the D188 strain, we constructed a mutant lacking three identified mycoredoxin-encoding genes, which was then used as a recipient for a recombinant plasmid carrying an Mrx1-roGFP2 construct. The resulting in-frame fusion protein, Mrx1-roGFP2, enabled the creation of a modified microorganism capable of detecting oxidative stress-inducing molecules. Given that *R. fascians* belongs to the *Actinomycetota* phylum, these findings could be extrapolated to other actinobacterial phytopathogens, offering a novel approach for identifying oxidative compounds as potential alternatives for controlling actinobacteria-driven plant diseases.

P-14-003
Targeting human dihydroorotate dehydrogenase in antiviral therapies: structural insights for a green drug discovery

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The pyrimidine biosynthesis pathway (PBP) is an essential enzymatic cascade, crucial for DNA and RNA synthesis. Among the enzymes acting in *de novo* PBP, human dihydroorotate dehydrogenase (*h*DHODH) catalyzes the rate-limiting step by converting dihydroorotate into orotate through a ping-pong redox mechanism which involves the reduction of FMN, later regenerated by electron transfer to ubiquinone. *h*DHODH has been extensively studied as a drug target for various diseases, including cancer and autoimmune disorders[1] and significant efforts have focused on developing a detailed SAR-model for the rational design of small molecules targeting the ubiquinone binding site. In line with the growing awareness on "green drug discovery", we are currently investigating arzanol, a natural compound extracted from *Helichrysum microphyllum*. This natural molecule, already characterized for its antiviral and anti-inflammatory properties[2], has never been subjected to target validation, leaving the molecular target responsible for these effects unknown. Considering both the arzanol structure and the implication of *h*DHODH in antiviral research, we propose *h*DHODH as the molecular target of arzanol, mediating its antiviral properties. In this study, we demonstrated that arzanol competes with ubiquinone cofactor, inhibiting *h*DHODH with a potency in the low μM range. We then proved its antiviral activity when tested against SARS-CoV-2 infected cells, unveiling its putative molecular target. Finally, through co-crystallization experiments, we have successfully obtained structural data of the protein-inhibitor complex, providing valuable insights for the development of innovative green therapeutic agents based on this original molecular scaffold. Our study can also benefit research in the oncology field, where *h*DHODH is emerging as a moonlighting protein.[3] [1]Sainas, S. *et al.* (2022) *JMC* 65, 12701–12724. [2]Appendino, G. *et al.* (2007) *J. Nat. Prod.* 70, 608–612. [3]Zhang, Q. *et al.* (2025) *Cell rep.* 44, 115327.

Regulation of gene expression

P-15-001
Molecular biological characterization of the hypoxia sensitivity of human stearyl-CoA desaturases

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Stearyl-CoA desaturase (SCD) enzymes, which catalyze the formation of unsaturated fatty acids, play a pivotal role in protecting cells against lipotoxicity. The two human isoforms, SCD1 and SCD5, are subject to regulation at multiple levels. The modulating role of the low-oxygen (hypoxic) environment has recently been proposed. The central element of the hypoxia signaling pathway is the transcription factor (TF) Hif1α, which regulates the expression of numerous genes in order to adapt to low oxygen concentrations, including a proto-oncogene, ETS1 TF. This study aimed to investigate the regulatory mechanisms underlying the hypoxia-sensitive expression of SCD1 and SCD5 and the impact of human genetic variants in an *in vitro* cell model. *In silico* analysis of the functional promoters of SCD1 and SCD5 revealed multiple ETS1 binding sites and a single Hif1α consensus sequence. Moreover, polymorphisms were identified that disrupt ETS1 binding sites, thereby affecting transcriptional regulation. In order to model hypoxic conditions, HEK293T and SK-N-FI cells were treated with 100 μM desferrioxamine (DFO) for 4-24 hours. The alterations in expression levels were monitored by immunoblot and qPCR. The activities of the wild-type and polymorphic promoters of SCDs were quantified in a pGL3-β-based luciferase reporter system

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under both normoxic and hypoxic conditions. DFO treatment significantly increased the expression of ETS1, SCD1, and SCD5 at both the mRNA and protein levels. In normoxic conditions, polymorphisms that disrupt the ETS1 binding site resulted in a significantly reduced promoter activity compared to wild-type promoters in SCDs. The results of this study provide new insights into the regulation of SCD1 and SCD5 in hypoxic conditions and highlight the impact of reduced oxygen levels on the lipid composition of cells. Additionally, they illustrate the influence of individual genetic variants. This work was supported by FK_138115, PD142709, and EKÖP-2024-II grants.

P-15-002
Effect of missense mutations on the human NADH cytochrome b5 oxidoreductase enzyme

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Inadequate functioning of the enzyme systems responsible for maintaining the optimal saturated : unsaturated fatty acid (FA) ratio has been identified as a key factor in the development of numerous metabolic diseases. Electrons necessary for desaturase activity are provided by the cytochrome b5-reductase and cytochrome b5 enzymes, or presumably by the cytosolic NADH cytochrome b5 oxidoreductase (NCB5OR). Despite its role in FA metabolism, natural genetic variation of NCB5OR expressed in all tissue types has been insufficiently studied. In this study, 16 novel missense variants were selected from the NCBI database, occurring at least three times in different populations. Potential impact of these mutations was evaluated using a range of online prediction tools. Four mutations (Q15L, G31D, D285V and N428S) were identified as having a notable impact on the enzyme's functionality. *In silico* predictions were validated in an *in vitro* cellular system for all 16 NCB5OR mutations. This involved examining untagged, GluGlu-tagged and EGFP-fused NCB5OR variants in two cell lines. To ascertain whether the selected variants exerted an influence on protein expression at the protein and/or mRNA level, we employed immunoblotting and qPCR. Furthermore, the expression of specific markers associated with ER stress (XBP1, CHOP, BiP, PDI) was monitored. The intracellular localization of the protein was determined by examining cells transfected with EGFP-fused constructs using fluorescence microscopy. The *in vitro* experiments yielded evidence that the G31D, R140H, T180I and N428S mutations result in notable alterations in the expression, function and/or localization of NCB5OR. These findings contribute to the growing understanding of the role of NCB5OR and its natural variants in human disease, and provide a foundation for the development of new, effective preventive and therapeutic protocols for metabolic diseases. This work was supported by the 2024-2.1.1-EKÖP and FK138115 grants.

P-15-003
Identification of cell cycle-dependent expression and localization of dUTPase isoforms

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The enzyme dUTPase has a prominent role in maintaining genomic integrity via the hydrolysis of dUTP to dUMP. On one hand, it prevents dUTP misincorporation into the DNA, while on the other hand it produces dUMP substrate for thymidilate synthesis. Recently, it has been published[1] that next to the well-described nuclear (DUT-N) and mitochondrial (DUT-M) isoforms of dUTPase, it has two other isoforms in human cells. Although the mRNA levels of these novel isoforms were described in several human cell lines, localization and cell cycle-dependent expression remains to be elucidated. Cell cycle-dependent gene expression analysis is particularly important as numerous genes show tightly regulated expression patterns. For cancer cells, analysis of cell cycle related events is of paramount significance since tumorigenesis is characteristically coupled to cell cycle perturbations. Therefore, we aimed to investigate cell cycle dependent expression of dUTPase isoforms in U937 and MOLT4 human leukemia cell lines. Cell cycle phase distribution of cells was analyzed with FACS, protein expression was investigated with western blot and mRNA expression levels were determined with RT-qPCR. In contrast to the data available in the literature[2], we found that DUT-M expression is not constitutive, however it is S-phase dependent similar to DUT-N. Furthermore, the novel DUT-3 isoform has constitutive expression through the cell cycle, although it shares the same promoter as DUT-M. The expression of DUT-4 was found to be different from the other isoforms. Moreover, we sought to identify cellular localization of the novel isoforms using fluorescence microscopy. We found that the novel DUT-3 isoform is retained in the cytosol, while DUT-4 localizes to the nucleus. Our findings underline the importance of dUTPase expression during the whole cell cycle and in different subcellular regions. References: 1 Rácz G et al. (2023) Scientific Reports 13(1), 7760. 2 Ladner R et al. (1997) J. Biol Chem 272(30), 19072–19080.

P-15-004
Investigating the influence of estrogen receptor signaling on the SNHG noncoding gene family in MCF7 breast cancer cells

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Estrogen receptor (ER) signaling is crucial in cellular regulation, influencing coding and non-coding gene expression. Long non-coding RNAs (lncRNAs) are pivotal in cancer biology, affecting gene expression and cellular processes like proliferation and metastasis. Despite the limited number of known associations, ER's impact on lncRNAs remains underexplored in breast cancer. This study explores the time-dependent effects of estradiol (E2) on the lncRNA transcriptome in ER-positive

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MCF7 cells to identify differential expression and transcript isoforms. We applied direct RNA sequencing to assess the impact of E2 on the non-coding transcriptome and detected E2-induced differential gene and transcript expressions in various non-coding genes. Among the ncRNAs, 19 members of the small nucleolar RNA host gene family (SNHGs) were affected by E2 induction. We focused on one member of SNHGs consistently induced by E2, SNHG9, known for its oncogenic features in glioblastoma, pancreatic cancer, and ovarian cancer. Functional assays demonstrated that SNHG9 overexpression in ER-positive MCF7 cells led to a decrease in proliferation rates, suggesting a potential regulatory role in estrogen signaling pathways. Luciferase reporter assays indicated that SNHG9 has multiple potential miRNA binding regions with varying responses. Our findings suggest that SNHG9 may function as a microRNA sponge in breast cancer progression, potentially through estrogen signaling pathways. The shared regulatory elements in SNHG promoters hint at a common regulation mechanism by estrogen. Currently, we are investigating the specific miRNA binding regions on SNHGs and the potential transcriptional regulation of the SNHG gene family by ER signaling through promoters. Our results will likely provide further insight into understanding E2-induced changes in the transcriptome through lncRNAs in breast cancer cells.

P-15-005
Investigation of the effect of pulsed electromagnetic field application on PAD2 gene expression in PC12 cells

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Neurogenesis, the development of neurons from neural stem cells, plays a vital role in brain repair and function. This intricate process heavily depends on the peptidyl-arginine deiminase (PAD) enzyme family, which converts arginine into citrulline through a modification called deimination, impacting neuron growth and survival. Disruptions in deimination, especially involving PAD2, are associated with neurodegenerative diseases. Pulsed electromagnetic field (PEMF) therapy, utilizing low-frequency EMFs, is gaining recognition as a non-invasive treatment for neurological conditions such as depression, Parkinson's, and Alzheimer's disease. PEMF has demonstrated potential in stimulating neurogenesis and tissue repair. Since the PC12 cell line exhibits neuronal properties, they are invaluable for investigating neuronal differentiation, function, and signaling pathways. Within this framework, this study explores the relative expression levels of *PAD2* in PC12 cell lines before and after PEMF treatment (PT). For this purpose, first an in-house PEMF system was constructed. Then, cells were exposed to PEMF for 15, 30, and 60 minutes and their viability was assessed using the XTT assay, which confirmed that PT did not adversely affect cell survival. RNA for quantitative real-time PCR analysis was isolated before and after PT. According to the preliminary results of our project, *PAD2* expression increased progressively with the duration of PEMF exposure: 2.53-fold after 15 minutes, 2.51-fold after 30 minutes, and 6.31-fold after 60 minutes. In conclusion, PT may boost neurogenesis by regulating PAD2 expression, thereby corroborating previous findings suggesting that PEMF could facilitate neurogenic processes. This contribution expands our understanding of PEMF's potential benefits in managing neurodegenerative diseases. This work was supported by TUSEB (32133). *The authors marked with an asterisk equally contributed to the work.

P-15-006
The role of the transcription factor pita in chromatin organization and boundary function in *Drosophila melanogaster*

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Pita is an architectural C2H2 protein with zinc finger clusters (C2H2 domains). It contains a ZAD domain at the N-terminus, essential for homodimerization and long-distance interactions. The C-terminal cluster, consisting of 10 C2H2 domains, binds DNA motifs predominantly near transcription start sites. In cooperation with the architectural proteins dCTCF and Su(Hw), Pita forms boundaries/insulators between regulatory domains that control the expression of three Hox genes in the Bithorax complex (BX-C). Within the unstructured linker between the ZAD and C2H2 domains, a 12-amino acid (aa) region interacts with the BTB domain of CP190, a protein associated with many architectural proteins. Using Cas9, we replaced the first exon of the *pita* gene with the attP site for φC31-mediated recombination, generating the null pitaattP1 mutation. In the pitaattP1 platform, we obtained transgenic lines expressing mutant Pita variants under the control of the Ubi-p63E promoter. Pita is essential, and pitaattP1 null mutants die at the late embryonic stage. Unexpectedly, PitaΔZAD (ZAD deletion) partially restores viability and fertility; however, mutant flies live less than 10 days. In BX-C, PitaΔZAD lacks boundary function, highlighting ZAD's role in insulator activity. The PitaΔCp1mutant (deletion of 12 aa interacting with CP190) also shows reduced insulator activity. While PitaΔCp1 and PitaΔZAD are expressed normally, simultaneous deletion of the ZAD and CP190-interacting regions results in an almost completely unstable *in vivo* PitaΔZAD + ΔCp1 protein. Addition of the ZAD-like domain from the human protein ZNF276 fully restores the functional activity of PitaΔZAD + ΔCp1. These results suggest that the ZAD and CP190-interacting regions have similar and partially redundant functions in Pita activity. The mammalian ZAD-like domain may perform similar functions in Drosophila architectural proteins. Funding: This work was supported by the Russian Science Foundation (19-74-30026-P to P.G.).

P-15-007
Investigation of NFIB regulated genes in SH-SY5Y neuroblastoma cells

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Key developmental events such as correct proliferation and differentiation of stem cells, require precise control of gene expression and integrated actions of transcription factors. One example is the nuclear factor one (NFI) of the transcription factor family that comprises four members: NFIA, NFIB, NFIC and NFIX. Misregulation of NFIs have been implicated in various cancers including those of neural origin such as glioblastoma and neuroblastoma (NB). Specifically, NFIB expression is enriched in NB cells while low NFIB expression

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correlates with better patient survival. Moreover, the NFIB locus has been identified among H3K27 acetylated super-enhancers in various NB cell lines, including SH-SY5Y cells which represent the adrenergic subtype. Conducting cell viability and colony formation assays on this cell line, we have previously found that silencing NFIB decreases cell survival and proliferation [Uluca B et al. (2022) Biochim Biophys Acta Gene Regul Mech 1865, 194798]. Interestingly, while NFIB transcriptionally represses CDON, a cell surface protein which can act as a pro-apoptotic factor, knock-down of *CDON* does not rescue the effects of silencing NFIB. To investigate NFIB's role further, we set out to identify NFIB regulated genes in SH-SY5Y cells. To this end, we transduced SH-SY5Y cells with NFIB targeting shRNA encoding virus. Knock-down efficiency was assessed by RT-qPCR and western blotting. Next, these samples will be subjected to mRNA sequencing. The obtained raw sequencing data will be used to identify differentially expressed genes and to conduct pathway and ontology analyses. While ChIP-seq experiments are required to uncover direct NFIB targets, this initial work will help illuminate the gene expression program regulated by NFIB in adrenergic NB cells. Acknowledgements: This study was supported by TÜSEB (38207).

P-15-008
Elk-1 and mitochondrial gene regulatory networks in neurodegenerative diseases

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Elk-1 is a member of the ETS transcription factor family, playing a critical role in cellular growth, differentiation, and survival. The overexpression of Elk-1 has been reported to provide neuroprotective effects in models of Parkinson's disease (PD) and Huntington's disease (HD). Elk-1 associates with the mitochondrial permeability transition pore complex in neurons and has been shown to localize around mitochondria in cell somas and dendrites. Its involvement in the pathogenesis of neurodegenerative diseases suggests Elk-1 has potential as a therapeutic target. In this study, we investigated the relationship between Elk-1 and mitochondrial gene expression through models of Huntington's disease (HD), amyotrophic lateral sclerosis (ALS), and Parkinson's disease (PD). Using data from a previous RNAseq assay in Luhmes-based PD model, as well as public datasets from GEO (HTT and ALS) that were analyzed via Galaxy Server, we performed differentially expressed genes (DEG) and Gene Ontology (GO) analyses on these three disease models, with a focus on mitochondrial-related genes. The expression changes of mitochondrial genes were compared to Elk-1 level through correlation analysis. Using RStudio and the PANDA package, gene regulation relationships between Elk-1 and mitochondrial genes were evaluated, and corresponding networks were visualized using Cytoscape. The potential interaction network signifies a critical link between Elk-1 and the regulation of mitochondrial function and stress responses. Our results indicated that Elk-1 has a role in the pathogenesis of disease states via energy generation pathways and the regulation of oxidative metabolism in mitochondria. The relationship of Elk-1 to energy metabolism, mitochondrial dynamics, and stress reactions highlights its therapeutic potential as a novel target for approaching neurodegenerative disorders. *The authors marked with an asterisk equally contributed to the work.

P-15-009
Investigation of lncRNA modulation by apigenin in breast cancer therapy

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Apigenin, belonging to the flavone subclass, is a natural flavonoid widely found in plants. It exhibits some biological properties such as anticancer, antioxidant, and anti-inflammatory effects in cancer biology. It has potential as a therapeutic agent in breast cancer treatment. Apigenin could influence the expression of cancer-related long non-coding RNAs (lncRNAs), involved in gene regulation. This study aimed to investigate the therapeutic potential of apigenin in breast cancer and its effect on the expression of some breast cancer-related lncRNAs. MCF-10A was used as a healthy breast epithelial cell line, and MCF-7, an ER- and PR-positive luminal A breast cancer cell line, was used as the cancer model. MCF-7 and MCF-10A cells were treated with apigenin at varying concentrations (0–100 μM) for 24, 48, and 72 hours. The half-maximal inhibitory concentration (IC50) of apigenin was determined using the MTT (3-[4,5-dimethylthiazol-2-yl]-2,5 diphenyl tetrazolium bromide) assay. Total RNA was extracted from the treated cells and converted to cDNAs by reverse transcription. For the expression analysis, cDNA samples were analyzed by quantitative Real Time PCR (qPCR) method. The Ct results that obtained from the qPCR of targeted lncRNAs were examined by 2−Ct method. It was found that apigenin exhibits therapeutic potential in breast cancer by modulating the expression of breast cancer-related lncRNAs.

P-15-010
Post-translational regulation of NFIB by its interaction partner PIN1

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Nuclear factor one (NFI) family of transcription factors plays specific and essential roles in embryonic development and pathologies arising from dysregulation of developmental pathways. Two family members, NFIA and NFIB, have been shown to be required for the maintenance of the core transcriptional program in glioblastoma (GBM) subtypes. As NFI family members carry a highly conserved N-terminal DNA binding domain, they may bind and regulate similar target genes. However, specificity may arise from post-translational modifications such as phosphorylation. Such control mechanisms or NFIs' upstream regulators have not been well characterized. To gain more insight, we carried out a yeast two-hybrid screen which identified PIN1 as an NFIB interaction partner. Co-immunoprecipitation and GST pull-down experiments confirmed this interaction which requires PIN1's WW domain. PIN1 also binds other NFIs, and NFI N-terminal domains are necessary and sufficient for PIN1 interaction. PIN1 suppresses the transcriptional activity of NFIB on the GFAP and *HES1* promoters, while substrate-binding deficient PIN1 is unable to do

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so. Interestingly, functional repression of NFIB does not require PIN1 isomerase activity, although NFIB-PIN1 interaction is phosphorylation-dependent. Paradoxically, cycloheximide chase experiments found stabilization of NFIB by PIN1 which implies that PIN1 represses NFIB function not by regulating its abundance but by inducing a conformational change. These results identify NFIB as a novel target of PIN1; PIN1-mediated post-translational regulation of NFIB may present an additional control mechanism of gene expression in embryonic development and tumorigenesis [Saritas Erdogan et al. (2024) FEBS Lett 598, 2910-2925]. As *GFAP* and *HES1* are important NFI targets and are implicated in GBM, we are currently investigating the downstream effects of PIN1-NFIB interactions in GBM model cell lines. This study was supported by TUBITAK (115Z524, 120Z994) and ITU BAP.

P-15-011
Investigation of the role of interferon regulatory factor 4 on programmed cell death in melanoma cells

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The interferon regulatory factor 4 (IRF4) was first identified as a transcription factor involved in the development of immune cells. Our studies and those of others have shown that IRF4 assumes critical roles in various immune cell-derived cancers and normal skin cells. Melanoma has the highest lethality rate of all skin cancers. In our recent work, we have shown that IRF4 has also critical functions in melanoma cells [previously published in: Sobhiafshar U et al. (2024) Mol Oncol 18, 2423-2448]. Apoptosis is a form of programmed cell death that plays an important role in normal developmental processes and cancer. Based on data from our group and public databases indicating IRF4 control of a critical apoptosis regulatory BCL2 family member gene in melanoma cells, we set out to investigate in melanoma cell lines the potential role of IRF4 on the expression of this BCL2 family member and the apoptosis process itself. Accordingly, here we provide our findings on the mRNA expression and protein levels of the relevant apoptosis regulator, and on apoptotic cell death in melanoma cells upon experimental manipulation of IRF4 expression. In addition, we discuss IRF4's role in modulating the effect of a BCL2 family-targeting chemotherapeutic drug. These results provide a compelling rationale for further exploration of IRF4 and offer potential strategies for improving treatment outcomes in this highly resistant cancer type.

LB-R-15-01
Unravelling circadian regulation of gene expression in a multicellular and differentiated cyanobacterium

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Cyanobacteria are a large and morphologically diverse group of photosynthetic bacteria, including unicellular and multicellular strains. The presence of a circadian clock in cyanobacteria allows them to anticipate and adapt to daily environmental changes. In unicellular strains, circadian clock is driven by the core oscillator KaiC which harbors a 24-h phosphorylation oscillation. The phosphorylated KaiC regulates downstream clock-controlled genes via the master transcription factor RpaA. The circadian clock is well-known at the molecular level in unicellular strains. How it functions in multicellular organisms remains poorly understood. Among numerous filamentous species, *Anabaena* PCC 7120 is a key genetic model, particularly for studying multicellularity, cellular differentiation, and nitrogen metabolism. Previously, DNA microarray studies were conducted on bulk cultures, identifying groups of genes that exhibit 24-hour oscillatory behavior and their oscillations are dependent on the KaiC clock [previously published in: Kushige H et al. (2013) J Bacteriol 195:1276–1284]. However, the molecular mechanisms underlying the circadian clock in *Anabaena* remain poorly understood. In this study, we confirmed circadian gene expression in *Anabaena* at the population level by RT-qPCR experiment and single cell level by time-lapse microscopy. In addition, we showed that response regulator RpaA is required to generate these circadian transcriptions in *Anabaena* and that RpaA phosphorylation oscillates with a 24-h period. This data indicate that RpaA is also the regulator that transmits the signal from the clock to target genes. Interestingly, we observed that increased light intensity abolished the oscillatory pattern of RpaA phosphorylation. Consistent with this finding, the rhythmic expression of candidate genes was also disrupted under high-light conditions. These results motivate further investigation into the interplay between circadian regulation and dynamic light responses.

LB-R-15-02
Hypoxia-driven changes in MATN3 expression and EMT associated marker profiles in osteosarcoma

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The extracellular matrix (ECM) is a dynamic, heterogeneous network of macromolecules that enables the connection of structural proteins, glycoproteins, and polysaccharides. The ECM regulates biochemical signals critical for cellular homeostasis, migration, and proliferation. Matrilins are an ECM protein family that plays a role in the cellular network. They connect collagen-associated fibrils and aggrecan, COMP, Collagen-I -II, -IX, and small leucine-rich proteoglycans. Matrilins have been found in melanoma, cervical, and hepatocellular cancer. The oncogenic role of MATN3, particularly in gastric cancer, is highlighted in many studies. A bioinformatic study identified EMT-related gene signatures in osteosarcoma (OS) and reported that MATN3 expression is downregulated in metastatic tissues compared to primary tissues. The high metastatic ability and heterogeneity of OS are the main concern of therapy. Therefore, it is important to identify new molecular targets that participate in OS metastasis for novel therapeutic approaches. Here, we initially determined MATN3 expression at mRNA (qRT-PCR) and protein (western blot) levels at normoxia in OS cell lines (MG63, SaOS-2, 143B, and U2OS). The highest MATN3 mRNA expression was observed in

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Saos-2 and U2OS cells. The MG63 cell line displayed the lowest MATN3 expression. Because the hypoxic environment is critical for EMT and tumor metastasis, the expression pattern of the MATN3 was also investigated in chemically induced hypoxic conditions. EMT markers Vimentin, E-Cadherin, N-Cadherin and MMP-9 expressions were studied. These results will provide the information to compare MATN3 expression and EMT related markers under hypoxia in different OS cell panels having different characteristics. This study was partially financially supported by Dokuz Eylul University, BAP Department (Project ID: 3588) and TUBITAK 2210/C National MSc/MA Scholarship Program in the Priority Fields in Science and Technology (1649B022416337). *The authors marked with an asterisk equally contributed to the work.

LB-R-15-03
Modulation of the E3 ubiquitin ligase UHRF2 (ubiquitin like with PHD and ring finger domains 2) by low oxygen conditions in tumor cells

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Osteosarcoma (OS) is a malignant bone tumor characterized by osteoid formation. Current treatment approaches don't provide the desired survival rates. Therefore, it is crucial to investigate the molecular, genetic, and epigenetic mechanisms of OS. The *UHRF2* gene is an E3 ligase involved in ubiquitination and plays a regulatory role in the cell cycle, and functions as either a tumor suppressor or an oncogene in various cancer types. The *UHRF2* gene has been found to contribute to the progression of colon and breast cancers while it acts as a tumor suppressor in leukemia, esophageal squamous cell carcinoma, and non-small cell lung cancer. Bioinformatic studies have shown overexpression of *UHRF2* in OS. The hypoxic tumor microenvironment is one of the most important drivers of tumor progression, promoting tumor aggressiveness and poor prognosis. Low oxygen conditions fulfill these effects by altering the expression of specific genes. So, identifying genes whose expression is altered under hypoxic conditions is essential to identify new targets that contribute to OS progression. In the current study, we aimed to determine the expression pattern of UHRF2 under physical and hypoxic conditions in OS models. 143B cells had the highest UHRF2 mRNA and protein level. The effect of hypoxia on UHRF2 mRNA (qRT-PCR) and protein levels (western blot) was evaluated in different osteosarcoma cells. The results will contribute to understanding the role of expression changes in the UHRF2 gene under hypoxic conditions in OS. Sedanur CAN is supported by the 2211-International Graduate Scholarship Program of the Scientific and Technological Research Council of Turkey (BIDEB) (1649B022416339). This study was financially supported by Dokuz Eylul University Scientific Research Projects Coordination Unit, Project Number: 3676. *The authors marked with an asterisk equally contributed to the work.

RNA biology

P-16-001
Microbial metabolite preQ1 regulates host cell physiology through selective tRNA degradation and translation regulation

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The microbiome interacts with its eukaryotic host through various metabolites to affect cell physiology. One such direct molecular interaction is the incorporation of microbial metabolite queuine at the wobble anticodon of host tRNA (to synthesise queuosine), regulating the host cell translation and tRNA stability. Microbes also produce the precursor metabolite pre-queuosine1 (preQ1) in the queuosine biosynthesis pathway; however, its effects on host cell biology and the underlying mechanisms have not been explored. Here we show that preQ1 significantly represses the human and mouse cell proliferation; however, this effect is suppressed or reversed by queuine and depends on the same host enzyme that installs the queuosine modification. PreQ1 is present in the tissues and plasma of mice and is incorporated into the tRNAs of both *in vitro* mammalian cells and in mice. Importantly, we have demonstrated that preQ1 treatment suppresses tumor growth in mouse cancer xenograft model. Mechanistically, preQ1 reduces the cognate tRNA levels specifically and the translation of housekeeping genes in a highly codon-dependent manner. We identify the inositol-requiring enzyme (IRE1) ribonuclease as the enzyme responsible for the selective degradation of preQ1-modified tRNAs on the translating ribosomes. Our results show a novel pathway by which microbiota affect host cell physiology through preQ1, highlighting its potential role in regulating cell growth and tumour suppression. *The authors marked with an asterisk equally contributed to the work.

P-16-002
Evaluation of the cardiopreventive machanism of metformin in a mouse model of autoimmune myocarditis

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Dilated cardiomyopathy (DCM) is a severe inflammatory disease resulting from myocarditis, often leading to poor prognosis and fatal outcomes. It is characterized by significant infiltration of inflammatory cells into cardiac tissue. Metformin, a synthetic

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compound derived from guanidine, has potent glucose-lowering and anti-inflammatory properties. Its cardioprotective role is known; however, the mechanisms behind that are still to be elucidated. The aim of this project was to explore the molecular mechanisms of metformin's cardioprotective role during autoimmune myocarditis progression. We utilized a mouse model of experimental autoimmune myocarditis (EAM) in which BALB/c mice are immunized twice with αMyHC peptide and complete Freund's adjuvant to induce the inflammation. Metformin was administered from day 0 of the experiment. Our preliminary results show that metformin administration reduced myocardial inflammation, as evidenced by HE staining of paraffin-embedded heart sections. Therefore, we have isolated single nuclei from hearts at day 21 of EAM, both treated with metformin as well as from control animals. Single-nuclei cDNA libraries were prepared using 10× Genomics technology, and sequencing was performed on the Illumina NovaSeq 6000 platform, targeting 50 000 reads per cell. We have identified 11 major cell populations that include cardiomyocytes, fibroblasts, endothelial cells, T and B lymphocytes, and myeloid lineages. Our single-cell RNA sequencing data comparing healthy and inflamed hearts combined with metformin administration at the onset of the disease revealed that metformin exerts its cardioprotective function through activation of the AMPKα pathway. A deeper comprehension of alterations in transcriptomic signatures at the single-cell level may facilitate the formulation of innovative therapeutic strategies for inflammatory heart disease. Funded by the NCN, Poland: 2023/51/D/NZ7/00609.

P-16-003
Expression profiling of a novel intronic RNA and its potential interactions with miRNAs

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Breast cancer is the most frequently diagnosed cancer in women, characterized by genetic, epigenetic, and phenotypic heterogeneity, highlighting the need for targeted therapies. Advances in RNA sequencing have revealed the involvement of long non-coding RNAs (lncRNAs) in cancer progression. However, introns are often excluded from gene expression analyses, limiting the identification of intron-derived transcripts. In this study, we investigated the expression of a previously unannotated intronic RNA in various cancer cell lines and explored its potential interactions with miRNAs. Paired-end RNA sequencing was performed on three breast cell lines: HCC1143 (triple-negative breast cancer), BT474 (ER+/PR+/HER2+), and MCF10A (non-tumorigenic control). We developed a Nextflow-based bioinformatics pipeline to identify unannotated intronic RNA candidates and used cap analysis of gene expression (CAGE) peaks and polyadenylation signals (PAS) to define transcript boundaries. One candidate was selected for further analysis, and its 5′ and 3′ ends were mapped using rapid amplification of cDNA ends (RACE). To assess the expression of this intronic RNA across 1019 cell lines from 40 tumor types, we utilized Transipedia, a k-mer based platform for transcript expression analysis across multiple databases. Potential miRNA interactions were predicted using miRBase, revealing putative interactions with hsa-miR-619 and hsa-miR-7851 precursors. Notably, the intronic RNA was expressed in multiple cancer cell lines, including those derived from breast, lung, and kidney, suggesting potential biological significance. These findings indicate a possible regulatory role for this novel intronic RNA in cancer.

P-16-004
The regulatory role of specific noncoding RNAs in brain pathophysiology

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While there has been extensive research on noncoding RNAs (ncRNAs) in physiological mechanisms, the precise functions of specific classes of ncRNAs, including small nucleolar RNAs (snoRNAs) and their subset, small Cajal body-specific RNAs (scaRNAs), in brain pathophysiology remain largely unknown. In this study, we observed a significant upregulation of a particular scaRNA in the cortical region of 5xFAD mouse model of Alzheimer's disease (AD) compared to other snoRNA genes. Based on this observation, we hypothesize that this scaRNA may play a role in the pathophysiological process of AD. To investigate the regulatory mechanisms involving this scaRNA, we performed high-throughput total RNA sequencing and analysis on a neuroblastoma cell line overexpressing this scaRNA. Differential expression analysis, comparing gene datasets from 5xFAD mice and the scaRNA-overexpressing cells, revealed a common set of genes. Functional annotation of these genes indicated enrichment for immune system-related pathways. These findings could provide that the identified scaRNA may serve as an important mediator in AD progression and represent a potential therapeutic target.

P-16-005
The role of non-coding RNAs in nucleolar reorganization under stress

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After heat shock and extracellular acidosis, the nucleolus transforms into A-bodies – amyloid-like membraneless organelles consisting of the two types of non-coding RNAs (rIGSRNAs) and hundreds of proteins in the amyloid state. A-bodies have similarities with pathological amyloids, but these structures are physiological, reversible, and perform significant biological functions. They are important as models for studying both pathological and physiological formations. We studied the structure of the components of A-bodies and presented a refined model of formation, in which the "driver" of the formation of these amyloid structures is rIGSRNA. We determined an increase in rIGSRNA expression in MCF-7 cells after heat shock and acidosis using bioinformatics. Overexpression of rIGSRNA was confirmed by quantitative PCR (RT-PCR). More precisely, the transcript length was determined by RT-PCR. Using the T7 transcription, rIGSRNA was obtained and isolated. Using the circular dichroism, we showed that the two types of rIGSRNA has a different secondary structure. We also visualized the formation of rIGSRNA clusters in the nucleoli of MCF-7 cells using the fluorescence *in situ* hybridization. Bioinformatics analysis of the proteomic composition of the two types of

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structures showed that A-bodies formed after acidosis have more pronounced liquid-droplet properties than those formed after heat shock. Taken together, our studies reveal unknown features of A-body biogenesis. A-body formation begins with the stage of specific interaction of rIGSRNA with proteins, with further formation of liquid-droplet "foci" that form a mature structure. The specificity of the formation of different types of A-bodies is due to the secondary structure of ncRNA expressed under different types of stress. The reorganization of nucleoli into A-bodies is a cellular stress response pathway in which protein immobilization by ncRNAs is a specific post-translational regulatory mechanism.

P-16-006
Alternative splicing complexity results in a very diverse repertoire of circular RNAs in multiple myeloma, as revealed by nanopore sequencing

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Circular RNAs (circRNAs) are regulatory RNA molecules influencing cancer onset and progression. In this study, we used nanopore sequencing for circRNA profiling in multiple myeloma (MM). Total RNA extracts from 10 MM cell lines were subjected to rRNA depletion, RNase R treatment, and rolling-circle reverse transcription. Next, nanopore sequencing was conducted using the PromethION 2 Solo platform. Data were analyzed using CIRI-long 1.1.0 and *in-house*-developed algorithms. Thus, the circRNA-enriched fractions yielded 94 048 full-length circRNA sequences. Interestingly, these circRNAs are produced from 14 313 genes, indicating complex alternative splicing patterns, including the presence of novel exons and intron retention. Exemplary cases are *CCND1*, a target of t(11;14) translocation, which yields 18 circRNAs, *NSD2*, affected by t(4;14) translocation, producing 36 circRNAs, and *BRAF*, being transcribed into 49 circRNAs. In addition, many identified circRNAs are clustered in chromosomal hotspots for MM, such as chr11q13.1, chr16q22.1, and chr9q34.11. Notably, enrichment analysis of gene sets from the Molecular Signatures Database showed that many parental genes are involved in cell cycle regulation and are upregulated during unfolded protein response or through the activation of mTORC1 complex, all hallmarks of MM pathogenesis. Moreover, a great heterogeneity was observed between cell lines; 63,4% of the identified circRNAs were expressed in 1 cell line only, whereas 149 circRNAs were found in all 10 cell lines. Of note, pathway analysis revealed that 103 out of 149 circRNAs are produced from genes implicated in mitophagy, highlighting a pivotal process in the development and drug sensitivity of MM. In conclusion, we successfully characterized the wide diversity of the circular transcriptome in MM, laying a foundation for studying its role in MM pathogenesis. Acknowledgement: Funded by the EU Horizon Europe research and innovation programme (Project 101097094 – ELMUMY).

P-16-007
Determination of circFAM120A/miRNA/mRNA interaction in human lung adenocarcinoma cells (A549) under hypoxia conditions

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circFAM120A is a lung tissue-specific circular RNA thought to play a role in adaptation to hypoxic conditions. Owing to its stability and specificity, it has potential as a biomarker for lung cancer. Certain circRNAs contain miRNA-binding sites, which act as sponges to regulate miRNA activity. Database analyses suggest that circFAM120A may interact with hsa-miR-30d-3p and hsa-miR-148b-3p, both of which are upregulated under hypoxia, and that JMY may be one of their target genes. This study aimed to investigate the circFAM120A/miRNA/mRNA interactions in A549 cells under hypoxic conditions. qPCR was used to compare the expression levels of circFAM120A, hsa-miR-30d-3p, and hsa-miR-148b-3p in normal lung epithelial (BEAS-2B) and lung cancer (A549) cells. After 2–8 h of hypoxic incubation, mRNA levels of JMY, p53 cofactor, p53, and HIF-1α were measured in A549 cells. The results showed that circFAM120A expression was 238.1-fold higher ($P \leq 0.01$) in A549 cells. miRNA analyses revealed that hsa-miR-30d-3p expression was 11.41-fold higher ($P < 0.0001$) in BEAS-2B cells, while hsa-miR-148b-3p was 7.1-fold higher ($P \leq 0.01$). In normoxia, circFAM120A expression increased in A549 cells, whereas hsa-miR-30d-3p and hsa-miR-148b-3p levels decreased significantly. After 4 h of hypoxia, circFAM120A expression decreased 0.02-fold ($P = 0.0068$), hsa-miR-30d-3p remained unchanged, and hsa-miR-148b-3p increased 7.07-fold ($P \leq 0.01$). Under hypoxia, circFAM120A suppression correlated with a 1.62-fold increase in JMY mRNA and a 0.16-fold decrease in p53 mRNA. HIF-1α mRNA expression was upregulated 5.8-fold after 30 min. Under hypoxic conditions, the decrease in circFAM120A expression was correlated with an increase in hsa-miR-148b-3p, which was associated with the suppression of p53 mRNA. Overall, these findings indicate that under hypoxic conditions, the expression levels of circFAM120A, hsa-miR-148b-3p, and p53 mRNA exhibit an inversely correlated regulatory relationship.

P-16-008
Re-targeting ribosomal rRNA 2′-O-methylation in human cells through CRISPR/Cas9-mediated snoRNA guide editing

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Small nucleolar RNAs are a subclass of small non-coding RNAs participating in post-transcriptional modification of ribosomal RNA (rRNA) as well as displaying an array of non-canonical functions in eukaryote cells. In particular, box-C/D-snoRNAs (SNORDs) in complex with their protein partners (snoRNPs) contribute to 2'-O-methylation due to the specific structural elements such as conservative boxes and a guide region which

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determines the modification area in rRNA. Current studies focus on the strategy for re-targeting SNORD activity through alterations in guide region. Previously authors have demonstrated the possibility of editing single and multiple *GAS5* intron-encoded box-C/D-snoRNA genes (*SNORD*s) in human cells with CRISPR/Cas9 and its effects on host gene maturation [Published in: Filippova JA et al. (2019) Front Pharmacol, Nov 4;10:1246; Matveeva A et al. (2023) Int J Mol Sci, 24(24):17621]. SNORD81 was selected as a model snoRNA and multiple sites in 18S and 28S rRNA were chosen as new potential modification targets. AlphaFold 3 was used to generate models for altered snoRNPs in order to assess the maintenance of a functional structure. 293FT human cells were subjected to CRISPR/Cas9-mediated homologous recombination to obtain cell lines carrying modified *SNORD81* gene. Cells displayed the expression of mature SNORD81 variants containing novel guide regions of interest. Reverse transcription (RT) termination in the presence of low dNTPs concentrations was used as a method to investigate 2'-O-methylation sites. The results obtained demonstrate RT termination at desired positions within rRNA, indicating the formation of unique methylated sites. This suggested strategy might be adopted for applications requiring the site-specific modification of the RNA of interest. This work was supported by the Russian state-funded project for ICBFM SB RAS (grant number 125012300660-2). Gene editing strategy was supported by the Russian Science Foundation (grant number 21-64-00017).

P-16-009
Novel brain-specific long non-coding RNA associated with cancer

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Long non-coding RNAs (lncRNAs) are often dysregulated in different types of tumors and are therefore widely considered as promising diagnostic or prognostic biomarkers. In general, lncRNAs are also more tissue-specific than proteins, which opens up opportunities for their use as specific cancer-associated molecules. In this study, we identified a novel lncRNA, CABR2, which is exclusively expressed in normal brain tissues as well as in different brain tumors. Analysis of TCGA data revealed that high CABR2 expression strongly correlates with longer overall survival in patients with low-grade gliomas, suggesting its potential oncosuppressive role. We observed high expression of CABR2 in a panel of primary glioma cell cultures, as well as in cell lines SH-SY5Y and SK-N-MC. We determined the exact transcripts of CABR2 by 3' and 5' RACE method combined with nanopore sequencing and validated two alternative isoforms, which were also observed in RNA-Seq data of normal brain tissues. These isoforms share a large identical region within the first three exons and differ only at the 3' end. Additionally, we discovered a shorter transcript with an alternative first exon, which is specific for several primary glioma cell cultures. We demonstrated that CABR2 is predominantly localized in the cytoplasm and hypothesized its involvement in microRNA sponging. To explore the functional role of CABR2, we generated a series of overexpression lines in the CABR2-deficient cells IMR-32 and U-87 MG, as well as in SK-N-MC. We also performed CABR2 knockdown experiments using specific siRNAs. Further comparative transcriptomic analysis revealed a set of differentially expressed genes upon CABR2 knockdown, which were validated by RT-qPCR. Our findings confirmed the tumor suppressive role of CABR2, primarily through the positive regulation of SESN3 and ZMAT3 expression. This work is supported by the Ministry of Science and Higher Education of the Russian Federation [grant No. 075-15-2024-536].

P-16-010
Effects of *Lactobacillus acidophilus* probiotic on the expression of fecal microRNA in rat colon cancer model

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Our study aims to investigate the impact of *Lactobacillus acidophilus*-supplemented gut microbiota on fecal miRNA expression in rat colon cancer model. Fifty rats were randomly assigned to five groups (n = 10): control (GK), *L. acidophilus*-fed (G1), DMH-induced colon cancer (G2), pre-treatment cancer group with *L. acidophilus* (G3), and post-treatment cancer group with *L. acidophilus* (G4). Fecal samples were collected every three weeks for microbiota analysis, and bacterial composition was determined via next-generation sequencing (NGS). At the end of the 18th week, 72 fecal miRNAs were isolated from fecal samples collected from all groups, and fecal miRNAs' gene expression was evaluated by RT-PCR. Total bacteria, total *Lactobacillus* spp., and *L. acidophilus* were measured in fecal samples collected from the animals during the study. In the G1 group, the highest *Lactobacillus* spp. ratio was observed in week 3. In the G2 group, the ratio of *Lactobacillus* spp. to total bacteria was found to be quite low at the beginning. In the G3 and G4 groups, a slight decrease in the ratio of total *Lactobacillus* to total bacteria was determined at week 9, while an increase was observed in both groups at weeks 15 and 18. However, in the G2 group, compared to the control group, 56 miRNAs were down-regulated, 5 of which were significant and 11 miRNAs were up-regulated, only 2 of which were significantly. In group 3, compared to G2, 14 miRNAs were down-regulated, and 1 of them was significant; 48 miRNAs were up-regulated, and only 1 was significantly. In G4, 63 miRNAs were down-regulated compared to G2, and 14 of them were significantly, while 4 miRNAs were up-regulated, and no significant difference was found in any of them. Our findings suggest that *L. acidophilus* supplementation reduces certain oncogenic miRNAs and increases tumor suppressor miRNAs compared to the non-probiotic cancer group.

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P-16-011
eIF6 N106S mutation may rescue a translation defect in Shwachman-Diamond Syndrome

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Shwachman-Diamond Syndrome (SDS) is a rare autosomal recessive disorder caused by loss-of-function mutations in the *SBDS* gene, leading to defects in protein synthesis and resulting in pancreatic insufficiency and bone marrow failure. eIF6 regulates mRNA translation initiation by binding to 60S ribosomal subunits, preventing premature 80S ribosome formation. SDS patients have an increased risk of myeloid neoplasms, and eIF6 mutations have been identified only in patients with better prognosis. Indeed, eIF6 mutations rescue the lethality of *SBDS* KO in yeast but this proof lacks in human. The eIF6 N106S missense mutation, the most frequent in SDS, was modeled in HEK293T cells using Prime Editing. The mutation was introduced with ~25% efficiency in heterozygosis but not in homozygosis, suggesting a loss of function due to impaired binding, as confirmed by western blot. A mild reduction in protein synthesis supports this, while polysome profiling indicates a defect in translation initiation rather than ribosome biogenesis. Upon *SBDS* knockdown by RNAi, eIF6 N106S mutant cells exhibit a marked decrease in SBDS protein levels respect to control. SBDS depletion leads to 80S monosome accumulation, implicating defects in translation initiation or ribosomal recycling. Puromycin incorporation revealed a significant reduction in protein synthesis under SBDS-depleted conditions. All this suggests a role for N106S eIF6 in overcoming the stress caused by protein synthesis impairment. Preliminary RiboSeq experiments across all genotypes confirm a termination defect when SBDS is reduced, which is rescued by the eIF6 N106S mutation. Ongoing RNAseq analysis, integrated with RiboSeq data, will identify specific gene expression in pathological conditions, providing deeper insights into SDS and the mechanism of rescue by eIF6 mutation. These findings lay the groundwork for targeting eIF6 as a potential therapeutic approach.

P-16-012
N4BP1 RNase uses tandem KH domains to associate with EDC4 and mRNA decapping factors in P-bodies

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N4BP1 (Nedd4 Binding Protein 1) is a ribonuclease composed of two K-homology (KH) domains, a ubiquitin associated (UBA) domain, a NYN (N4BP1, YacP-like nuclease) domain, and a CoCUN (cousin of CUBAN) domain. It plays an important role in modulating immunological responses, NFκB signal transduction, and viral mRNA degradation. In this study, we demonstrate that N4BP1 interacts with EDC4, a key scaffolding protein of the mRNA decapping complex, as well as other proteins associated with 5′-cap hydrolysis in processing (P) bodies, including DCP1A, DCP2, and XRN1. To elucidate the molecular basis of the N4BP1-EDC4 interaction, we identified the two tandem KH domains (KH-1 and KH-2) as essential for this interaction. Mutational analysis and domain deletion studies revealed that the interaction between N4BP1 and EDC4 was disrupted upon deletion of the KH domains, mutations in KH-1 and KH-2 domains GxxG loops or RNase treatment, implicating an RNA-mediated mechanism. Moreover, we showed that interaction of N4BP1 with EDC4 is necessary for the association with other decapping factors. In conclusion, our findings highlight the critical role of RNA in mediating the interaction between N4BP1 and EDC4. This study provides valuable insights into N4BP1's new functions within RNA-protein complexes and its involvement in mRNA decay pathways.

LB-R-16-01
3'UTR variants of amyotrophic lateral sclerosis (ALS)-linked RNAs modify subcellular and cellular phenotypes

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While most human genes express mRNA 3'untranslated region (3'UTR) variants of different lengths, their impact on cell physiology and disease remains largely unknown. Here, we studied 3'UTR length heterogeneity in amyotrophic lateral sclerosis (ALS) and determined that three ALS-linked transcripts exhibit lengthening of their 3'UTRs in patient samples. We investigated phenotypical effects in a neuronal cell line expressing these 3'UTRs and observed that expression of these unique 3'UTRs induces morphological changes at different levels. Among the most expressed 3'UTRs variants in ALS, *NEFH* 3'UTR-Long induces the formation of nuclear RNA clusters and *SOD1* 3'UTR-Long diminishes filopodia in the plasma membrane. *SQSTM1* 3'UTR-Long did not show major changes in nuclear RNA clusters or filopodia. This is the first report that suggests that 3'UTRs may function independent of the coding region and modify the phenotype of a cell, further expanding the impact of alterations in mRNA biogenesis in ALS. *The authors marked with an asterisk equally contributed to the work.

Signal transduction

P-17-001
BbKI peptide improves insulin sensitivity in skeletal muscle cells subjected to lipotoxicity via enhancing GLUT4 translocation

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Type 2 diabetes (T2D) is a chronic metabolic disorder defined by inadequate insulin production and peripheral tissue insulin resistance. Many natural compounds have shown antidiabetic effects. Notably, peptide protease inhibitors derived from the plant *Bauhinia bauhinoides*, including BbKI, have demonstrated promising glucose-lowering potential but the mechanism of its action is poorly understood. Thus, the aim of this study was to

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evaluate the impact of BbKI peptide on insulin sensitivity in skeletal muscle cells. Experiments were performed *in vitro* on C2C12 myotubes, treated with BbKI alone or in combination with palmitate (16:0) to induce lipotoxicity. Expression levels of genes and proteins regulating ER stress response and GLUT4 translocation such as AMP-activated protein kinase (AMPK), protein kinase B (AKT) or AS160 were assessed by RT-PCR, cell fractionation and western blot analysis. We found that BbKI peptide stimulates phosphorylation of AS160 at Thr642, Akt at Ser473 and AMPK at Thr172, thereby enhancing insulin signaling in palmitate-treated insulin resistant C2C12 myotubes. This was followed by increased GLUT4 translocation to the plasma membrane in C2C12 cells exposed to BbKI. Additionally, BbKI administration reduced abundance of ER stress markers, including CHOP, BiP and phospho-PERK in palmitate-stressed C2C12 myotubes. In summary, our results indicated that BbKI may potentially improve response to insulin in peripheral tissues under both normal conditions and lipotoxic insult.

P-17-002
Discovering the molecular basis of the many-to-one function in fungal His-phosphotransfer proteins

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Phosphorelay systems are complex His-Asp phosphotransfer systems in prokaryota and eukariota, except in animals. They comprise a hybrid histidine kinase (hHK), a histidine phosphotransferase (HPt), and a response regulator, and are involved in the celular response and adaptation to environmental changes. In fungi, the number of hHK varies in species, but only one HPt. Our study tries to shed light on the mechanism of this many-to-one phosphotransfer role of the HPt in fungal phosphorelay systems. For that purpose, we have produced from *Chaetomium thermophilum* receiver domains (REC) of five hHK of belonging to different groups (REChHK6, REChHK3 and REChHK5) and the HPt. We phosphorylated REC domains and checked phosphotransfer to Ct_HPt using native gels that occurred in less than a minute. Interaction between these REC domains to HPt, measured with microscale thermophoresis, showed K_D values in the low micromolar range, indicating low-affinity binding. Also, we determined the structure of HPt in complex with REChHK6 bound to the phosphomimetic BeF3-. Our structure reveals the absence of the Y-T mechanism of phosphoryl stabilization in REChHK6, concomitant with the lack of a Leu-Thr switch, indicative of low phosphoryl binding stability. We determined the structures of REChHK3 and RECCal_Sln1 from *Candida albicans* lacking phosphomimetic, indicative of similar low phosphoryl binding stability. Finally, our HPt structure revealed differences in the length of the N-terminal and loop αD-αE between HPt of fungi. We analyzed Ypd1 from *C. albicans* obtaining an envelope structure by SEC-SAXS which showed a flexible long loop αD-αE and a longer N-terminal than HPt. Phosphotransfer from RECCal_Sln1 to Cal_Ypd1 wild-type and a mutant without loop αD-αE was observed. Our study proposes a transient binding of the phosphoryl group in fungal REC-1 domains, and that the long loop αD-αE of Cal_YPD1 does not appear to be involved in the phosphotransfer mechanism.

P-17-003
Novel regulation of signalling through Arf G-proteins: exploring the phospho-Arf interactome

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ADP-ribosylation factor (Arf) proteins are a family of small GTPases in the Ras superfamily that have important roles in vesicular transport and actin remodelling. Ordinarily, these proteins operate as two-state switches oscillating between GDP- and GTP-bound forms that interact with different upstream regulators and downstream effectors to drive signals and actions. Some Ras-family G-proteins have been found to be phosphorylated by protein kinases, and it is thought that this might influence their localisation, function, or both. Recently, we have identified tyrosine phosphorylation of Arf as a potential mechanism for creating a four-state switch (two guanine nucleotide states each +/- phosphorylation) capable of more complex signalling. To identify any novel interactions, we have stably expressed in both HeLa and HEK293T cells a series of inducible Arf1 and Arf6 proteins fused with TurboID (wild-type, phospho-mimetic, Y→E and phospho-site knockout Y→F forms) to differentially label nearby binding partners. Through recovery and enrichment of biotinylated proteins, subsequent LC-MS/MS analysis and screening out common and well-known contaminants we identified candidate interactors involved in trafficking at the Golgi and endoplasmic reticulum, F-actin-binding, and cancer progression. Of particular interest were metabolic enzymes, a significant number of which locate to mitochondria. We are now exploring these specific interactions to confirm their biological authenticity.

P-17-004
12-Lipoxygenase selective inhibitor, flavonoid baicalein, suppresses Ca^{2+} responses induced by immunomodulators glutoxim and molixan in macrophages

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Disulfide-containing drugs glutoxim® (disodium salt of oxidized glutathione with d-metal at nanoconcentration) and molixan® (complex of glutoxim with nucleoside inosine) are used as broad-spectrum immunomodulators and cytoprotectors in the complex therapy of bacterial, viral and oncological diseases. Clinical studies have shown that molixan is effective in the prevention and treatment of COVID-19 infection; it leads to a more rapid regression of the disease severity to a milder form. Earlier, we have discovered for the first time that glutoxim and molixan induce an increase in intracellular Ca^{2+} concentration, $[Ca^{2+}]i$, due to Ca^{2+} mobilization from thapsigargin-sensitive Ca^{2+} stores and subsequent store-operated Ca^{2+} entry in rat peritoneal macrophages. 12-lipoxygenases (12-LOX) are known to play an important role in the functioning of peritoneal macrophages. Recently, 12-LOX and their products have been found to be involved in the pathogenesis of a wide range of

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human acute and chronic inflammatory diseases: cardiovascular, neurodegenerative and oncological diseases, diabetes and severe pneumonia in COVID-19 patients. To elucidate the involvement of 12-LOX pathway of arachidonic acid (AA) oxidation in the effect of glutoxim and molixan on $[Ca^{2+}]i$ in rat peritoneal macrophages, we used a specific 12-LOX inhibitor, flavonoid baicalein (5,6,7-trihydroxyflavone). Baicalein has anti-inflammatory, neuroprotective, antitumor and antioxidant effects. Using Fura-2AM microfluorimetry we have shown that 5 mM baicalein significantly suppresses both Ca^{2+} mobilization from Ca^{2+} stores and subsequent store-operated Ca^{2+} entry, induced by glutoxim or molixan in macrophages. The data obtained invite us to suggest the involvement of 12-LOX and (or) products of 12-LOX pathway of AA metabolism in the complex signaling cascade triggered by glutoxim or molixan and leading to $[Ca^{2+}]i$ increase as well as in the modulation of store-operated Ca^{2+} entry in macrophages.

P-17-005
Glycolysis-driven metabolic feedback sustains TGF-β-induced EMT in lung epithelial cells

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Chronic respiratory diseases (CRDs) are characterized by persistent inflammation, airway remodeling, and progressive airflow limitation. Transforming growth factor-beta (TGF-β) plays a central role in CRD pathogenesis by driving epithelial-to-mesenchymal transition (EMT), which contributes to fibrosis and airway dysfunction. While TGF-β is well known for its pro-fibrotic effects, its influence on cellular metabolism, particularly glycolysis, in lung epithelial cells and its association with EMT remains unclear. We investigated the role of glycolysis in TGF-β-induced EMT using A549 and BEAS-2B lung epithelial cells. Immunoblotting, RT-qPCR, metabolic flux analysis, lactate quantification, glucose uptake assays, chromatin immunoprecipitation (ChIP), and siRNA-mediated gene silencing were employed to examine the link between glycolysis and EMT signaling. TGF-β induced EMT, as indicated by increased cell motility and changes in epithelial and mesenchymal markers. This was accompanied by enhanced Smad3 phosphorylation and transcriptional activation of glycolytic enzymes. Pharmacological inhibition of glycolysis with 2-deoxy-D-glucose (2-DG) suppressed Smad3 activation and EMT progression. Smad3 knockdown reduced TGF-β-induced glycolytic enzyme expression, including 6-phosphofructo-2-kinase/fructose 2,6-bisphosphatase 3 (PFKFB3). Furthermore, lactate supplementation reinforced Smad3 phosphorylation, suggesting a metabolic feedback loop that sustains TGF-β signaling. These findings reveal a vicious cycle wherein TGF-β enhances glycolysis, leading to lactate accumulation and sustained Smad3 activation, further promoting EMT. Targeting metabolic reprogramming may provide a novel therapeutic strategy for airway remodeling, and TGF-β-associated CRDs.

P-17-006
A heterocellular signaling facilitates the inactivation of hepatic stellate cell

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Liver fibrosis is a complex process involving dynamic interactions among liver-resident cells. During fibrogenesis, hepatocytes and macrophages release signaling molecules, including damage-associated molecular patterns and inflammatory cytokines, which promote transdifferentiation of hepatic stellate cells (HSCs) into myofibroblasts. While reversion of HSC transdifferentiation plays a crucial role in liver fibrosis regression, its molecular mechanisms remain poorly understood. Here, we show that HSC inactivation is facilitated through an intercellular communication. Through adopting an hepatic HSC engraftment model, we observed a markedly accelerated rate of HSC inactivation in livers recovering from fibrosis compared to normal livers. As the mechanistic basis, we found that hepatocytes in recovering livers secrete intercellular signaling molecules to facilitate HSC inactivation, in co-culture and conditioned media systems. Our study proposes the first rational mechanism for HSC inactivation during liver fibrosis regression, highlighting a hepatocyte-driven signaling pathway as a critical driver. Promoting this signaling pathway will provide a novel antifibrotic strategy.

P-17-007
CK1ε-DVL in Wnt signaling: more than a mere kinase-substrate relationship

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Casein kinase 1 epsilon (CK1ε) is a positive regulator of the phospho-protein Disheveled (DVL), the central hub of the Wnt/β-catenin signaling pathway. In cells, DVL has been shown to phase separate into punctate structures, known as "puncta," which dissolve upon DVL phosphorylation mediated by CK1ε. Interestingly, CK1ε activity is also regulated by autophosphorylation of its long C-terminal tail. However, the cascade of CK1ε (auto)phosphorylation events leading to DVL puncta disassembly is still unclear. Here, we explored the molecular factors that control DVL phosphorylation by CK1ε. We used NMR in a binary system to investigate how multisite phosphorylation of the enzyme and/or the substrate modulates their interaction. We identified a DVL2 epitope, conserved across the Dishevelled isoforms, that mediates the binding to CK1ε. Notably, the interaction is not dependent on the phosphorylation events, suggesting a novel scaffolding function for the kinase. The kinetic analysis revealed that the enzyme-substrate (auto) phosphorylation within the DVL-CK1ε complex occurs synchronously. Indeed, CK1ε autophosphorylation does not exclude DVL processing and *vice versa*. Last, we pinpointed a single residue in DVL as the critical determinant of CK1ε-DVL complex formation. Mutating this residue completely abolished CK1ε binding *in vitro* and disrupted DVL phosphorylation in cells, ultimately affecting the distribution of the puncta. Our

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findings unveil a DVL phosphorylation mechanism in Wnt signaling that depends on the scaffolding function of Ck1ε.

P-17-008
Understanding molecular mechanisms in cyclic nucleotide-dependent protein kinases

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The second messengers cyclic adenosine monophosphate (cAMP) and cyclic inosine monophosphate (cIMP) can partially activate the cyclic guanosine monophosphate (cGMP) dependent protein kinase I (PKG I). The two splice variants of PKG I, PKG Iα and PKG Iβ, differ only within the first 100 amino acids and are completely identical within the catalytic and cyclic nucleotide binding domains. Despite this high similarity, both isoforms respond differently to cyclic nucleotides. The binding of cGMP in a competitive fluorescence polarization assay towards PKG Iα can be described by a single sigmoidal curve, while the binding process is more complex in PKG Iβ. For PKG Iα, the binding of cAMP and cIMP cannot be distinguished as their affinity is a factor of 100 weaker than the cGMP binding. This difference is just a factor of ten for the high-affinity binding in PKG Iβ. The low-affinity replacement in PKG Iβ of cAMP is a non-cooperative event, while the cIMP and cGMP binding is cooperative. This selectivity is also found in a kinase activity assay where activation constants are in the nM-range with cGMP and in the μM range for cIMP (5 μM) and cAMP (10 μM). Size exclusion chromatography shows a difference of 5 Å in Stokes' radii between PKG Iα and PKG Iβ without cGMP, while the Stokes' radii in the presence of cGMP are indistinguishable. Employing switchSENSE technology, conformational changes can be studied in detail. For cGMP, PKG Iα shows a biphasic process where an initial conformational change is triggered by sub-nanomolar cGMP. The PKG Iβ isoform shows a transient transition between an open and a closed conformation. Both isoforms show a transient transition for cAMP and cIMP. These experiments help to understand basic molecular mechanisms how PKG I is activated, which control smooth muscle contraction/relaxation in the human body. Methods developed here can also be applied to study disease associated mutations and help to identify potential drug targets.

P-17-009
Trace amine-associated receptors *in vitro* pharmacology

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The trace amine-associated receptors (TAAR) belong to a large group of aminergic G-protein coupled receptor (GPCR) proteins discovered in 2001. Among these, TAAR1 is the most extensively studied and is considered a promising therapeutic target for schizophrenia. The remaining five human TAARs are thought to function as a novel class of olfactory receptors, though emerging evidence suggests they may also have roles in the central nervous system and peripheral tissues. A significant challenge in studying TAARs is their low membrane expression in heterologous systems *in vitro*, likely due to their retention in the endoplasmic reticulum and subsequent degradation by the proteasome. One contributing factor to this poor expression may be the absence of N-terminal glycosylation, as demonstrated in TAAR1. To enhance plasma membrane expression, we have tested various N-terminal tags derived from other GPCRs, such as rhodopsin, β2-adrenergic, and glutamate receptors. Additionally, we evaluated chaperones (RTP1S, RTP2), Golf, Ric8b cotransfection commonly used in olfactory receptor deorphanization that are also difficult to express in a heterologous system. In our project, we utilized a BRET biosensor to measure cAMP accumulation and a NanoBiT complementation assay to study TAAR–G protein interactions. Our findings revealed that receptors, such as TAAR9, exhibited activity even without tags or chaperones, though the beta2-adrenergic N-terminal tag enhanced the signal. TAAR5, however, required the rhodopsin N-terminal tag to induce signaling, while TAAR6 activation was only observed with chaperone cotransfection. Through this approach, we identified two novel ligands for TAAR6. These results suggest that cells expressing TAARs possess an intrinsic molecular mechanism that supports the proper transport and folding of these receptors to the cell surface. This work was supported by the St. Petersburg State University, St. Petersburg, Russia (project ID: 117033714).

P-17-010
Melatonin preconditioning enhances the anti-fibrotic potential of mesenchymal stem cells by modulating cytokine and miRNA profiles

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Melatonin (MT), an antioxidant and anti-inflammatory hormone, is a promising preconditioning agent to enhance mesenchymal stem cells (MSCs) for regenerative medicine. While MSCs promote tissue repair, their therapeutic potential is limited by oxidative stress and reduced function in fibrotic environments. MT may improve MSC resilience and efficacy under these conditions. This study investigates the impact of MT preconditioning on MSCs' anti-fibrotic potential. Bone marrow MSCs (BM-MSCs) and umbilical cord MSCs (UC-MSCs) were treated with 5 μM MT for 24 h. Doubling time, migratory capacity, cytokine profile and fibrosis-related miRNA expression were investigated. A fibrosis score was calculated based on differentially expressed miRNAs (≥1.5 FC) associated with either fibrotic (-1) or anti-fibrotic (+1) properties. MT preconditioning significantly reduced MSC doubling time. In Scratch assays, UC-MSCs exhibited reduced closure rates at 8 h, normalizing by 16 h, while BM-MSCs showed a dynamic response suggesting a time-dependent interaction with MT. Cytokine array analysis showed that in UC-MSCs, the pro-fibrotic TIMP-1 and TIMP-2 were downregulated (1.79–2.24 fold), while pro-inflammatory IL-6 and MCP-1 were upregulated (2.44 fold), suggesting a modified inflammatory response. In BM-MSCs, IL-6, IL-7, and MCP-1 had milder increases, while VEGF-A and BDNF decreased (1.33–1.89 fold) potentially impacting angiogenic regulation in fibrosis. miRNA profiling indicated that BM-MSCs had a superior anti-fibrotic signature (fibrosis score = 8.97) than UC-MSCs (1.78), suggesting greater potential for fibrosis resolution under MT preconditioning. These findings suggest that MT preconditioning enhances MSC therapeutic potential for fibrosis by modulating cytokine secretion and shifting miRNA expression

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toward an anti-fibrotic profile. This study is funded by TUSEB-2022-B-03-27707.

P-17-011
Uncovering the role of OPN3, a non-visual opsin receptor, in cellular signaling

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Opsin 3 (OPN3) is a member of the opsin family, a subclass of G-protein-coupled receptors (GPCRs) characterized by their retinal-dependent, light-sensitive properties. While traditionally associated with non-visual photoreception, OPN3 has recently been implicated in diverse metabolic processes, particularly in the pathophysiology of type 2 diabetes, obesity, and cancer. However, despite its emerging physiological significance and potential therapeutic relevance, no pharmacological agonists or antagonists have been identified. Given that GPCRs constitute one of the most successful classes of drug targets, elucidating modulators of OPN3 activity could pave the way for novel therapeutic strategies in metabolic disorders. To investigate the functional role of OPN3, we first confirmed its expression in HEK293T cells by immunoblotting and demonstrated its localization to the plasma membrane using semi-quantitative immunocytochemistry. We demonstrated by functional assays that the expression of OPN3 has regulatory effects on adenylyl cyclase-mediated cAMP accumulation. Notably, this process remains unaltered, even when canonical G-protein pathways are subject to inhibition through the use of pertussis toxin (PTX), $G\alpha_o$, $G\alpha_z$ and $G\alpha_i$ inhibiting toxin (OZITX), and the phosphorylation catalyzed by G-protein-coupled receptor kinases 2 and 4 (GRK2 and GRK4). Intriguingly, OPN3 was found to enhance signaling through the transcription factors serum response element (SRE) and nuclear factor erythroid 2-related factor 2 (NRF2), pathways associated with proliferative and cytoprotective anti-oxidative/anti-inflammatory responses, respectively. These findings underscore OPN3's role in modulating intracellular signaling cascades and highlight its potential as a druggable target.

P-17-012
Marked cell-dependent effects of purinergic signaling on the proliferation of breast cancer cell lines

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Purinergic receptors are membrane proteins that bind purines or pyrimidines. They include P1 (adenosine-binding) and P2 receptors, which are further divided into P2X (ligand-gated ion channels) and P2Y (G-protein-coupled receptors) classes. Despite the widespread expression of purinergic receptors and their involvement in various diseases such as cancer, their role in breast tumor progression is uncertain. We studied purinergic receptor expression and function in SKBR-3, JIMT1, MDA-MB-231, and MCF-7 breast cancer cell lines. P2X4 and P2Y2 receptors, selected based on expression profiles available in The Human Protein Atlas, were labeled with fluorescent antibodies and analyzed by flow cytometry. Cell proliferation was assessed following ATP, 2-MeS-ATP, and 2-thio-UTP treatment in the absence or presence of POM-1, an ATP hydrolysis inhibitor. ATP levels were measured using a luciferase assay. ATP treatment inhibited the proliferation of three out of the four cell lines, and only MDA-MB-231 cells responded with enhanced proliferation to ATP. Experiments with POM-1 revealed that the whole ATP effect is due to breakdown products in MCF-7 cells, while ATP degradation products do not significantly contribute to the effect observed in the other three cell types. JIMT-1 and MDA-MB-231 cells release ATP to the medium according to the luciferase assays. Experiments with 2-MeS-ATP, showing preference for P2X4 over P2Y2 receptors, and with 2-thio-UTP, a selective agonist of P2Y2 receptors suggest that P2X and P2Y receptors exert opposite effects on cell proliferation. Receptor expression analysis showed that both P2X4 and P2Y2 were expressed at roughly similar levels across cell lines. Purinergic signaling has significant and largely variable effects on cell proliferation calling for further, deeper analysis.

P-17-013
The role of p53-p21-DREAM pathway in ATF4 transcriptional repression by p53-inducing agents

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Integrated stress response (ISR) and p53-dependent signaling are the major signal transduction networks maintaining cellular homeostasis. A short-time exposure to the mitochondrial electron transport chain (ETC) complex III inhibitor myxothiazol results in the transcriptional activation of ATF4, one of the ISR key players, whereas longer incubations result in ATF4 downregulation accompanied by p53 induction. p53 induction by myxothiazol is mediated by pyrimidine depletion, and uridine supplementation has been shown to prevent p53 accumulation and ATF4 suppression. One of the mechanisms underlying p53-dependent trans-repression is mediated by the p53-p21-DREAM pathway. The ATF4 promoter contains the degenerate DREAM-binding element, implying that this pathway might mediate ATF4 suppression by p53. We tested the influence of various p53-inducing compounds on the ATF4 level in colon and lung cancer cells with wild-type p53 and p53- and p21-deficient cells using RT-qPCR. The studied compounds included topoisomerase I and II inhibitors, various DNA-damaging agents, inhibitors of pyrimidine biosynthesis, and specific p53-inducing compounds that prevent its proteasomal degradation. Indeed, wild-type cells, most of the tested compounds caused a drop in the basal ATF4 mRNA level as well as upon ATF4 activation by the ETC inhibitors (piericidin A, the combination of myxothiazol and uridine). The effects were less pronounced in case of ATF4 activation by the ER-stress inducing molecules. Next, we studied the roles of p53 and p21 in these effects by evaluating ATF4 suppression in p53- and p21-deficient cells. Both p53 knockout and p21 knockdown caused an increase in the ATF4 basal mRNA level and largely prevented ATF4 suppression by

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doxorubicin. Noteworthy, p21 depletion completely prevented ATF4 downregulation by specific p53-inducing agents. Taken together, these findings demonstrate that p53 and p21 modulate ATF4 transcription.

LB-R-17-01
Flexibility of the mammalian integrated stress response

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Increased phosphorylation of the eukaryotic translation initiation factor eIF2a (eIF2α-p) coupled with decreased guanine nucleotide exchange activity of eIF2B is a hallmark of the "canonical" integrated stress response (c-ISR). It is unclear whether impaired eIF2B activity in human diseases including leukodystrophies which occurs in the absence of eIF2α-p induction is synonymous with c-ISR. We have characterized a previously unknown mechanism triggered by decreased eIF2B activity, distinct from c-ISR, which we term "split" ISR (s-ISR). S-ISR is characterized by translational and transcriptional programs that are different than those observed in c-ISR. While c-ISR involves induction of the core proteins ATF4, CHOP and GADD34, s-ISR involves only ATF4 induction. The mechanisms of induction of ATF4 in c-ISR and s-ISR are also different. ATF4 synthesis in c-ISR is independent of the cap-binding protein eIF4E. In contrast, s-ISR requires eIF4E-dependent translation of the upstream open reading frame 1 and subsequent stabilization of the *ATF4* mRNA. This is followed by altered expression of a subset of metabolic genes (e.g., *PCK2)* resulting in metabolic rewiring required to maintain cellular bioenergetics when eIF2B activity is attenuated. Overall, these data demonstrate hitherto-unappreciated plasticity of the mammalian ISR, whereby the loss of eIF2B activity in the absence of eIF2α-p induction, activates the eIF4E/ATF4/PCK2 axis to maintain energy homeostasis. Chien-Wen Chen et al. (Nature, 2025), online ahead of print. *The authors marked with an asterisk equally contributed to the work.

LB-R-17-02
β-Eudesmol increases HAS2 expression in human dermal fibroblasts mediated by TGF-β expression

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Human dermal fibroblasts (NHDFs) are abundant cells in the dermis. NHDFs are responsible for keeping skin homeostasis by producing key components of the extracellular matrix, such as elastin, collagen, and hyaluronic acid. Skin aging is the result of a loss of extracellular matrix and proliferative capacity. Increased production of extracellular matrix may lead to a new anti-aging strategy. Hyaluronic acid is known to be synthesized only by hyaluronan synthase (HAS). HAS has three isoforms, HAS1, HAS2, and HAS3, which differ in enzyme activity, hyaluronan molecular weight, and location of expression. NHDFs mainly express HAS2. In our previous study, we screened herbal medicine extracts that increase HAS2 expression levels and the amount of hyaluronic acid in NHDF. We have found that β-eudesmol, extracted from *Atractylodes lancea* rhizome, a type of herbal medicine, increases HA production by promoting the expression level of HAS2 in NHDFs. In addition, analysis of the pathway of action of β-eudesmol with inhibitors revealed that HAS2 expression levels are upregulated in an upstream pathway of TGF-β-receptor. However, the effect of β-oedesmol on TGF-β expression levels has not been investigated. The purpose of this study was to measure the TGF-β expression levels of NHDF by the addition of β-eudesmol. cDNA was produced from NHDF after addition of β-eudesmol and qPCR was performed to measure expression level of TGF-β. The results showed that the expression of TGF-β was significantly increased in NHDFs supplemented with β-eudesmol compared to control. The results suggest that β eudesmol increases HAS2 expression level and amount of hyaluronic acid in NHDF by mediating increase in TGF-β expression. *The authors marked with an asterisk equally contributed to the work.

Structural biology

P-18-001
Structural basis of AHP1 and ARR1 interaction in cytokinin signaling

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Cytokinin signaling involves a cascade of phosphorylation events initiated when the hormone binds to the sensory domain of a hybrid histidine kinase. This kinase then phosphorylates one of the phosphotransfer proteins (designated AHPs in *Arabidopsis thaliana*), which subsequently translocates to the nucleus to phosphorylate and activate a response regulator (ARR). Once activated, the ARR can induce downstream responses by either binding to promoter regions of cytokinin-inducible genes or acting as a negative feedback regulator. In this study, we present the structural and biophysical characterization of the AHP1/ARR1 complex. The proteins were recombinantly expressed in *E. coli* and purified using immobilized metal affinity chromatography where the two proteins formed a stable complex with a shorter retention volume compared to either one. After several crystal screenings, we obtained an adequate crystal that diffracted X-rays to 2.9 Å resolution. Solving the structure revealed the interface between the two proteins. Furthermore, using bio-layer interferometry, we measured the interaction between ARR1 and AHP1-5 (five active AHPs). The results show that ARR1 can bind all AHP1-5 with moderate binding affinities of hundreds of nM (94–174 nM). The binding curves show characteristics of a transient interaction, typically found in signaling cascades. These results are consistent with the moderate binding interface we observed in the complex structure AHP1/ARR1.

P-18-002
Analysis and engineering of KIT.METRNL signalling to boost heart tissue regeneration after myocardial infarction

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KIT, a receptor tyrosine kinase, is dimerized at the cell surface by the cytokine stem cell factor (SCF) to initiate fundamental

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signaling pathways, including hematopoiesis, gametogenesis, and melanogenesis [Previously published in: Verstraete K et al. (2012) Science 12, 753–766]. However, recent studies have shown that KIT can also bind meteorin-like protein (METRNL) to trigger heart tissue regeneration [Previously published in: Reboll MR et al. (2022) Nat Rev Cancer 376, 1343–1347]. Ischemic heart diseases are the leading cause of mortality and morbidity worldwide. Therefore, revealing the molecular principles underlying this completely new function of KIT might open new therapeutic opportunities for maximizing post-infarction recovery. However, the field is currently plagued by a paucity of mechanistic details of the KIT.METRNL complex assembly. Here, we provide critical new data towards elucidating the structure-function landscape of the novel interaction between KIT and METRNL and a blueprint for how KIT can be activated by two structurally distinct ligands. The gained knowledge is a necessary prerequisite to guide us to rationally engineer METRNL and/or design a *de novo* protein binder towards improving its interaction with KIT to elicit intensified signaling that can lead to amplified heart tissue regeneration. Collectively, the project will impact basic and applied cardiovascular research and promises to provide new possibilities for alleviating the enormous socioeconomic burden associated with these diseases.

P-18-003
Same domains, same shape, different unfolding and binding: characterization of protein fusions of PDZ3_ZO-1 with GA88

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PDZ domains are globular protein–protein interaction domains found in many vital proteins and their ability to bind short specific peptide motifs has already been employed for practical uses in several synthetic enzyme scaffolds. In some cases (e.g., PDZ3_ZO-1) it is widely recognised that their correct function relies on the presence of their neighbouring domains via dynamic allostery mechanisms. Following previously published results of *in silico* modelling, protein fusions of PDZ3_ZO-1 and artificial domain GA88, FD5A (PDZ3-GA88) and FD6A (GA88-PDZ3) were prepared and characterized. During the secondary (circular dichroism) and tertiary (e.g. analytical ultracentrifugation) structure determination, no significant differences between the two proteins were observed. In contrast, when measuring thermal denaturation (thermal shift assay), the two proteins showed melting temperatures varying by almost 10°C, implying great distinctness of stability. Interactions of the fusions with known PDZ3 peptide ligands revealed additional diverging properties. The more stable FD5A, in which a disturbance in PDZ3 dynamics near the binding cavity was predicted, underwent ligand concentration-dependent three-state thermal unfolding in the presence of some partners, suggesting binding-induced independent unfolding of its domains. No such behaviour was observed with FD6A. Upon further inspection by isothermal titration calorimetry, definitive differences in binding strength and thermodynamics were also found between the fusions' interaction with a novel tight binder, TET3 peptide, with FD6A showing KD lower by an order of magnitude. These results further attest to the non-modular nature of the PDZ3_ZO-1 acting as a silent disruptor, invisible in structure, yet influencing stability and function greatly. Further attention to this issue is crucial since a deeper understanding leading to creative exploitation of this phenomenon could advance future artificial protein design.

P-18-004
Structural characterization of a novel Arp2/3 iso-complex with enhanced actin nucleation efficiency

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Cellular motility is a fundamental process in biology, enabling tissue development, immune responses, and wound healing in multicellular organisms. Actin cytoskeleton remodelling is central to this process, with the Arp2/3 complex playing a key role in the nucleation of branched actin filaments. This multi-protein complex is composed of seven subunits (Arp2, Arp3, ARPC1–5). Remarkably, Arp3, ARPC1, and ARPC5 have isoforms that impart distinct cellular functions and mechanisms to the Arp2/3 complex. Iso-complexes of Arp2/3, formed by alternative subunit isoforms, expand its functional diversity and adaptative capabilities. Here, we report the structural and functional characterization of a newly identified Arp2/3 iso-complex containing an isoform of the Arp2 subunit and exhibits significantly higher actin filament nucleation activity compared to previously described Arp2/3 iso-complexes. This enhanced nucleation efficiency suggests a specialized mechanism underlying its activity. Remarkably, this iso-complex demonstrates tissue-specific expression and is involved in a unique cellular process that distinguishes it from other Arp2/3 variants. To elucidate the molecular basis of its enhanced functionality, we aim to determine the high-resolution structure of this iso-complex. Structural insights will shed light on the mechanisms by which this complex contributes to the increased efficiency of actin nucleation. Our findings highlight the functional specialization of Arp2/3 iso-complexes and underscore the importance of structural studies in unravelling their unique mechanistic properties. This work opens new ways for exploring how actin cytoskeleton dynamics are fine-tuned to answer certain requests of cellular processes.

P-18-005
Structural investigation of the wild-type and mutant EVH1 domain of the Homer1 protein in regards of Shank interaction

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The postsynaptic density (PSD) is a large protein network under the postsynaptic membrane. It is involved in memory formation and learning processes. Mutations of PSD proteins can result in different neurodegenerative disorders like autism spectrum

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disorder (ASD). Homer1 is a large scaffold protein in the PSD containing a long coiled-coil region and a globular EVH1 domain. EVH1 serves as a target-binding module, it can interact with proline-rich regions of different partner proteins like Shank. Mutations in the EVH1 domain can contribute to the development of ASD. We have determined the solution structures of the wild-type EVH1 and also investigated two of its mutants using NMR. Resonance assignment of the two mutants has been performed by CCPNMR. To complement their structural investigation, SAXS and CD measurements were also performed. Interaction with Shank peptides was characterized using biolayer interferometry and NMR titration. Our results suggests that the mutations primarily affect the stability and partner binding affinity of the Homer1 EVH1 domain.

P-18-006
Understanding the structure and function of human metabolic carbohydrate processing enzyme NagA

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N-acetylglucosamine-6-phosphate deacetylase (NagA) is a poorly characterised human enzyme exhibiting unique active site plasticity. The physiological implications of this unique substrate specificity are currently unclear. Human NagA is thought to catalyse a reverse step in the hexosamine biosynthetic pathway (HBP), regulating UDP-GlcNAc levels, a crucial intermediate involved in many downstream processes. However, human NagA may also act as a gatekeeper, preventing the incorporation of erroneous groups into the pathway. Using an ATP-recycling system, we synthesised novel glucosamine 6-phosphate derivatives and demonstrated that NagA processes these substrates with a comparable rate and affinity to N-acetylglucosamine-6-phosphate, the native bacterial substrate. X-ray crystal structures of NagA mutants revealed how these larger derivatives bind within its active site. Additionally, protein pulldown experiments identified interactions between NagA and human deaminases GNPDA1 and GNPDA2. Cryo-EM analysis revealed an interesting stoichiometry in the NagA-deaminase complex, suggesting a cooperative role in the HBP regulation. This study sheds light on NagA's enzymatic specificity and its potential physiological roles in metabolic regulation.

P-18-007
Shedding light on rabies virus neutralization via antibody engineering

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Rabies, caused by the zoonotic neurotropic rabies virus (RABV) within the genus *Lyssavirus* of the family Rhabdoviridae, is a lethal disease resulting in approximately 60 000 human deaths annually. The trimeric glycoprotein (G) spikes of RABV are the only surface-exposed proteins on the virion and mediate receptor recognition and host cell entry. RABV-G displays several epitopes which are targeted by antibodies, including neutralizing monoclonal antibodies (mAbs) that have been proposed as effective alternatives to traditional polyclonal rabies immunoglobulin treatment. In this study, one such neutralizing mAb, CR57, was engineered into a diabody to enable crystallization in complex with the targeted domain from rabies G. Here, we report the crystal structure of the RABV-G domain III in complex with the CR57 diabody at 2.70 Å resolution. The structure reveals the antibody epitope, where key interactions are targeted at a 6-residue hydrophobic peptide on RABV-G. We present an antigenic mapping of all known epitopes on the spike, which reveals two predominant sites of vulnerability to neutralization. Structural analysis suggests two potential mechanisms for CR57-mediated neutralization: steric hindrance of receptor binding and inhibition of fusogenic conformational changes. These findings were supported by cell-cell fusion assays, which demonstrated the ability of mAb CR57 to block fusogenic transitions. Our results provide a molecular description for rabies inhibition by one of the most potent antibodies against diverse rabies strains.

P-18-008
Pheromone-mediated response in Rap systems: deciphering the pheromone promiscuity of phage Phi3T

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In Firmicutes, quorum sensing (QS) is controlled by the RRNPPA family through pheromone-receptor signaling. The process begins with the secretion of an inactive propeptide, which is then cleaved by external proteases into an active pheromone (Phr) that is reabsorbed into the cell. In *Bacillus* species, Rap proteins, a subset of RRNPPA, are vital for regulating sporulation and competence. Pheromones are encoded within the propeptide sequence of the Rap receptor, displaying highly specific binding interactions. These sequences may feature multiple versions of the same or similar pheromones. Earlier studies on phage Phi3T demonstrated that different pheromones in one propeptide bind to receptors with varying affinities and kinetics, implying distinct biological roles within bacterial communities. This study further investigates this by altering the stability and affinity of Rap–Phr interactions in Phi3T and related systems. Biophysical techniques were employed to examine peptide-protein interactions and to identify the structural mechanisms behind pheromone recognition, aiming to understand the biological significance of 'partial selectivity' in Rap–Phr interactions.

P-18-009
Abstract withdrawn

P-18-010
Probing the role of DNA binding residues and acidic C-tail in β-pinwheel domain of DNA gyrase

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DNA topoisomerases play a crucial role in bacterial cell survival by altering the DNA topological state during replication, transcription and recombination. DNA gyrase constitutes a type

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II topoisomerase that introduces negative supercoils in DNA to relax positive helical turns for overcoming DNA overwinding. DNA wrapping is known to be responsible for introducing positive or negative supercoiling in DNA. The non-specific interactions occurring between the C-terminal domain of Gyrase A (GyrA-CTD) and DNA dictate the exact placement of the two DNA segments to prevent super-helicity and initiate DNA binding. Though mechanism of action of DNA gyrase in bacterial species is conserved, differences in their activity and specificities have been reported. Moreover, DNA wrapping and binding by DNA gyrase attributed to GyrA-CTD is not clearly elucidated. We determined the X-ray crystal structure of native C-terminal domain of DNA Gyrase A from *Salmonella enteric* serovar Typhi (*S.* Typhi) at 2.4 Å resolution. The key DNA binding residues were identified through structural and biochemical studies of both wild-type and mutant proteins. The effect of acidic GyrA-CTD on DNA binding was seen by electro-mobility shift assay and fluorescent anisotropy. The overall structure of GyrA-CTD comprises an antiparallel beta pinwheel possessing a six blade architecture. The GyrA-CTD acidic tail serves as a repressing element in the absence of DNA but moves apart when DNA gyrase forms the cleavage complex. We observed that the deletion of acidic tail of GyrA-CTD decreased supercoiling activity in comparison to wild-type protein. The currently employed antimicrobial agents targeting DNA gyrase QRDR region are losing efficacy due to emerging resistance. This work showcases the significance of GyrA-CTD in the functioning of DNA gyrase, and can be further explored as a new target for design of novel inhibitors. Previously published in: Sachdeva et al (2024) Int J Biol Macromol 261(Pt 1):129728.

P-18-011
The action of matrix metalloproteinase-9 as the first step toward efficient and safe degradation of amyloid

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The accumulation of ordered protein aggregates, amyloid fibrils, causes a wide range of systemic and localized amyloidoses and accompanies various neurodegenerative diseases, leading to impaired functioning of organs. A lot of effort has focused on finding agents for efficient and safe degradation of already formed pathologic amyloid deposits. For example, immunotherapy attempts to achieve amyloid destruction by activating proteolytic enzymes. Here, we examine poorly understood effects of an immune enzyme, extracellular matrix metalloproteinase-9 (MMP9), on amyloids associated with Alzheimer's and Parkinson's diseases, lysozyme, insulin, and dialysis-related amyloidoses. The universality of MMP9 action on various amyloids has been established, with its efficacy being highly dependent on the size of the fibrillar cluster. MMP9 causes irreversible loss of ordered structure of amyloid fibrils without fragmentation or increased cytotoxicity. The MMP9 induced degradation of amyloids is promoted by destabilization of intramolecular interactions, but intermolecular hydrogen bonds in the fibril backbone remain intact. The ability of MMP9 to "pre-loosen" amyloid clusters and thereby increase the availability of binding sites for other enzymes capable of fragmenting and cleaving the resulting degradation products to monomers can be used to develop a combined effective and safe anti-amyloid therapy. This work was supported by the Russian Science Foundation (NO. 23-74-10092).

P-18-012
Unraveling the stages of β-barrel transformation in fibrillogenesis of odorant-binding proteins

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The formation of amyloid fibrils accompanies the development of amyloidoses, including in neurodegenerative diseases. The inherent high amyloidogenicity of proteins with the structure of β-barrel renders them suitable subjects for the study of the of fibrillogenesis mechanisms and related pathological processes. For example, odorant-binding proteins (OBPs), responsible for binding and transport of water-insoluble odorants, demonstrate the ability to form fibrillar structures already at physiological temperature and pH, resulting in the loss of their functional activity. We analyze the sequence of β-barrel transformations in the fibrillogenesis pathway of OBPs applying spectroscopic (absorbance, fluorescence of intrinsic residues and different fluorescent probes, circular dichroism), microscopic (transmission electron and confocal laser scanning microscopy), electrophoretic methods, and molecular dynamics. We have shown the formation of a monomeric protein at the very beginning of the lag phase of dimeric OBPs' fibrillogenesis, before any changes in structure are manifested. We have demonstrated that further oligomerization of OBPs molecules triggers structural changes in their β-barrel. Two types of oligomeric/aggregated forms with only subtle changes in the β-barrel accumulated in the lag phase of fibrillogenesis have been identified. The structural change demonstrated include sequential a weakening of β1-strand contacts with neighboring strands, followed by its disorganization leading to β-barrel uncoupling. Significant reorganization of the β-barrel has been shown to occur in the exponential phase, involving the formation first of prefibrillar forms with evident toxicity to mammalian cells and then of mature amyloid fibrils. Thus, we have identified transitions in the OBPs' fibrillogenesis that may be targets for the development of drugs inhibiting this process. This work was supported by the Russian Science Foundation (NO. 24-24-00247).

P-18-013
Structural insight into the distinct regulatory mechanism of the HEPN–MNT toxin antitoxin system in *Legionella pneumophila*

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HEPN–MNT, a type VII TA module, comprises the HEPN toxin and the MNT antitoxin. HEPN–MNT acts as a nucleotidyltransferase which transfers the NMP moiety to the corresponding HEPN toxin, thereby interfering with its toxicity. Here, we report crystal structures of the *Legionella pneumophila* HEPN–MNT module, including HEPN, AMPylated HEPN,

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MNT, and the HEPN–MNT complex. Our structural analysis and biochemical assays suggest that HEPN is a metal-dependent RNase and identify its active site residues. We also elucidate the oligomeric state of HEPN in solution. Interestingly, *L. pneumophila* MNT, which lacks a long C-terminal α4 helix, controls the toxicity of HEPN toxin via a distinct binding mode with HEPN. Finally, we propose a comprehensive regulatory mechanism of the *L. pneumophila* HEPN–MNT module based on structural and functional studies. These results provide insight into the type VI HEPN-MNT TA system. Ref: Jin CL. et al. (2024) Nature Commun. 15: 10188.

P-18-014
Structural and biochemical characterization of anti-CRISPR protein AcrIE3

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The arms race between bacteria and bacteriophages (phages) has led to the evolution of diverse anti-phage defense systems and phage-encoded antagonists. Clustered regularly interspaced short palindromic repeats (CRISPR)-CRISPR associated (Cas) systems are bacterial defense mechanisms against invading phages and other mobile genetic elements. To neutralize the immunity, phages have evolved multiple anti-CRISPR (Acr) proteins that can inhibit the function of CRISPR-Cas systems via various mechanisms. AcrIE3, initially identified in *Pseudomonas* phage DMS3, disables the type I-E CRISPR-Cas system in *P. aeruginosa* by interacting with the Cascade complex. Here, we determined the crystal structure of AcrIE3 and identified its target Cas component in the type I-E CRISPR-Cas system of *P. aeruginosa*. We demonstrated that AcrIE3 binds to the Cas8e subunit of the Cascade complex through size exclusion chromatography and isothermal titration calorimetry. The crystal structure of AcrIE3 exhibits a novel helical fold with a negatively charged surface. Mutational analyses and AlphaFold modeling suggested that AcrIE3 interacts with the protospacer adjacent motif recognition site in Cas8e. Our findings provide the mechanistic insights into the function of AcrIE3, expanding the knowledge of Acr proteins against CRISPR-Cas immunity. Previously published in: Koo J et al. (2025) Structure 33, 160-170.

P-18-015
Structural and biochemical study of the anti-CRISPR protein AcrIE7

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CRISPR-Cas is a prokaryotic adaptive immune system that defends against bacteriophages and other invading genetic elements [1]. To evade CRISPR-Cas immunity, bacteriophages have evolved diverse anti-CRISPR (Acr) proteins. AcrIE7, identified in the mobile genetic element of *Pseudomonas* species, inhibits the type I-E CRISPR-Cas system in *Pseudomonas aeruginosa* [2]. In this study, we determined the structure of AcrIE7 using X-ray crystallography and NMR spectroscopy and analyzed its molecular interactions with Cas proteins of the *P. aeruginosa* type I-E CRISPR-Cas system through experimental and computational approaches. Both the crystal and solution structures revealed that AcrIE7 adopts a novel all-helical fold. Binding assays employing multiple biochemical techniques showed that AcrIE7 does not interact with Cas proteins in the type I-E Cascade complex or the CRISPR adaptation machinery. Instead, AlphaFold modeling predicted an interaction between AcrIE7 and Cas3 of the type I-E system. Collectively, our findings suggest that AcrIE7 inhibits the type I-E CRISPR-Cas system by targeting Cas3 and underscore both the utility and limitations of AlphaFold modeling in protein interaction analysis. [1] Deveau H et al. (2010) Annu Rev Microbiol 64, 475–493. [2] Marino ND et al. (2018) Science 362(6411), 240–242. *The authors marked with an asterisk equally contributed to the work.

P-18-016
Integrative approach for the characterization of the interaction landscape of LRPAP1 on the human LRP2 receptor

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LRP2, commonly known as megalin, is a large single-transmembrane glycoprotein (~1.2 MDa dimer) that mediates extracellular ligand uptake via clathrin-mediated endocytosis. It is primarily localized at the apical membrane of epithelial cells in the kidney, brain, and thyroid, where it mediates the internalization of various ligands, releasing them in endosomes upon vesicle acidification before recycling back to the membrane. Unlike other LDLR family members, LRP2 interacts with an exceptionally diverse range of ligands, including secreted proteins, hormones, and small molecules. However, it remains unclear how newly synthesized LRP2 avoids premature ligand binding during its trafficking through the secretory pathway. LRPAP1, an endoplasmic reticulum (ER) chaperone of the low-density lipoprotein receptor (LDLR) family, is hypothesized to prevent ligand interactions during secretion. Yet, the precise mechanism governing its interaction with LRP2 and its dissociation before reaching the apical membrane remains elusive. In this study, we combined cryo-electron microscopy (cryo-EM), crosslinking mass spectrometry, and biochemical analyses to characterize the interaction landscape and stoichiometry of LRPAP1 with human LRP2. Our findings reveal that at least four copies of LRPAP1 associate with LRP2, overlapping with its ligand-binding sites, suggesting a competitive inhibition mechanism. While LRPAP1 typically interacts with LDLR family members via positively charged residues binding to complement-type (LA) repeats, we identified one LRPAP1 copy engaging LRP2 through an alternative, previously uncharacterized mechanism. Finally, we propose a pH-dependent release model in which acidic conditions induce a conformational change in LRP2, leading to the autoinhibition of LRPAP1 binding. Our study provides new insights into the molecular regulation of LRP2 trafficking and ligand selectivity, highlighting the role of LRPAP1 in ensuring correct receptor maturation and function.

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P-18-017
Structural characterization of a unique member of the NaRJ subfamily, YcdY

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YcdY is a redox enzyme maturation protein that belongs to the NarJ subfamily. NarJ subfamily members, such as NarJ, DmsD, TorD, NarW, have been shown to contribute to protein folding or molybdenum cofactor insertion for complex iron sulfur molybdoenzymes. However, YcdY is considered a unique member of the NarJ subfamily because it has been proposed that YcdY is involved in the insertion of the zinc cofactor into the YcdX phosphatase, which does not have a molybdenum cofactor. To gain insight into the chaperoning function of YcdY based on its structural features, we determined the crystal structure of YcdY. YcdY presents a curved single-domain architecture with a helix-bundle structure decorated by α-helices. The concave side of the YcdY structure harbors an extensive dent that is generated by hydrophobic or conserved residues. Comparative analysis of the YcdY structure and its homologous structures suggests that YcdY uses the dent to accommodate its client protein. Moreover, we provide binding and enzymatic evidence that YcdY functions as a chaperone for proteins other than YcdX. *The authors marked with an asterisk equally contributed to the work.

P-18-018
Structural and interaction analysis of the bifunctional E3 ligase HERC5 with ubiquitin and ISG15 by NMR spectroscopy

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Ubiquitin and ubiquitin-like post-translational modification systems are essential components of the functional diversity in eukaryotic biochemical processes. The ISGylation system, a ubiquitin-like modification system integrated with the immune system, plays a crucial role in the defense of higher eukaryotic organisms against viral infections. Many enzymes of the ISGylation system, as well as the ISG15 protein, are encoded by interferon-stimulated genes. The key components of the ISGylation system include ISG15 (a ubiquitin-like protein), Uba7 (E1 enzyme), UbcH8 (E2 enzyme), and HERC5 (E3 enzyme). HERC5 is a unique E3 enzyme that binds to the ribosome and directly modifies proteins during translation. In this study, we optimized the production and purification of the C-lobe region of the HERC5 protein for structural studies. Using NMR spectroscopy, we determined its NMR structure and analyzed its interaction with the E2 enzyme binding site. Previous studies have also suggested that HERC5 is a bifunctional enzyme capable of participating in both ubiquitination and ISGylation pathways. To further investigate this, we examined its interactions with both ubiquitin and ISG15. Our findings indicate that while HERC5 interacts with both ubiquitin and ISG15, its interaction with ISG15 is significantly stronger, further supporting its role in ISGylation.

P-18-019
Exploring the structural properties of a novel metagenomic esterase for enhanced polylactic acid biodegradation

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Enzyme-catalyzed depolymerization of synthetic polyesters has advanced rapidly over the past decade, presenting promising opportunities for plastic recycling and addressing the growing plastic pollution crisis. In this study, we aimed to structurally characterize a novel metagenomic esterase, Mgy, and its distant ancestral variant, 118, both of which exhibit high catalytic activity toward DL-polylactic acid degradation. We employed size-exclusion chromatography (SEC), circular dichroism (CD) spectroscopy and X-ray crystallography for this characterization. SEC revealed a high level of soluble aggregates, prompting optimization of the buffer composition. AlphaFold modelling was used to examine surface properties, aiding in the engineering of more stable enzyme variants. We identified disordered regions at the N-terminus and additional cysteine residues, which may contribute to protein aggregation. To test this hypothesis, truncated versions of both enzymes, lacking the N-terminal region, were expressed, purified, and analyzed. SEC confirmed that all four enzyme variants (full-length and truncated) favor high ionic strength and reducing environments, minimizing soluble aggregates. The full-length proteins showed distinct oligomeric species distributions, predominantly in tetrameric form, while the truncated variants existed primarily as monomers. CD data indicated that the enzymes contain approximately 18% α-helices and 37% β-sheets, a pattern consistent across full-length and truncated forms. Preliminary X-ray diffraction data were collected for the full-length 118 at the XRD2 beamline, Elettra, Trieste up to 4 Å resolution. Ongoing efforts aim to optimize crystallization conditions for higher-resolution data. The 3D structure determination of this metagenomic esterase will provide critical insights into the structural properties underlying its plastic degradation activity, paving the way for engineering more robust enzymes for sustainable environmental applications.

P-18-020
Oligomeric states of AAA+ Lon protease involved in its regulation

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Lon is an AAA+ serine-lysine protease that plays a crucial role in protein quality control in both eukaryotic and prokaryotic organisms. Dysregulation of Lon is associated with various diseases. Despite its ubiquity, the mechanisms governing regulation of Lon remain poorly understood. While Lon is functionally active as a hexameric ring, evidence suggests that it

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can also form larger complexes, which may represent different regulatory states. Here we investigated the formation and structural basis of higher-order oligomers of *E. coli* Lon. Using cryoEM and mass photometry, we demonstrated the existence of distinct oligomeric states, including the dodecamer (12mer), undecamer (11mer), and decamer (10mer). High-resolution structural analysis reveals that the 12-, 11- and 10mer states are formed by the arrangement of hexameric and pentameric rings of the protomers as (6 + 6), (6 + 5), and (5 + 5) assemblies, respectively. The interfaces between these rings are mediated in oligomers by intricate interactions involving the N-terminal domain (NTD) of Lon. Notably, the NTD interfaces differ significantly among the three configurations. The 12mer adopts a symmetrical arrangement with a contiguous channel connecting the two hexamers, whereas the 11mer and 10mer exhibit an angular arrangement in which the NTD blocks the formation of a continuous channel. Additionally, we have determined the structures of these oligomers in complex with nanobodies that stabilize the flexible interface regions, leading to improved resolution and identification of low-populated states. We also demonstrated that Lon proteases from various organisms form similar oligomeric assemblies. Based on our experimental evidence we hypothesize that formation of higher order oligomers is driven by NTDs, and these oligomers represent transition states of Lons at various stages of their functioning, leading to regulation of Lon activity in cells. Supported by the intramural NIH funds and by RSF project 21-74-20154P.

P-18-021
Structural and functional characterization of selected Class 3 asparaginases found in pathogenic fungi

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Asparaginases, which catalyze the hydrolysis of L-asparagine to L-aspartate and ammonia, are widespread in microorganisms, plants and animals, forming a large and diverse superfamily of enzymes. According to a new nomenclature [1,2], asparaginases are classified into three unrelated Classes: bacterial-type (Class 1), plant-type (Class 2) and *Rhizobium etli*-type (Class 3). The diverse applications of asparaginases in medicine (as antileukemics), as targets for the development of fungicides (medicine and agriculture), and in food industry, have generated a long-standing scientific interest and motivated search for new variants of these enzymes. The primary goal of this work has been the biochemical and structural characterization of fungal asparaginases belonging to Class 3. We determined several crystal structures and investigated the basic physicochemical and biochemical properties of selected fungal asparaginases, for comparison with two previously characterized enzymes from *Rhizobium etli*, ReAIV [3] and ReAV [4]. We selected five asparaginases from different pathogenic fungal species – *Aspergillus candidus* (AcA), *Botryosphaeria parva* (BpA), *Colletotrichum fructicola* (CfA), *Fusarium oxysporum* (FoA) and *Penicillium expansum* (PeA) – and investigated their kinetic parameters, thermal stability, temperature and pH optimum, as well as salt profile and Zn^{2+} dependance. The biochemical experiments were supported with a set of high-resolution crystal structures of BpA, CfA, and FoA. References: 1. Loch JI and Jaskolski M, IUCrJ, 2021. 8: 514-531; 2. da Silva LS, et al., Biotechnol Appl Biochem, 2022. 69: 503-513; 3. Loch JI, et al., Acta Crystallogr D Struct Biol, 2023. 79: 775-791; 4. Loch JI, et al., Nat Commun, 2021. 12: 6717.

P-18-022
Targeting EF-P lysinylation by crystallographic fragment screening

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Multidrug resistance (MDR) in Gram-negative bacteria is a major global health concern, with pathogens like *Acinetobacter*, *Salmonella* and *Shigella* classified as high-risk threats. Translation elongation factor P (EF-P) is essential for overcoming ribosomal stalling at polyproline motifs, frequently occurring in proteins related to bacterial resistance such as efflux pumps. In bacteria, a posttranslational modification is required for EF-P activation. Most commonly a specific lysine residue undergoes β-lysinylation, catalyzed by the ATP-dependent EpmA ligase. Deletion of either the *efp* gene or its modifying enzyme was shown to be detrimental for *Salmonella enterica* resistance to environmental stresses such as the presence of antibiotics. Disrupting this modification could impair bacterial fitness and enhance antibiotic efficacy, yet no inhibitors of EpmA currently exist. This project employs crystallographic fragment screening to identify potential inhibitors targeting EpmA. This method detects low-molecular-weight compounds binding to key enzyme sites, providing a foundation for structure-guided drug development. Advances in high-throughput X-ray crystallography and computational tools, such as PanDDA, now enable the efficient screening of large fragment libraries. Preliminary studies on human hypusination enzymes demonstrated a high hit rate, validating the approach. By mapping the druggable sites of EpmA and screening homologous enzymes, this study will provide insights into EF-P regulation and bacterial adaptation. Ultimately, the identification of EpmA inhibitors could lead to novel antimicrobial strategies, addressing the urgent need for new treatments against MDR pathogens.

P-18-023
Identification of a novel fold in the H_2S-producing cystathionine beta-synthase enzyme from multidrug-resistant bacteria *Pseudomonas aeruginosa* and *Klebsiella pneumoniae*

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The infections caused by multidrug-resistant (MDR) bacteria are a leading cause of morbidity and mortality among those

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hospitalized and represent a critical threat to global health. The increasing emergence of MDR bacterial strains has rendered many conventional antibiotics ineffective, underscoring the urgent need for new therapeutic strategies. Among the MDR bacteria, *Pseudomonas aeruginosa* and *Klebsiella pneumoniae* are of particular concern. These pathogens can acquire and spread resistance mechanisms, employing strategies such as the production of β-lactamases, efflux pumps, and the modification of antibiotic targets. One novel approach to sensitizing persistent bacteria to the host immune response focuses on targeting the bacterial reverse transsulfuration pathway, which is essential for the synthesis of cysteine. The key enzyme that catalyzes this pathway, cystathionine-β-synthase (CBS) is present in both *P. aeruginosa* and *K. pneumoniae*. CBS catalyzes the conversion of homocysteine to cystathionine in the presence of L-serine. Through alternative reactions, CBS can also use cysteine to produce hydrogen sulfide (H_2S). H_2S decreases oxidative stress by scavenging reactive oxygen species and upregulating antioxidant enzymes, reducing the efficacy of antibiotics. Additionally, H_2S influences the expression of genes involved in antibiotic resistance, biofilm formation, and virulence. Accordingly, inhibiting bacterial H_2S synthesis by targeting CBS, sensitizes bacteria to oxidative stress and enhances the efficacy of ROS-generating antibiotics. We have biochemically, biophysically, and structurally characterized PaCBS and KpCBS. Our data proves that the regulatory Bateman module is essential for S-adenosylmethionine (SAM)-induced activation in both enzymes. Our three-dimensional structures reveal an unprecedented CBS fold that had not been described to date. These findings offer groundbreaking three-dimensional templates that could significantly advance drug development.

P-18-024
Strategies to target human D-aspartate oxidase and modulate endogenous D-aspartate content in schizophrenia

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D-aspartate (D-Asp) stimulates glutamatergic neurotransmission in mammal brain: while it is abundant at embryonal stage, it rapidly decreases at post-natal stages due to the activity of D-aspartate oxidase (DASPO). D-Asp severe depletion in correlation with DASPO overexpression occurs in schizophrenic patients causing neurotransmission reduction [previously published in Errico F et al. (2013) Journal of psychiatric research, 47.10: 1432-1437]. D-Asp administration improves neurotransmission, synaptic plasticity and dendritic length, thus suggesting the modulation of endogenous levels of D-Asp as a promising approach for new potential therapies. To this aim, DASPO has been recently considered as target of possible inhibitors and proteolysis targeted chimeras (PROTACs). Olanzapine was demonstrated to be an inhibitor of DASPO with IC_{50} = 23.4 μM; thus we synthesised new potential inhibitors starting from olanzapine or thiophene-related molecules, then their efficacy was tested in inhibition assays. Among a panel of 20 molecules, six had similar inhibition activity to that of olanzapine, and X-ray crystallography was used to investigate molecular interactions and understand how to improve inhibition. The structure of wild-type DASPO was solved by molecular replacement (PDB ID: 6RKF as model, previously published in Molla G et al. (2020) The FASEB Journal; 34: 1182–1197). The presence of inhibitors is currently under investigation exploiting information about their inhibition mechanisms, as they show non-competitive or competitive mechanisms of action. Alongside, we would like to promote DASPO degradation via proteasomes. To this aim, PROTAC molecules bearing olanzapine linked to lenalidomide or (VH032)-Me were synthetised and tested *in vitro* for the interaction with DASPO and E3 ligases (Cereblon or Von Hippel Lindau E3 ligases, respectively), necessary for DASPO proteasomal degradation. *The authors marked with an asterisk equally contributed to the work.

P-18-025
Structural and biochemical characterization of C3G-CrkL-c-Cbl complexes

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c-Cbl is a proto-oncogenic E3 ubiquitin ligase that targets proteins for degradation and regulates multiple signaling pathways. c-Cbl attenuates signaling by the small GTPase Rap1 through the guanine nucleotide exchange factor C3G and the Crk adaptor proteins (CrkL and CrkII). C3G and c-Cbl interact via CrkL in B and T cells, platelets, and chronic myeloid leukemia cells. C3G-CrkL-c-Cbl assemble by specific interaction domains: the SH2 domain of Crk proteins binds to phospho-Tyr sites (pY700 and pY774) in c-Cbl [Previously published in: C.E. Andoniou, et al. (1996) Oncogene 12, 5162–5186], while the SH3N domain recognizes Pro-rich motifs in C3G [Previously published in: A. Rodríguez-Blázquez, et al. (2023) Cell Commun Signal 21, 1, 30]. Despite their functional relevance, the molecular architecture and stoichiometry of these complexes remain unknown. Understanding their structural organization is crucial for elucidating their role in Rap1 regulation and ubiquitin-mediated protein degradation. We first employed isothermal titration calorimetry to determine the thermodynamic parameters of the interactions and the relative stoichiometry of the complex. The formation of ternary complexes required c-Cbl phosphorylation by Src family kinases. CrkL binds to pY700 and pY774 in c-Cbl with sub-micromolar affinity. CrkL binds to two Pro-rich of C3G with similar affinity. Using size-exclusion chromatography coupled to multi-angle light scattering, we determined the absolute stoichiometry for the complex. Our data supports the notion that these proteins form stable and well-defined complexes that are amenable for reconstitution and analysis *in vitro*. Collectively, our findings suggest that C3G, CrkL and c-Cbl form a stable complex that may serve as a critical molecular link between Rap1 signaling and ubiquitin-mediated protein degradation pathways. This knowledge paves the way to understand the molecular basis of diseases where these pathways are dysregulated, such as cancer.

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P-18-026
Understanding thraustochytrid PUFA synthases: the role of enoyl-ACP reductase domains

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Omega-3 polyunsaturated fatty acids (PUFAs) are essential nutrients in the human diet. These molecules are synthesized *de novo* by enzymes known as PUFA synthases (PFAs), which resemble microbial polyketide synthases (PKS), as well as mammalian and bacterial fatty acid synthases (FAS). PFAs are organized in three polypeptides in thraustochytrids and myxobacteria, or four in marine gammaproteobacteria. The enoyl-ACP reductase (ER) domain catalyzes the reduction of a carbon-carbon double bond during PUFA modification reactions. In gammaproteobacteria, a single ER domain is found in the megasynthase, as the standalone protein PfaD. In contrast, thraustochytrids possess two ER domains: one in PfaB (ERb) and another in PfaC (ERc), although their specific functions remain unclear. ER has been shown to form homodimers in gammaproteobacteria PFAs, bacterial FAS, and PKS. In this study, we investigated the PUFA synthase from the thraustochytrid *Schizochytrium* sp. and found that ERb and ERc can interact to form a heterodimer, as demonstrated by the 2.2 Å resolution crystal structure with flavin mononucleotide (FMN) bound. This suggests that ER domains may play a role in the dimerization of PfaB and PfaC. Molecular docking and structural alignments indicate that the enzyme could function through a ping-pong mechanism involving FMN and NADH. To our knowledge, this is the first crystal structure of a PUFA synthase from thraustochytrids, providing new insights into the mechanism of action of PFAs in these organisms.

P-18-027
Deciphering the unknown: structural characterization of NIF3 protein

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The NIF3 protein, a member of the DUF34 family, remains one of the most conserved yet poorly understood proteins across Bacteria, Archaea, and Eukaryotes. The specific function of the protein is still not understood. This project aims to elucidate the structural characteristics of eukaryotic NIF3L1. Using an integrative approach combining crystallographic fragment screening, cryo-EM, and various biophysical techniques, we aim to uncover the oligomeric states of NIF3L1 and its structural determinants. Preliminary data suggest NIF3 forms homohexameric and octameric assemblies. Detailed structural analysis will provide insights into the protein's architecture and its potential functional implications.

P-18-028
Structural and functional investigations on protein cancer targets involved in lipid metabolism

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Prostate cancer (PCa) relies on lipid metabolism for growth and survival, particularly under nutrient-deprived conditions. One key enzyme involved in this pathway is acetyl-CoA synthetase short chain family member 2 (ACSS2), which plays a crucial role in lipid biosynthesis and histone acetylation. ACSS2 is notably upregulated in PCa cells where it supports tumor cell survival and contributes to disease progression. ACSS2 has gained more clinical relevance in recent years, yet it remains structurally under-characterized, limiting the effort to develop target inhibitors. In this project, I have successfully expressed and purified recombinant human ACSS2 and established a reproducible coupled kinetic assay to characterize its enzymatic activity. Using this assay, I have evaluated a small library of compounds and identified at least one inhibitor that reduces ACSS2 activity *in vitro*. In parallel to this kinetic screening, I have utilized a DNA-encoding library (DEL) screening approach consisting of approximately 4 billion novel binders to expand and identify a pool of candidate inhibitors. For structural studies, I will fuse ACSS2 with a self-crystallizing partner protein to promote crystal lattice formation suitable for X-ray diffraction. Employing these approaches will integrate enzymology, chemical biology, and structural biology to provide insight into the functionality and druggability of ACSS2. This research can support the development of selective ACSS2 inhibitors as potential therapeutics for prostate cancer and other lipid-driven malignancies.

P-18-029
Crystal structure of SleC: an essential lytic transglycosylase for *Clostridium difficile* spore germination

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Clostridioides difficile (formerly *Clostridium difficile*) is an emerging human pathogen that forms resilient spores, enabling persistence and transmission. Spore formation enhances resistance to harsh environments and serves as a key virulence factor in *C. difficile* infection (CDI). Germination is a critical step in CDI, triggered by environmental signals in the host intestine. SleC, a germination-specific cortex-lytic enzyme, is essential for spore reactivation in *C. difficile* and *C. perfringens*. Its activation is initiated by the subtilisin-like protease CspB, regulated by CspC. Once activated, SleC degrades the spore cortex, facilitating germination. Initially synthesized as a precursor (pre-pro-SleC) in the mother cell, it undergoes sequential cleavage during sporulation, leaving the inactive zymogen (pro-SleC) within the spore. During germination, CspB removes the pro-domain, activating SleC. Active SleC functions as a bifunctional enzyme, exhibiting lytic transglycosylase and N-

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acetylmuramoyl-L-alanine amidase activities, targeting cross-linked tetrapeptide structures within the cortex. Here, we present the crystal structure of intact SleC, including its pre-domain, pro-domain, and hydrolase domain, in a tetrameric form at 2.17 Å resolution.

P-18-030
Algorithm-driven optimization of cysteine protease mutagenesis: GROMACS-based mutagen selection for enhanced solubility

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Cysteine protease (CysPC) DEK1 is a key regulator of plant development, but its poor solubility limits structural and functional studies. To address this, we developed a reinforcement learning-based computational framework integrating molecular dynamics (MD) simulations via Groningen MAchine for Chemical Simulation (GROMACS) with systematic mutagen selection to optimize DEK1 solubility while preserving its structural stability. Our approach employs Proximal Policy Optimization (PPO), a reinforcement learning algorithm that iteratively explores mutation combinations based on solubility-related physicochemical properties. Key input features extracted from GROMACS simulations include hydration free energy, radius of gyration, solvent-accessible surface area (SASA), secondary structure stability, and interaction energy with water molecules. These features guide the reinforcement learning model in selecting beneficial mutations that enhance solubility. The PPO-based strategy enables adaptive learning, allowing the model to refine mutation predictions dynamically. Validation through *in silico* free energy calculations and comparative molecular dynamics simulations confirms that PPO-driven mutagenesis selection improves solubility more effectively than heuristic-based approaches. This study introduces a data-driven, adaptive framework for protein solubility optimization, with potential applications in protein engineering, drug development, and structural biology. The integration of reinforcement learning with molecular dynamics paves the way for more efficient and scalable protein design methodologies.

P-18-031
Structural insights into regulation of apoptosis signal regulating kinase 1 (ASK1) by 14-3-3 protein

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ASK1, also known as MAP3K5, is a crucial stress sensor and upstream regulator of p38 and JNK signaling pathways. Dysregulation of ASK1 has been seen in cancer, inflammatory, cardiovascular and neurodegenerative diseases. Hence, ASK1 activity has to be strictly regulated to respond stress stimuli appropriately. In unstressed conditions, ASK1 is held in an inactive state termed as signalosome through binding of negative regulators, thioredoxin (TRX1) and 14-3-3. Recently, a cryo-EM structure of ASK1 revealed its dimeric and asymmetric architecture, suggesting possible ways to interact with 14-3-3. It is a highly conserved phosphoserine/phosphothreonine-binding scaffold protein and, despite many years of intensive research, there is no atomic resolution structure of multi-domain ASK1 in complex with 14-3-3 or thioredoxin, which has hindered functional and mechanistic understanding of ASK1 regulation. This project aims to unravel the structural and molecular basis of ASK1 regulation by 14-3-3 protein. The SV-AUC experiments revealed that ASK1 and 14-3-3 form a complex with apparent dissociation constant (K_D) of □90 nM. The sedimentation coefficient distribution c(s) also suggested that the presence of 14-3-3 induced the tetramerization of ASK1, i.e. a complex with a stoichiometry of 4:4 (two ASK1 dimers forming a tetramer stabilized by two 14-3-3 dimers). The formation of a 4:4 complex was subsequently confirmed by SEC and cryo-EM. Cryo-EM reconstruction showed that each of the 14-3-3 dimers stabilizes the tetrameric arrangement of ASK1 by binding the C-terminal segments of ASK1 chains from opposite ASK1 dimers. This suggests that tetramerization of ASK1 causes steric hindrance of the catalytic centers of the kinase domains, presumably also interactions between the kinase domains and MAP2K kinase substrate, thus explaining the inhibitory effect of 14-3-3 binding. Our results provide the first insight into the structural basis of 14-3-3 protein-mediated regulation of ASK1 kinase.

P-18-032
Deciphering the interaction of botulinum neurotoxin A light chain (LCA) with human septins

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Septins are a large family of proteins that bind to guanine nucleotides and interact with each other to form cytoskeletal filaments, which can organize into higher-order structures. These filaments are considered the biologically active form of septins and play crucial roles in several cellular processes. Consequently, the absence or dysfunction of these proteins is associated with various pathologies. Previous studies on septins have revealed their ability to interact with proteins from other families, expanding the scope of investigation into their biochemical and functional significance. One such protein is the light chain of botulinum neurotoxin type A (LCA), a metalloprotease capable of cleaving proteins essential for neurotransmitter release, ultimately leading to muscle paralysis. In this study, several experimental approaches were employed to investigate and characterize in detail the interaction between LCA and human septins. To this end, LCA and various septin complex constructs were expressed, purified, and used to evaluate interactions through pull down assays, microscale thermophoresis, and structural modeling. The results indicated that LCA interacts directly with septins 6 and 7, specifically at their C-terminal domains. Furthermore, mutations in specific LCA residues confirmed their importance for stable complex formation. Structural modeling enabled the prediction of the LCA C-terminal conformation in interaction with septins, allowing the identification and evaluation of other residues critical for this association and supporting the proposed model. In conclusion, this study advances the understanding of the LCA-septin interaction and proposes a plausible model for their association.

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P-18-033
Dynamic sidechain interactions in disease-associated mutants of the Shank1 PDZ domain

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Shank1 (SH3 multiple ANK repeats protein 1) is a member of the Shank family, an abundant scaffold protein of the postsynaptic density (PSD) of glutamatergic synapses. Shank proteins consist of multiple protein–protein interaction domains, through which, they connect to the transmembrane receptors via other scaffolds such as GKAP and Homer, and also communicate with the cytoskeleton through actin-binding proteins. Loss of Shank function is associated with diminished size of dendritic spines and symptoms of autism spectrum disorders, while peripherial expression of Shank (normally limited to the central nervous system) had been found in various cancers, where Shank acted as an oncogene by facilitating cell growth. In this study, we analyzed five disease-associated point mutations of the Shank1 PDZ domain, and their effect on selected known Shank1 PDZ interactions. Dissociation constants were obtained using multiple experimental methods. Currently, there is a lack of data available on the dynamics of the domain, since all models deposited in the PDB are X-ray crystal structures with gaps at mobile segments. Structural flexibility was suggested to be important for partner selectivity, especially since Shank1 PDZ domains contain a unique, conserved, highly mobile loop segment. To bridge this gap, we performed docking and MD simulations on models of the complexes and found dynamic behaviour and multiple novel sidechain interactions not described previously, which serve as an explanation for the experimental results observed on the point mutants. Additionally, we conducted solution NMR measurements on the apo wild-type Shank1 PDZ domain and one of the point mutants, and obtained experimental data that support the results of the MD simulations. Additionally, we utilized AlphaFold to model the complexes and simulate their competitive binding.

P-18-034
Structural variance in barley *in silico* panproteome

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Proteins have a central role in translating the genetic information encoded in the genomes into a wide variety of phenotypic traits. At the German Federal Ex situ Gene Bank, hosted by the IPK, the phenotypic diversity of plants is preserved as extensive collections of accessions, many of which can now be genotyped thanks to advancements in sequencing technologies. The recent advances in artificial intelligence, along with AlphaFold's breakthrough [1], has made it possible to predict reliable structural models for all the proteins in a species – its proteome – directly from genomic data. Here, we present the initial results of a panproteome analysis of *Hordeum vulgare* (barley), a globally significant crop valued for its economic importance in agriculture and brewing, as well as its ability to withstand environmental stresses. Our objective is to identify protein structural features that are either conserved or have diverged among different barley variants, assessing the degree of structural variability. The most recent barley pangenome [2] contains information regarding 76 genotypes, whose genes encode over 850 000 unique amino acid sequences, 75.6% of which are functionally annotated. So far, we have predicted approximately 25% of the unique protein structures in the proteome, excluding those annotated as unknown or as transposable elements. These predicted proteins were then clustered based on their structural similarity features [3], revealing conserved and variable aspects that can provide insights into phenotypic differences among barley variants. [1] Jumper, J et al. (2021) Nature 596, 583–589. [2] Jayakodi, M et al. (2024) Nature 636, 654–662. [3] Barrio-Hernandez, I et al. (2023) Nature 622, 637–645. *The authors marked with an asterisk equally contributed to the work.

P-18-035
Targeting inter-duplex junctions in catenated DNA with bidirectional bis-intercalators for enhanced anticancer activity

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Targeting DNA inter-duplex junctions with bis-intercalators presents a promising strategy for enhancing anticancer effects. In this study, we employed the self-complementary d(CGTATACG) sequence, which forms a tetraplex base-pair junction resembling DNA-DNA contact sites, as a model for investigating two alkyl-linked diaminoacridine bis-intercalators, DA4 and DA5. Cross-linking at the junction site by these bis-intercalators induced substantial DNA structural rearrangements, transitioning the B-form helical end-to-end junction into an overwound, side-by-side inter-duplex conformation with A-DNA-like characteristics and curvature. These structural alterations facilitated the insertion of DA4 and DA5 in a propeller-like geometry between adjacent duplexes. Notably, the addition of a single carbon to the DA5 linker introduced a bend that aligned its chromophores with CpG sites, enabling continuous stacking interactions and specific water-mediated contacts at the inter-duplex interface. Furthermore, we demonstrated that the distinct topological alterations induced by DA4 and DA5 resulted in the inhibition of topoisomerase II activity, potentially contributing to their anticancer effects. Our findings establish a foundation for designing bis-intercalators that selectively target biologically relevant DNA-DNA contact structures, offering new avenues for anticancer drug development.

P-18-036
Connecting the dots in the postsynapse: interaction between GKAP and Shank scaffold proteins

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Intricate and subtle molecular mechanisms are behind the processes of memory, learning and synaptic plasticity. The immense molecular network responsible for efficient and adaptive

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signal transduction in the acceptor side of excitatory synapses is called the postsynaptic density (PSD). Essential PSD proteins include NMDA receptors, PSD-95, GKAP, Shank and Homer. Disruption in any of these proteins may lead to a cascade of consequences resulting in diverse neuronal diseases. The protein GKAP (guanylate kinase associated protein) is a mostly disordered multivalent scaffold molecule. Several SLiMs (short linear motifs) have been described in GKAP to interact with other PSD proteins. One of these motifs is the C terminal QTRL motif, recognized by the Shank PDZ domain. We have shown previously (in the case of the LC8 dynein light chain binding motif of GKAP) that neighboring residues of SLiMs can contribute to complex formation. We performed NMR measurements to acquire data about the chemical environment of a longer GKAP C terminal region to describe the structural properties in its free form and when bound to the Shank PDZ domain. Supporting experiments include mass spectrometry, circular dichroism spectroscopy and analytical size exclusion chromatography. Uncovering residue-level, subtle details of this interaction will lead us to the better understanding of the postsynapse assembly.

P-18-037
Using Cryo-electron microscopy to understand the mechanisms of antimicrobial resistance to DNA gyrase in *Mycobacterium tuberculosis*

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Among the drugs used for susceptible and resistant tuberculosis treatment, fluoroquinolones, particularly moxifloxacin (MFX), have been vastly used in the last few years. The target of this drug is the enzyme DNA gyrase (DNAG), encoded by the *gyrA* and *gyrB* genes. Among the resistance mechanisms against this drug in *Mycobacterium tuberculosis*, include missense mutation in both genes for DNAG. However, little is known about the impact of these mutations on the molecular mechanism of bacterial (MTB) resistance to MFX. Therefore, we selected from the recent literature a series of missense mutations observed in *gyrA* and *gyrB* genes that lead to resistance to MXF in MTB clinical isolates. All the selected mutations have not yet been biophysically and biochemically characterized. To date, several new mutant DNAG proteins have been constructed through site-directed mutagenesis. The thermal stability of the enzymes was investigated using differential scanning fluorimetry (DSF) in the presence of MFX or DNA substrate. We optimized a biochemical assay to assess the degradation of a DNA substrate in the presence of MFX, comparing DNA cleavage between WT and mutant enzymes. We are currently working to obtain cryo-electron microscopy structures for selected DNAG protein mutants to understand better the differences in the binding mode of MFX, DNA, and ATP in mutant enzymes. Our biochemical data indicates that, for some DNAG mutants, there is a difference in the enzyme's capacity to cleave DNA, which suggests there is resistance to MFX. The thermal stability assays suggest that, for some mutants, there could be a lower affinity to MFX, but that is not clear for others, which could suggest that there are different resistance mechanisms that have not been described before. Therefore, with this work, we propose insights into novel molecular resistance mechanisms for MFX resistance caused by single point mutations in DNAG, which have not been fully described in the literature to date.

P-18-038
Characterization of the structural components of jumbo phage phiK601 using cryo-electron microscopy

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Jumbo bacteriophages have attracted significant attention due to their unique properties, which may enable their effective use against antibiotic-resistant bacteria. Their large capsids can package genomes more than of 200 kbp, a size comparable to the smallest bacteria. This allows versatile infection mechanisms, complex metabolic processes and a better capacity to evade bacterial antiviral defense systems. Understanding the structural composition of virion particles and the molecular organization of individual proteins is essential for understanding the mechanisms underlying the phage lifecycle. In recent years many phage structures were solved with cryo-electron microscopy, which allows the *de-novo* study of protein molecular structures forming the bacteriophage virion. However, structural data on jumbo phages remain very limited. In our study we conducted a structural study of the intact phiK601 *Pseudomonas aeruginosa* bacteriophage isolated from a water sample collected from Powai Lake in Mumbai. Using cryo-EM, we reconstructed the main components of the virion at a resolution sufficient to resolve molecular structures. Additionally, we developed an optimized workflow that significantly improves the data processing pipeline for jumbo phage molecular structures. Comparison of our results with recently published jumbo phage structures revealed common structural patterns, particularly the presence of an inner body correlated with minor proteins located within the capsid shell. The obtained density maps provide previously unpublished structural data on jumbo phage components. Our findings provide new insights into the molecular composition of the phiK601 virion and contribute to understanding of jumbo phage virion architecture. The work was supported by funding from the RSF (24-44-02003) and the Department of Science and Technology, India (DST/INT/RUS/RSF/P-79/2023).

P-18-039
Molecular architecture of the post-synaptic density: structural insights into PSD95 and SAP97 proteins

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The nervous system operates via the transfer of signals within and between electrically active cells known as neurons. This signal transfer typically occurs at cellular junctions called

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chemical synapses, where ionotropic receptor channels on the receiving membrane elicit an electrical response. These channels are regulated by the post-synaptic density (PSD), a complex structure that includes hundreds of proteins with both structural and signalling roles. A major component of the PSD is the multi-domain PSD-95 protein. This study investigates the molecular architecture of the PSD scaffold, focusing on the interactions between PSD95 and SAP97 protein domains with lipid membranes. Our initial experiments utilized SAXS, which provided valuable structural insights into the roles of PSD95 and its PDZ12 domain in scaffolding the PSD. Following SAXS, we employed neutron reflectometry to study the incorporation of PSD95 into asymmetric lipid bilayers, revealing the formation of a defined protein layer at the membrane and suggesting non-covalent protein-protein interactions that contribute to the PSD scaffold model. We then conducted hydrogen/deuterium exchange mass spectrometry (HDX-MS) to examine the conformational dynamics of PSD95. The results revealed significant protection of the PDZ domains upon membrane binding, supporting its central role in stabilizing the PSD structure. Recently, we also carried out X-ray crystallography studies on SAP97, a PSD95 homolog, and are currently analysing the results. Preliminary findings suggest that SAP97 plays an important scaffolding role in PSD assembly, which will be further explored as the data is finalized. Together, these combined techniques offer a multi-dimensional view of PSD architecture, emphasizing the essential roles of PSD95 and SAP97 in organizing the synaptic membrane. These findings advance our understanding of the molecular interactions that govern PSD structure and their implications in neuronal function and disease.

P-18-040
Structural landscape of mutant form NEIL2 DNA glycosylase obtained by HDX-MS

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DNA base damage, particularly oxidation, represents an inevitable yet mutagenic process requiring timely recognition and repair by the base excision repair (BER) system. The bifunctional DNA glycosylase NEIL2 (Nei Endonuclease VIII-like 2) is one of 11 human glycosylases responsible for initiating the removal of oxidized purines and pyrimidines, exhibiting broad substrate specificity. A unique structural feature of NEIL2 is the intrinsically disordered proline-rich motif (Leu58–Arg128) adjacent to Lys50, a critical catalytic residue. To investigate the role of Lys50 in different steps of lesion recognition, binding, and excision, we decided to study mutant NEIL2 form with K50A substitution. NEIL2 conformational domain flexibility and interdomain disordered region complicate its structural resolution via X-ray crystallography. To study its structural dynamics during DNA interactions, we employed hydrogen-deuterium exchange coupled with mass spectrometry (HDX-MS), a solution-phase method that reveals protein conformational dynamics by measuring exchange rates between amide protons and solvent deuterium. We optimized HDX-MS experimental conditions and generated deuterium uptake time courses. Peptide mapping for the NEIL2 K50A mutant achieved > 85% sequence coverage. Challenges arose in analyzing the unstructured loop (residues 59–115), which contains 10 proline residues that hindered pepsin cleavage. Deuterium uptake rates varied across protein regions, correlating with secondary and tertiary structural features characteristic of NEIL2 glycosylase. The study highlights HDX-MS as a powerful tool for probing NEIL2 dynamic architecture and provides new information about its repair mechanism.

P-18-041
Sea anemone Kunitz peptide HCIQ2c1: structure, modulation of TRPA1 channel, anti-inflammatory activity and suppression of nociceptive reaction *in vivo*

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TRPA1 is a homotetrameric non-selective calcium-permeable channel. It contributes to chemical and temperature sensitivity, acute pain sensation and development of inflammation. HCIQ2c1 is a peptide from the sea anemone *Heteractis magnifica* which inhibits serine proteases. Here, we showed that HCIQ2c1 significantly reduces AITC- and capsaicin-induced pain and inflammation in mice. The subplantar administration of HCIQ2c1 at 0.1 mg/kg dose to mice significantly reduced carrageenan-induced paw edema by a factor of two, which is comparable to the effect of diclofenac at 1 mg/kg dose. The peptide has a strong anti-inflammatory potential by the attenuation of systemic and local inflammatory effects through the inhibition of intracellular Ca^{2+} release, the production of ROS and pro-inflammatory cytokines, and enzymes involved in arachidonic acid metabolism. Electrophysiology recordings in *Xenopus* oocytes expressing rat TRPA1 channel revealed that HCIQ2c1 binds to open TRPA1 and prevents its transition to closed and inhibitor-insensitive 'hyperactivated' states. NMR study of the ^{15}N-labeled recombinant HCIQ2c1 analog described a classical Kunitz-type structure and revealed two dynamic hot-spots (loops responsible for protease binding and regions near the N- and C-termini) that exhibit simultaneous mobility on two timescales (ps–ns and μs–ms). In modelled HCIQ2c1/TRPA1 complex, the peptide interacts simultaneously with one voltage-sensing-like domain and two pore domain fragments from different channel's subunits, and with lipid molecules. The model explains stabilization of the channel in the open conformation and the restriction of 'hyperactivation', which are probably responsible for the observed analgetic activity. HCIQ2c1 is the third peptide ligand of TRPA1 from sea anemones and the first Kunitz-type ligand of this channel. HCIQ2c1 is a prototype of efficient analgesic and anti-inflammatory drugs. This research was funded by the Russian Science Foundation (#22-14-00326).

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P-18-042
Nucleotide-dependent interrogation of damaged DNA during bacterial nucleotide excision repair

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Efficient elimination of DNA lesions by the nucleotide excision repair (NER) pathway is critical in all organisms. In bacteria, the early steps of NER are implemented by the successive action of three proteins, UvrA, UvrB, and UvrC via a series of large and dynamic multi-protein complexes. These complexes operate in three major stages during NER's early steps. During stage 1, a large (300-400 kDa) complex of the UvrA and UvrB proteins (AB) identifies lesion-containing DNA. This process requires rapid binding and release of DNA. Damaged DNA must be specifically recognized and distinguished from native DNA, even though the relevant lesions induce widely varying DNA structures. Lesion-containing DNA is stably bound by a dimeric form of UvrA within the AB complex (Stage 2). A major reorganization ensues wherein UvrA is lost from the ensemble, concomitantly, UvrB becomes localized at the site of damage (Stage 3). Additional events lead to excision of the damage on one strand, and repair of the resulting single-stranded gap. Here, we focus on nucleotide dependent discrimination of damaged DNA from native by UvrA, loading of UvrB at the lesion site, and the nucleotide dynamics driving these processes. Using cryogenic electron microscopy (Cryo-EM), we determined structures of UvrA bound to DNA damaged by three distinct lesions. We found that UvrA mounted a similar response against chemically distinct lesions. The dozens of structures in our study captured UvrA's proximal and distal sites filled with ADP, ATP, or ATPγS. dsDNA in our complexes was found in several configurations compared to B-form DNA; distortions in the DNA were directly linked to UvrA's nucleotide state. Our collection of structures could be grouped based on configurations of protein and DNA and arranged into a sequence of intermediates along the NER reaction coordinate.

P-18-043
Mechanistic understanding of the nucleotide excision repair: structural bases of damage detection process by UvrA and lesion hand-off to UvrB

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Nucleotide excision repair (NER) represents one of the major molecular machineries that control chromosome stability in all living species. In Eubacteria, the initial stages of the repair process are carried out by the UvrABC excinuclease complex. Despite the wealth of structural data available, some crucial details of the pathway remain elusive. In this study, we present a structural investigation of the *Mycobacterium tuberculosis* UvrAUvrB complex and of the UvrA dimer, both in complex with damaged DNA[1]. Our analyses have yielded new insights into the DNA binding mode of UvrA, showing an unexplored conformation of Insertion Domains (IDs), underlying the essential role of these domains in DNA coordination. Furthermore, we observed an interplay between the ID and the UvrB Binding Domain (UBD): after the recognition of the damage, the IDs repositions with the concomitant reorganization of UBD, allowing the formation of the complex between UvrA and UvrB. These events have been observed along the formation of the uncharacterized UvrA2-UvrB-DNA and the UvrA2-UvrB2-DNA complexes which we interpreted as hierarchical steps initiating the DNA repair cascade in NER pathway, resulting in the formation of the pre-incision complex. Thanks to the structural characterization, we were able to develop a model of DNA damage recognition, supported by biochemical data. 1) Marianna Genta, Giulia Ferrara, Riccardo Capelli et al., 04 November 2024, PREPRINT available at Research Square [https://doi.org/10.21203/rs.3.rs-5098223/v1]. Accepted by Nature Communications on 03/2025. *The authors marked with an asterisk equally contributed to the work.

P-18-044
How to be different: the structure of a non-canonical septin (Sep7) from *Magnaporthe oryzae*

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In most species, septins polymerize into hetero-oligomeric filaments involved in important cellular events which often require membrane remodelling. Over recent years we have provided crystal and cryo-EM structures of septin complexes from a wide range of different species. These have shed considerable light on how filaments are assembled and interact to form higher order structures. All reported structures are composed of "canonical" septins, classified into four different groups. These share a common fold which provides the two surfaces necessary for forming the interfaces (NC and G) required for polymerization. A "non-canonical" group of septins (group 5) has been described in some species but little is known about their role in filament assembly and subsequent downstream processes. Here we describe the structure of Sep7, a group 5 septin from *Magnaporthe oryzae*, a fungus responsible for rice blast disease which leads to a loss of 10–30% of harvests worldwide. Septin filaments form at the base of the appressorium, a structure which uses turgor pressure to invade the leaves of plants. Crystals of Sep7 could only be obtained after engineering a mutant which lacked 49 residues predicted to form a disordered internal loop. The resulting structure reveals radically different G and NC interfaces when compared to canonical septins, principally due to the insertion of a helix at the G-interface which destroys normal intersubunit contacts and generates novel ones. The interface is largely stabilized by a network of aromatic residues from a distorted 3-stranded meander at the base of the subunit. As a result, the interface is more open than normal and if a sep7 dimer were to incorporate

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into a canonical filament it would inevitably induce filament curvature. Alternatively, a compensating opening at the NC-interface could lead to homomeric linear filaments as seen in the crystal structure. Studies to investigate these possibilities are currently underway.

P-18-045
Structural biology technologies and how to access them

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Instruct-ERIC is a pan-European distributed research infrastructure making high-end technologies and methods in structural biology available to users. Instruct promotes innovation in biomedical science and operates on a non-economic basis within the scope of the ERIC Regulation. Instruct-ERIC is currently comprised of 17 Member Countries and Organisations: Belgium, Czech Republic, EMBL, Finland, France, Germany, Greece, Israel, Italy, Latvia, Lithuania, Netherlands, Portugal, Slovakia, Slovenia, Spain and United Kingdom. Researchers worldwide can apply for access to structural biology technologies and services at any of Instruct's 11 centres of excellence across Europe, whilst researchers in these 17 members can receive funded access through Instruct. Instruct-ERIC specifically supports research that uses integrated approaches and technologies. It operates with the following principles: a) scientific excellence is our priority in the services we provide and the supported research; b) transparency, equality and legality is the cornerstone of the operational model. In addition to access to high-end technologies for integrated structural biology, Instruct focuses on progressing basic research, such as sample preparation, protein production, and crystallisation for advanced structural analysis. Supporting the work of early-career researchers is a priority for Instruct; the highly successful internship programme is a central part of the organisation, providing a platform for PhD students and early-stage postdoctoral fellows to spend 3–6 months in a new laboratory in a new country to learn a new structural biology technique, to bring the expertise back to their home lab. Instruct also funds several training courses each year, focusing on different but fundamental integrated structural biology methods and techniques. Instruct also looks to provide funding for R&D, with schemes for early-career and advanced structural biology methods development.

P-18-046
Functional and structural studies of insect NavPas channel

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Voltage-gated Na^+ (Nav) channels of insects are the target of new insecticides. Despite their great importance for agriculture, there is still a lack of information on the structure and regulation of Nav channels. Eukaryotic Nav channels are formed by a core α-subunit, which is a single-polypeptide chain containing four homologous pseudo-subunits surrounding a central pore. Previously, it was reported that the function of the American cockroach NavPas channel could not be studied by electrophysiology in *Xenopus laevis* oocytes. Here, we studied the expression of this channel fused with the fluorescent protein tagRFP in eukaryotic HEK cells and observed a preferential accumulation of NavPas in intracellular compartments. Only at high expression the channel was observed on the cell membrane surface. Usage of tagRFP to visualize cells with surface expression of NavPas allowed us to study the channel activity using the whole-cell patch method. The structure of the NavPas channel was studied by cryo-EM in lipid-protein nanodiscs. The preferred orientation of the particles made it difficult to obtain a high-resolution structure. To address this issue, we collected over 23 000 images and extracted ~6 million particles. After 2D classification, 1 million "good" particles were selected, and after 3D classification, 327 000 particles were used for structure determination. Finally, a 2.9 Å structure was obtained for the monomeric form of NavPas in the nanodiscs. Compared with the previously published structure in digitonin micelles, the intracellular domains were found to be pressed against the surface of the nanodisc membrane. The obtained data provide new insights into the structure and function of the NavPas channel and are valuable for the future development of new insecticides. The work was supported by Guangdong Province Key Special Project #2023ZDZX2072. *The authors marked with an asterisk equally contributed to the work.

P-18-047
Evolution of pyridoxal kinase activity in bacterial vitamin kinase family: a kinetic, molecular and structural approach

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The biosynthesis of thiamine requires the phosphorylation of 4-amino-2-hydroxymethyl-5-methylpyrimidine (HMP) to HMP-PP with HMP-P as an intermediate. In bacteria, two homologous enzymes catalyze the phosphorylation of HMP: 1) ThiD2-ccPLK/HMPK which also phosphorylates pyridoxal (PL), and 2) ThiD-HMPPK kinases, which further phosphorylates HMP-P to produce HMP-PP. Crystal structures of an ancestral ThiD-HMPPK from Enterobacterales reveal that residues H179 and T211, essential for HMP-P phosphorylation, are missing in ThiD2-ccPLK/HMPK. Conversely, the crystal structure of *Staphylococcus aureus* ThiD2-ccPLK/HMPK shows a hemithioacetal intermediate formed between PL and the strictly conserved residue C111. Phylogenetic analyses suggest that ThiD2-ccPLK/HMPK enzymes evolved from ThiD-HMPPKs, leading to the loss of HMP-P kinase activity and the emergence of PL kinase activity as a novel function. To explore this transition, we reconstructed ancestral sequences corresponding to the last common ancestor of ThiD-HMPPK (ancThiD), an intermediate ancestor (ancC), and the last common ancestor of ThiD2-ccPLK/HMPK (ancThiD2). *In silico* analyses suggest that T211A was the first evolutionary step, followed by Q44M, A111C, and H179S, which reshaped the active site. Biochemical characterization demonstrated that ancThiD exclusively phosphorylates HMP and HMP-P, while ancC shows a wider substrate specificity, phosphorylating HMP, HMP-P, and PL. In

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ancThiD2, the PL kinase activity was optimized, making it the most efficient activity, whereas HMP-P phosphorylation remained as a vestigial activity. Molecular dynamics simulations indicate enhanced active site flexibility in ancC and ancThiD2, which increased in the presence of PL and site-directed mutagenesis show that ancThiD2^{C111A} mutant presents reduced catalytic efficiency for PL. In contrast, the ancC^{A110C} mutation had no effect, suggesting that other substitutions contributed to the optimization of PL phosphorylation. FONDECYT 1221667.

P-18-048
Crystal structure of Hispidin-3-hydroxylase from *Armillaria ostoyae* sheds light on its activity-based mutagenesis

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Hispidin-3-hydroxylase (H3H) is a FAD-dependent monooxygenase that catalyzes the formation of fungal luciferin. Here we present the first crystal structure of H3H from *Armillaria ostoyae* (aoH3H) in complex with FAD determined at 3.8 Å resolution. Despite several attempts to optimize crystal growth, the resolution was difficult to improve. Therefore, to predict the location of the substrate-binding sites and identify tentative catalytic residues, we exploited structure homology-based molecular docking. The position of NAD(P)H within the binding pocket was estimated based on mutagenesis data, describing that replacement of three residues resulted in change of cofactor specificity from NADPH to NADH [Previously published in: Tong Y. et al.(2020) J Biol Chem, 16013–16022]. To further elucidate the proposed models of substrate binding, we generated a series of H3H mutants, each containing a single residue replacement with alanine or methionine within the presumable active site. Subsequently, we measured Michaelis constant and apparent catalytic constant for the mutants. These measurements revealed that the substitution of selected residues impacts the enzyme kinetics considerably, thereby validating our structural considerations. An immeasurably low activity of the Y244A H3H mutant indicated that the Y244 residue might be particularly important for catalysis. Furthermore, a pronounced effect was observed when L219 and R391 were replaced with alanine, leading to a two-order-of-magnitude increase in K_M. This suggests that L219 and R391 participate in substrate binding. At the same time, S328, which was predicted to interact with NAD(P)H, affects enzyme activity when substituted with methionine. This observation supports the docking-derived model of H3H-NAD(P)H interaction. This work was supported by the Russian Science Foundation grant 24-74-10087.

P-18-049
Structural and functional characterization of the beta-barrel assembly machinery complex from the Lyme disease causing agent *Borrelia burgdorferi*

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Lyme disease, caused by the spirochete *Borrelia burgdorferi*, poses a significant health threat in Europe and the USA. Understanding the molecular mechanisms underlining its pathogenesis is crucial for developing effective therapeutic strategies. This study focuses on the β-barrel assembly machinery (BAM) complex, an integral component of the outer membrane in Gram-negative bacteria, including *B. burgdorferi*. In *B. burgdorferi*, the BAM complex consists of the central protein BB0795 (BamA) and at least two accessory proteins, BB0028 (BamB) and BB0324 (BamD). Although the components of the *B. burgdorferi* BAM complex are generally known, the exact nature of their interactions in forming complex remains unclear. The BAM complex plays a vital role in the folding, assembly, and export of outer membrane proteins. This study aims to provide valuable insights into the spirochete's biology by unraveling the mechanisms behind BAM complex formation and function, with a focus on protein–protein interactions and 3D structure determination. We have crystallized two essential BAM components, BamB and BamD. The crystal structure of BamB has been determined, revealing a β-propeller structure composed of seven β-sheets arranged in a circular conformation, with each β-sheet consisting of four anti-parallel β-strands. Using pull-down assays, gel-filtration chromatography, and microscale thermophoresis, we have characterized the interactions between the BAM complex proteins. Furthermore, based on AlphaFold 3.0 protein-protein complex predictions, we have evaluated the interactions between BamA and the accessory proteins BamB and BamD using site-directed mutagenesis. This knowledge may contribute to potential drug development by targeting this readily accessible outer membrane component. This work was supported by the ANM grant Nr. 26/BMC/PG.

P-18-050
Panproteome structural diversity showcases conformational states

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A panproteome gathers the proteomes from different organisms of a species. The panproteome of barley currently comprises 76 variants, with distinct phenotypes, and completely sequenced genomes [1]. Most of the time, these variants share the same protein, with the exact same sequence, but different proteoforms often appear. They showcase a natural diversity, which may or may not be significant to function. With the latest developments of AI protein structure prediction, we have access to an unprecedent amount of protein structures. How to analyse these data is a state-of-the-art challenge. In our group, we are predicting the structures for the complete panproteome of barley. By examining specific proteins or protein families, we uncover proteoforms with potentially different functional properties. Two compelling examples are the MATE family of multidrug resistance transporters and PAIR1, a protein

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involved in the pairing of homologous chromosomes during meiosis. Despite the high sequence similarity, some of their proteoforms appear in different conformational states. Members of the MATE family appear in a closed or in an open state, typical functional states for opening and closing the channel of this transporter. And some PAIR1 proteoforms present a beta sandwich in a stretch of a long-disordered region, where others are completely disordered, possibly enhancing the interaction between these molecules during meiosis. Using computational modelling, we can mutate a proteoform to exhibit one state or the other. This helps identify crucial residues involved in conformational changes. By tracing the abundance of specific proteoforms across barley variants, we link structural diversity to phenotypic traits. With these two examples, and others, we harness the naturally occurring diversity of proteoforms to deepen our understanding of protein function.

P-18-051
Investigating the stability of Orb2 glutamine-rich domains associated with long-term memory formation

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Long-term memory formation relies on intricate molecular mechanisms that regulate protein synthesis at synapses. In *Drosophila melanogaster*, the cytoplasmic polyadenylation element-binding (CPEB) protein Orb2 plays a key role in memory consolidation by controlling mRNA transport and translation. The glutamine-rich domain of Orb2 is essential for self-assembly, but its structural stability and oligomerization mechanisms remain poorly understood. To investigate the stability of Orb2 oligomers and their hydrogen-bonding interactions, molecular dynamics (MD) simulations were performed. The 2- and 3-subunit models were derived from the 6VPS crystal structure, while the 4- and 10-subunit models were predicted using AlphaFold3. Each subunit consists of two β-sheets, which facilitate hydrogen bonding between adjacent subunits and enhance complex stability. One-microsecond MD simulations were conducted in GROMACS using the CHARMM36 force field, with stability assessed through inter-protein hydrogen bond (H-bond) analysis based on geometric criteria. The 2-subunit model was unstable, with early subunit dissociation. The 3-subunit model demonstrated improved cohesion, likely due to internal H-bond formation. Higher-order assemblies exhibited enhanced stability, with average H-bond counts of 20.3 ± 1.9 (2-subunit), 30.5 ± 1.5 (3-subunit), 31.3 ± 1.6 (4-subunit), and 41.3 ± 0.9 (10-subunit). These results suggest that two β-sheets per subunit promote stable inter-subunit interactions, favoring ordered oligomer formation. These findings provide structural insights into Orb2 self-assembly and its potential role in long-term memory regulation.

P-18-052
CryoRhodopsins: a comprehensive characterization of a group of microbial rhodopsins from cold environments

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Microbial rhodopsins are omnipresent on Earth but the vast majority of them remain uncharacterized. Here we describe a new rhodopsin group found in microorganisms from cold environments, such as glaciers, denoted as CryoRhodopsins (CryoRs). A distinguishing feature of the group is the presence of a buried arginine residue close to the cytoplasmic face. Combining single-particle cryo-electron microscopy and X-ray crystallography with rhodopsinactivation by light, we demonstrate that the arginine stabilizes a UV-absorbing intermediate of an extremely slow CryoRhodopsin photocycle. Together with extensive spectroscopic characterization, our investigations on CryoR1 and CryoR2 proteins reveal mechanisms of photoswitching in the newly identified group. Our data suggest that CryoRs are sensors for UV irradiation and are also capable of inward proton translocation modulated by UV light.

P-18-053
Structural characterization of the pioneer transcription factor Sox with the nucleosome

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Sox-2, a pioneer transcription factor, plays a crucial role in chromatin remodeling and transcriptional regulation, particularly in cellular development and reprogramming. Understanding its molecular interactions with chromatin at the atomic level is essential for elucidating its functional roles in physiological processes. To achieve this, we resolved the high-resolution (2.7 Å) structure of the *in vitro* reconstituted complex between the Sox-2 HMG domain and the nucleosome using cryo-electron microscopy (cryo-EM). Our findings reveal that Sox-2 binding to its target sequence within the nucleosome induces stark DNA deformation at the Sox cognate site (SHL 2), widening the minor groove through clamping and stretching of the DNA backbone – a mechanism suggestive of chromatin invasion. This study provides critical insights into the molecular interactions between Sox-2 and nucleosomes, shedding light on its function under physiological conditions. Given the implications of Soxopathies, where mutations in the HMG domain lead to developmental disorders and cancers, this work enhances our understanding of chromatin dynamics and gene regulation, ultimately advancing knowledge of disease mechanisms. *The authors marked with an asterisk equally contributed to the work.

LB-R-18-01
Hepatitis C, SARS-CoV-2 and West Nile infection and antiviral treatments monitored by multimodal imaging

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Recent advancements in virology have revealed a common feature among positive-strand viruses: the alteration of cellular membranes to form replication complexes. Despite variations in origin and structure, these membranous compartments, classified as double-membrane vesicles (DMVs) or invaginated vesicles (IVs), are observed across diverse viruses, including hepatitis C virus (HCV) and SARS coronavirus. Notably, West Nile virus (WNV) generates IVs, suggesting a conserved strategy across distant viruses. In this study we have correlated cutting-edge imaging techniques – infrared microscopy, confocal immunofluorescence and correlative cryogenic light-soft X-ray

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tomography (CLXT) – in the water window photon energy range to investigate in whole, unstained cells, the morphology of the membranous rearrangements induced by WN, HCV and SARS-CoV-2 infection and after antiviral treatments in near-native conditions. These infection alterations could be reverted by combination of different antiviral treatments and the healing process could be monitored by multimodal imaging techniques. In addition to providing structural insights into the cellular aspects of viral pathogenesis, our study illustrates how cryo-SXT is a powerful three-dimensional wide-field imaging tool for the assessment and understanding of complex cellular processes in a setting of near native whole hydrated cells. Our results also constitute a proof of concept for the use of cryo-SXT at ALBA synchrotron and at lab-scale soft X-ray microscope (SXM) as a platform that enables determining the potential impact of candidate compounds on the ultrastructure of the cell that may assist drug development at a preclinical level. This study was funded by ALBA Synchrotron proposals 2022065884, 202102489920, 23087667 and for the CoCID EU project and by CLEXM (EU MSCA-DN).

LB-R-18-02
Cryo-EM structure of vacuolar ALMT9 reveals a lipid-regulated activation mechanism

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Vacuolar ALMTs control anion accumulation in plant cells. In guard cells, they regulate stomata aperture. The activation of vacuolar ALMTs depends on voltage and cytosolic malate, but the underlying molecular mechanisms remain elusive. Here we report the cryo-EM structures of ALMT9 from *Arabidopsis thaliana* (AtALMT9), a malate-activated vacuolar anion channel, in plugged and unplugged lipid-bound states. In all these states, membrane lipids interact with the ion conduction pathway of AtALMT9. We identify two unplugged states presenting two distinct pore width profiles. Combining structural and functional analysis we identified conserved residues involved in ion conduction and in the pore lipid interaction. Molecular dynamics simulations revealed a peculiar anion conduction mechanism in AtALMT9. We propose a voltage-dependent activation mechanism based on the competition between pore lipids and malate at the cytosolic entrance of the channel.

LB-R-18-03
Exploring PARP15 as a new pharmacological target: preliminary analysis for structural and biochemical characterization

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The poly ADP-ribose polymerases (PARPs) family comprises 17 members that catalyze the covalent attachment of ADP-ribose units on target proteins as a post translational modification. PARPs consume NAD as substrate and, according to their activity, they can be divided in mono-ADP ribosylating (MARylating) and poly-ADP ribosylating enzymes (1). NAD-consuming enzymes govern critical steps of tumorigenesis and the ADP-ribosylation signal has a role both in virulence and immunity as a regulatory mechanism leading to a therapeutic relevance that needs to be clarified (2). The MARylating enzyme PARP15 has yet to be structurally and biochemically characterized; moreover, too little is known about its biological role within the cell but, considering the relevant evidence of PARP family members in tumor background, it might potentially cover a crucial role and be considered a possible target. The crystallographic structure of the only PARP15 catalytic domain has already been obtained and it is reported to be a dimer; however, little is known on longer protein constructs. In this study we focused our efforts on a truncated form of recombinant PARP15, devoid only of the first 77 amino acids at N terminus. Recombinant expression in *E. coli* and purification protocols have been optimized to obtain a pure and homogeneous sample. The His-SUMO tagged PARP15 structure is here investigated through a Cryo-EM explorative analysis. Mass photometry and Small-Angle X-ray Scattering analysis are planned in order to confirm potential dimerization. Stability and protein folding assessment are currently ongoing as well as activity assays set up. With this purified protein we plan to perform binding affinity tests with the substrate NAD and to test available PARP inhibitors that may help us clarify tasks and features of PARP15. 1. Nizi M.G. et al. (2022) J. Med. Chem. 65, 11, 7532–7560. 2. Suskiewicz Marcin J et al. (2023) Cell. 186, 21 4475-4495.

LB-R-18-04
Structural and functional stability study of PTPN3 protein and its somatic variants in tumoral tissues

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The human protein tyrosine phosphatase non-receptor type 3 (PTPN3) is a phosphatase that has been implicated in various cancers, acting either as a tumour suppressor or promoter depending on the context. PTPN3 is an α/β protein composed of a FERM domain, a PDZ domain, and a PTP catalytic domain. While PDZ-binding residues have been already identified, the structural interface of the PTPN3-PDZ, especially PTP contributing residues, remains poorly defined. Mutations of PTPN3, have been identified in cancer patients, as catalogued in the COSMIC (Catalogue Of Somatic Mutations In Cancer) database. The aim of our study was to assess how cancer-associated mutations could affect the protein's structure and activity, and, eventually, to investigate the PDZ-PTP interactions. We conducted a variant impact analysis of all missense mutations reported in COSMIC for the catalytic domain, using bioinformatics tools, including PDB, Pymol, Mafft, PolyPhen-2, ClinVar, CoeViz and Consurf database. Then, the catalytic domain of wild-type PTPN3 and a panel of missense variants (R675Q, G691E, N692H, D694Y, R749Q, R751Q and E777K) were expressed in *E. coli*, purified and characterized by near and far-UV circular dichroism and intrinsic fluorescence spectroscopy. Structural models of the PTPN3 catalytic domain and PDZ domain, were generated using AlphaFold (v.2 and v.3). Molecular dynamics simulations with GROMACS were performed to study

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the dynamic behaviour of the wild-type PTPN3 catalytic domain and selected mutants (G691E, N692H, R749Q, R751Q), as well as on the PTP domain-loop-PDZ domain assembly. Some PTPN3 variants exhibit changes in tertiary and secondary structures. The catalytic activity as well as the thermal stability of some PTPN3 variants differ from those of the wild type. These findings highlight how PTPN3 missense mutations can alter the protein's structure and activity, showing the value of an integrated approach to assess the consequences of cancer-associated mutations.

LB-R-18-05
Von Hippel-Lindau E3 ligase as a molecular target – structural and biochemical studies with ligands and PROTAC

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Advancements in targeted therapies have significantly transformed modern oncology. Among them, proteolysis-targeting chimeras (PROTACs) represent a novel class of molecules designed to induce the selective degradation of oncogenic proteins (Hsia O et al. (2024) Nature 627, 204-211). PROTACs are bifunctional molecules composed of two ligands – one targeting a protein of interest and the other an E3 ligase, bound by a flexible linker. Recruitment of the E3 ligase enables ubiquitination and subsequent proteasomal degradation of the target protein. One such E3 ligase, the von Hippel-Lindau (VHL) protein, is currently under preclinical investigation for its therapeutic potential. In our study, we successfully obtained the VHL-ElonginB-ElonginC (VBC) complex, confirming its proper folding and stability using nano-differential scanning fluorimetry (nanoDSF) complemented by dynamic light scattering (DLS). We then employed Microscale Thermophoresis (MST) to characterise the binding interactions between VBC and a panel of small-molecule ligands. This included the known ligand VH032 and the PROTAC XY028-133, designed to target cyclin-dependent kinases 4 and 6 (CDK4/6), key regulators of the cell cycle. We also assessed the formation and stability of the complexes involving VBC, PROTAC, and CDK4/6. To gain structural insight into these binary interactions, we initiated co-crystallization trials with selected compounds (ongoing activity). Our findings provide a foundation for understanding how small molecules and PROTACs engage with the VBC complex. This knowledge will support structure-guided design and optimization of PROTACs, particularly in fine-tuning linker properties, thus advancing the development of more effective cancer therapies.

LB-R-18-06
Unraveling DED assembly for cell fate decisions in death receptor signaling

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FADD, procaspase-8, and cFLIP assemble through death-effector domains (DEDs), directing death receptor signaling toward either cell survival or apoptosis. Despite their crucial roles, understanding the 3D regulatory mechanisms of these interactions has been limited by the absence of atomic coordinates for their ternary DED complex. Using X-ray crystallography and cryogenic electron microscopy (cryo-EM), we solved the structures of the FADD-procaspase-8-cFLIP and procaspase-8-cFLIP complexes, revealing structural insights into these critical interactions. The ternary structure of FADD-procaspase-8-cFLIP illustrates how FADD and cFLIP orchestrate the assembly of caspase-8-containing complexes and provides mechanistic explanations for their roles in promoting or inhibiting apoptotic and necroptotic signaling. Additionally, a helical procaspase-8-cFLIP heterodimer appears to promote limited caspase-8 activation for cell survival. Significantly, we unexpectedly obtained the binary structure of the procaspase-8-cFLIP complex, discovering that cFLIP and procaspase-8 autonomously form a binary DED complex, independent of FADD. This finding uncovers an unconventional, reversed cFLIP-procaspase-8 assembly mechanism. The complex formed in this manner has the ability to recruit FADD, thereby allosterically modulating cFLIP assembly and partially activating caspase-8 for RIPK1 cleavage. This process is reminiscent of how several tumor cells maintain relatively high levels of cFLIP to achieve immortality. Our structure-guided mutagenesis assays provide critical insights into these regulatory mechanisms, shedding light on the resistance to apoptosis and necroptosis that contribute to tumor cell survival and immortality. Finally, this research presents a unified model for the intricate, bidirectional, hierarchy and reverse hierarchy-based processes that govern cell fate decisions through multiprotein helical assembly. *The authors marked with an asterisk equally contributed to the work.

Chemical biology

P-19-001
Using genetic code expansion to monitor the localization of acetylated nucleophosmin in cultured mammalian cells

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Post translational modifications (PTMs) regulate protein function and cellular localization through the addition of chemical groups to the side chains of certain amino acids. Lysine acetylation is one of the main PTMs that occur in eukaryotes. It fine-tunes the functioning of the nucleolar protein nucleophosmin (NPM1), which shuttles into the nucleoplasm in response to cellular DNA damage [previously published in: González-Arzola K et al. (2022) Nat Struct Mol Biol 29, 1024–1036]. It has been proposed that SIRT7-mediated deacetylation of lysines 27 and 54 from NPM1 takes place upon UV-induced genotoxic stress [previously published in: Ianni A et al. (2021) Proc Natl Acad Sci USA 118, e2015339118]. However, we wonder whether deacetylation of these sites drives the delocalization of NPM1 to the nucleoplasm or is a concomitant occurrence involved in other aspects of the

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DNA damage response (DDR). Herein we show that the localization of NPM1 is independent of lysine acetylation at positions 27 and 54. *In silico* molecular dynamics simulations revealed negligible structural changes between acetylated and deacetylated NPM1. *In vitro* assays confirmed the pentameric state of acetylated NPM1 although the resulting pentamer is less stable than the one assembled by wild-type protein. Additionally, the *in cell* incorporation of genetically encoded acetyl-lysine [previously published in: Cohen S et al. (2016) ChemBioChem 17, 1008–1011] in positions 27, 54 and both simultaneously, revealed nucleolar and nucleoplasmic localization of NPM1 in control and UV-irradiated cells, respectively. In other words, the (de)acetylation of Lys27 and Lys54 at NPM1 by SIRT7 is not the molecular switch that shifts NPM1 out of the nucleolus, albeit its prominent role under DDR. We anticipate that other PTMs, such as phosphorylation, could collaborate with the deacetylation of these sites or could be entirely responsible for the nucleolus-nucleoplasm shuttling of NPM1. *The authors marked with an asterisk equally contributed to the work.

P-19-002
Biophysical and biochemical characterization of human metallothionein MT1HL1

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Metallothioneins (MTs) are small, cysteine-rich proteins crucial for metal homeostasis, detoxification, and oxidative stress response. While many human MT isoforms are well-studied, the MT1HL1 isoform is poorly characterized [previously published in: Krezel A et al. (2021) Chem Rev 121, 14594–14648]. Encoded by a gene on chromosome 1q43, contrary to others (16q13), MT1HL1 shares high sequence similarity with other MTs but has unique structural and functional features. This study comprehensively characterizes MT1HL1 biophysically and biochemically, focusing on its metal-binding affinity and selectivity for Zn(II), Cd(II), and Cu(I) ions. MT1HL1 was expressed in bacteria and purified using an intein-based method to avoid extraneous amino acids that could interfere with analyses. The stoichiometry of Zn(II) and Cd(II) binding was assessed through UV-Vis spectroscopy, mass spectrometry, and reactions with PAR and DTNB, indicating coordination of 5–7 mol. equiv. of Zn(II) per protein molecule. Competition assays showed that the affinity of MT1HL1 for Zn(II) binding sites is lower than that of other human MT isoforms. Notably, Zn(II)-saturated MT1HL1 transferred 2 mol. equiv. of Zn(II) to PAR, compared to one for the MT2A isoform [previously published in: Pomorski A et al. (2023) Metallomics 15, mfad027]. These findings confirm that MT1HL1 has binding sites with lower slightly affinity relative to other MTs. Future work will determine the exact dissociation constants using Zn(II) fluorescence and Cu(I) chromophoric probes. This research aims to elucidate MT1HL1's biological role and its implications for cellular metal homeostasis and stress response, suggesting that MT1HL1 may function as a specialized metallothionein variant due to its unique gene localization and sequence differences. This research was supported by the National Science Centre of Poland (Opus grant no. 2021/43/B/NZ1/02961 to AK). *The authors marked with an asterisk equally contributed to the work.

P-19-003
Exploring the therapeutic potential of *Volvopluteus gloiocephalus* extracts: anti-inflammatory activity, aldo-keto reductase inhibition, and steroid receptor binding affinity

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Fungi are increasingly recognized as sources of biologically active compounds with therapeutic potential, including antimicrobial, anti-inflammatory, and anticancer activities. These compounds range from high-molecular-weight polysaccharides and enzymes to low-molecular-weight phenolics and terpenoids, offering viable alternatives to synthetic drugs. This study evaluated the anti-inflammatory potential, aldo-keto reductase (AKR) inhibition, and steroid receptor binding affinities of three extracts – 80% methanolic (MeOH), 70% ethanolic (EtOH), and polysaccharide (PSH) – from *Volvopluteus gloiocephalus* sampled in Novi Sad (Serbia). Polysaccharides were identified via ^{1}H NMR spectroscopy, revealing hexoses (e.g., glucose or mannose), acetylated or methylated sugars, and conjugated phenolics. Signals suggested β-1,3 or β-1,6 linkages in β-glucans. *Ex vivo* assays assessed cyclooxygenase (COX-1) and lipoxygenase (12-LOX) metabolite inhibition, while *in vitro* tests evaluated binding affinity to ligand-binding domain (LBD) of estrogen receptor α (ERα), estrogen receptor β (ERβ), androgen receptor (AR) or glucocorticoid receptor (GR), and AKR1C3/AKR1C4 inhibition, targets relevant for hormone-dependent cancer therapy. The PSH extract exhibited the highest inhibition of LOX-derived metabolites 12-HETE (68.34%) and 12-HHT (77.40%), while the EtOH extract showed the greatest inhibition of COX-derived PGE2 (88.30%). The strongest GR-LBD binding was observed for EtOH and PSH extracts, supporting their potential as anti-inflammatory agents. None of the extracts exhibited estrogenic or androgenic activity. All extracts, except MeOH, significantly inhibited AKR1C3, with PSH exhibiting 96.17% inhibition, exceeding ibuprofen. Additionally, all extracts inhibited AKR1C4 (> 70%). These findings highlight *V. gloiocephalus* extracts, particularly polysaccharides, as promising candidates for novel natural anti-inflammatory and anticancer therapeutics.

P-19-004
The role of C-terminal cysteine cluster in MTF-1 transcription factor – a biophysical perspective

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Mammalian metallothioneins are small, cysteine-rich proteins with a high affinity for heavy metals. The transcription of metallothioneins is regulated by metal transcription factor-1 (MTF-1), which translocates from the cytoplasm to the nucleus in response to heavy metal stress signals. In the nucleus, MTF-1 binds to metal response elements (MREs) in the promoter region

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of target genes, activating their transcription. MTF-1 activates gene expression in response to metals and oxidative stress, but the mechanisms underlying this activation remain unclear. Many reports indicated that increased labile Zn(II) concentrations activate MTF-1, with zinc finger (ZF) saturation playing a key role [previously published in: Laity JH et al. (2007) Arch Biochem Biophys 463, 201–210]. However, it is uncertain whether these responses are related to labile Zn(II) concentration or other domains within MTF-1. The C-terminal CxCxCxC cysteine cluster mediates homodimerization, and mutations in these cysteine residues reduce transcription, although it is unclear whether this reduction is due to Zn(II) binding or cysteine oxidation [previously published in: Günther V et al. (2012) Biochim Biophys Acta Mol Cell Res 1823, 476–483]. Since metallothionein expression is induced by heavy metals such as Zn(II), Cu(I), and Cd(II), it is crucial to thoroughly understand all potential binding sites and clarify their roles. This study focuses on the biophysical characterization of the C-terminal cysteine cluster in a synthetic peptide model containing the CxCxCxC motif, as well as the determination of Zn(II) and Cu(I) binding stoichiometry. The research also investigates the effects of cysteine residue mutations on binding geometry and overall affinity. The results suggest that the cysteine cluster may play a role in MTF-1 activation and its translocation to the nucleus. This research was supported by the National Science Centre of Poland (Opus grant no. 2021/43/B/NZ1/02961 to A. Krezel).

P-19-005
Quantitative determination of DNA nucleosides by mass spectrometry in HEK293-FT cells

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Quantitative analysis of the full set of DNA nucleosides, including their modified forms, is a crucial task requiring a rapid, highly sensitive, and reproducible method. Accurate knowledge of nucleoside concentrations enables assessment of oligonucleotide synthesis quality, investigation of cellular metabolic processes, and evaluation of gene expression levels. Non-canonical nucleosides such as 5-methylcytidine, 5-hydroxymethylcytidine, and 5-formylcytidine could be used as markers of epigenetic modifications, as their levels vary during cell differentiation, ageing, and correlate with tumour aggressiveness. One of the most effective approaches for identifying both canonical and modified nucleosides and quantifying them precisely is liquid chromatography coupled with mass spectrometry (LC-MS). The detection of both molecular and fragment ions provides structural information, enhancing reliability of compound identification. This study aimed to determine the level of 5-methylcytidine in eukaryotic HEK293-FT cells using LC-MS. An optimised enzymatic hydrolysis protocol was developed to obtain a mixture of mononucleosides from nucleic acids. Chromatography/mass spectrometric analyses enabled the detection of characteristic m/z values: m/z 228.0979 (dC), 240.0990 (mC), 266.0895 (dG), 241.0830 (dT), and 250.0946 (dA). The use of fragment ions also facilitated structural confirmation: m/z 112.0507 (dC), 126.0661 (mC), 152.0564 (dG), 127.0695 (dT), and 136.0616 (dA). Calibration curves were constructed using hydrolysates of double-stranded oligonucleotides with known composition, enabling accurate quantification via Xcalibur 4.0 software. The method provides both qualitative and quantitative analysis of DNA nucleosides in the 10^{-6} to 10^{-9} M range, ensuring high sensitivity and precision. The determined DNA methylation level in HEK293-FT cells was 3–4%. This work was supported by the Russian state-funded project for ICBFM SB RAS No. FWGN-2025-0020.

P-19-006
Formation and stability of mixed Cu(I)/Zn(II) complexes in MT4

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Zinc and copper ions are essential for the proper functioning of the human body. Despite their different functions and varying cellular concentrations, Cu(I) and Zn(II) ions differ in the complexes they form with metalloproteins. The primary role of metallothioneins (MTs) is to buffer labile concentrations of these metals. Mammalian MTs are small Cys-rich proteins (~30%) and have no aromatic residues. This protein family includes four main isoforms: MT1–MT4. Isolated MT3 and MT4, which contain bound Cu(I) in mixed Cu(I)/Zn(II) complexes, suggest that these isoforms are important for binding, distribution, and metabolism of these metal ions. MT3 has recently been extensively studied for its interactions with Cu(I) and Cu(II) ions, while research on MT4 remains limited [previously published in: Krezel A et al. (2021) Chem. Rev. 121, 14594–14648; Calvo JS et al. (2018) Metallomics 10, 1777–1791]. Human recombinant MT4 was synthesized in *E. coli* with a chitin-binding tag (IMPACT). The resulting protein was reduced, purified, and reconstituted with Zn(II) to form Zn_7MT4. The complexes formed were characterized using spectroscopic methods. Cu(I)-MT4 interactions were studied under anaerobic conditions to prevent oxidation of both Cu(I) and the protein. The affinity of Cu(I) ions for MT4 was assessed using copper chelators (BCA, BCS). Spectroscopic analysis revealed the binding of Zn(II), Cu(I), and Cu(II) ions to MT4 and the formation of mixed reduced and oxidized Cu(I)/Zn(II)-MT4 complexes. These complexes indicate the displacement of Zn(II) from MT4 and the binding of Cu(I) ions with varying affinities. Cu(I) binding to MT4 resulted in the formation of two clusters, Cu_6Cys_9 and Cu_7Cys_{11}, in the β and α domains, respectively. Understanding Cu(I) binding and complex formation is crucial for determining the role of MT4 in copper and zinc metabolism within cells. This research was supported by the National Science Centre of Poland (Opus grant no. 2021/43/B/NZ1/02961 to AK).

P-19-007
New advancements in the understanding of zinc and copper metabolism – interplay of Cu(I) and Zn(II) ions in mixed metallothionein-3 complexes

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Metallothioneins (MTs) are small cysteine-rich proteins crucial for maintaining Zn(II) and Cu(I) homeostasis. Among the

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human MT isoforms, MT3 has the most significant copper character and is regarded as a reservoir of excess copper. However, its precise role in copper homeostasis remains unknown [previously published in: Krezel A, Maret W. (2017) Int. J. Mol. Sci. 18, 1237]. Several Cu(I)/Zn(II)-MT3 forms have been isolated from human tissues, particularly the air-stable $Cu_4Zn_4MT3_{ox}$ [previously published in: Calvo JS et al. (2018) Metallomics 10, 1777-1791; Peris-Díaz MD et al. (2023) Anal. Chem. 95, 10966–10974]. Little is known about other mixed forms, especially reduced ones, including stoichiometry, structure, and stability. This information is crucial for understanding Cu_xZn_yMT3 complexes and their role in copper/zinc metabolism. For this purpose, MT3 was analyzed spectroscopically under anaerobic conditions. UV-Vis and CD titrations showed the displacement of Zn(II) by Cu(I) from Zn_7MT3. Even at higher Cu(I) occupancy, not all Zn(II) ions were displaced, forming mixed complexes. Both apo- and Zn(II)-saturated MT3 could bind the same amount of Cu(I), suggesting that Zn(II) influences the coordination of Cu(I) ions. A more sensitive technique using chromophoric (BCS, PAR) and fluorescent (ZnAF-2F) chelators allowed precise determination of the dissociation constants for Cu(I) and Zn(II) in copper and mixed complexes. This provided insights into thermodynamic and structural interactions between these metal ions. Zn(II) and Cu(I) influence each other, with affinities for these ions lower in mixed complexes than in homometallic complexes. Nevertheless, Cu_xZn_yMT3 complexes can act as metal buffers for these essential ions in the cellular environment. This research was supported by the National Science Centre of Poland (NCN) under the Opus grant no. 2021/43/B/NZ1/02961 (to A.K.).

P-19-008
Efficient Cu(I) transfer from the C-terminal tail of Ctr-1 to zinc metallothionein-2 and GSH – implications for cellular copper homeostasis

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Zinc and copper are essential elements for the proper functioning of cellular processes. The homeostatic control mechanisms for these metal ions differ significantly due to their distinct chemistries. Extracellular Cu(II) ions are reduced to Cu(I) ions and transported into cells via the Ctr-1 transporter, where specialized metallochaperones manage them to prevent Fenton-like reactions. Chaperones facilitate the transfer of Cu(I) to specific organelles and target proteins. The precise roles of metallothioneins (MTs) and glutathione (GSH) in copper homeostasis and the labile copper pool remain unclear. Here, we demonstrate the interaction of the C-terminal (cytosolic) tail of Ctr-1, which contains the HCH motif known to bind Cu(I), with GSH and Zn(II)-loaded and metal-free MT2. Spectroscopic studies on Cu(I)-loaded Ctr-1 fragment (13 AA) reveal that Cu(I) is efficiently transferred to both forms of MT2, with negligible differences in transfer efficiency. This indicates a high affinity of MT2 for Cu(I) and dynamic coordination of both partners, ensuring fast kinetics while maintaining tight binding. Reversible studies show almost no competition between various Cu(I)/Zn(II)-MT2 species and the Cu(I)-free Ctr-1 fragment. Cu(I) transfer to GSH is also efficient; however, reverse studies indicate some competition, suggesting a lower distinction in metal affinities. Transfer studies, confirmed by ESI-MS, indicate that mixed Cu(I)/Zn(II)-MT2 species play an essential role in Cu(I) handling. These data provide the first evidence that the Ctr-1/MT2 interaction occurs within the cellular kinetic window, shedding new light on the role of the mixed Zn(II)/Cu(I)-MT species in both metal ions homeostasis. Moreover, the data suggest that the Cu(I)-GSH complex may serve as a critical Cu(I)-cargo between macromolecules, thereby accelerating Cu(I) transfer. This research was supported by the National Science Centre of Poland (Opus grant no. 2021/43/B/NZ1/02961 to AK).

P-19-009
AIE properties of a newly synthesized furan-based derivative and its application in bioimaging

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Fluorescence imaging is a crucial technique for augmenting our comprehension of biological systems. It allows for understanding various biological phenomena through visualization of cellular structures and dynamic processes such as cell division or molecular trafficking. Moreover, highly sensitive, specific, and real-time visualization has a crucial role in the diagnosis of a variety of diseases. Early detection of cancer or other different pathologies significantly improves the results of therapy and/or the survival rates of patients. Bioimaging and biosensing tools are thus required to be highly sensitive and specific. Aggregation-induced emission (AIE) is a phenomenon in which molecules exhibit enhanced fluorescence emission in the aggregate state than in the solution state. AIE-based luminogenes (AIEgens) are excellent candidates for biomedical research, including sensing, imaging, and theranostics applications. In our work, a furan-based molecule was obtained by two-step synthesis based on Knoevenagel condensation and the Williamson reaction. Its AIE properties were studied using spectroscopic methods. Preliminary biological experiments were performed on a model of glioblastoma cell line T98G. We evaluated the viability of selected cells in the presence of the compound using the SRB technique. Time-dependent accumulation of the newly synthesized luminogen in the cells was studied by spectrofluorimetric technique, and its ability to stain intracellular structures was assessed using fluorescence microscopy. Moreover, 2-photon excited lasing (2PEL) indicated the potency of the compound for vital imaging of biological systems. Our results showed that furan-based AIEgen may be a useful biosensor characterized by high photostability and non-linear properties. Further studies on the molecular target and selectivity of the compound will be continued.

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P-19-010
Phenolic changes in pollens during *in vitro* digestion and cytotoxic effects on human cancer cell lines

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The aim of this study was to evaluate the compositional changes and bioaccessibility of phenolics and antioxidants in pollen during *in vitro* digestion, as well as the cytotoxic effects of digested pollen on various cancer cell lines. Three pollen samples from Bayburt (B1-3), Burdur (ME2-3) and Aydın (A2-2) were subjected to *in vitro* oral, gastric and intestinal digestion. Both digested and undigested samples were analyzed for total phenolic, flavonoid and antioxidant activities. The cytotoxic effects of the digested samples were evaluated on lung (A549, H1299), skin (A431), liver (Hep-G2) and colon (Caco-2) cancer cells as well as fibroblast (Bj) cells. All three pollen samples were found to have total phenolic (~2500 ± 300 mg GAE/100 g) and flavonoid (~400 ± 35 mg QE/100 g) contents before digestion. After digestion, these contents changed and ME2-3 pollen had the highest content of phenolic and flavanoid compounds. TEAC and CUPRAC antioxidant results ranged between 128.97-143.28 and 54.83-76.76 μmol TE/g, respectively. The release of p-coumaric, gallic, trans cinnamic acids and rutin increased gradually from the oral to the intestinal stage [previously published in: Mutlu C and Erbas M. (2023) Food BioSci 53, 102805]. The cytotoxic effects of the samples on cell lines were ranked as A549 > Caco-2 > BJ > A431 > H1299 > Hep-G2 according to IC50 results. Pollen has been recognized as a natural medicine for centuries and numerous studies have investigated its bioactive components. However, no previous study has examined changes in the phenolic composition of pollen samples during digestion or its cytotoxic effects on cancer cells. Therefore, this study provides a new perspective and approach to the literature on this subject. Acknowledgment: The authors would like to thank the Scientific and Technological Research Council of Türkiye (TUBITAK, project number: 116O711) for supporting this research.

P-19-011
Transmembrane domains 1 and 2 of human formylpeptide receptor 2 are dispensable for the calcium flux by PD150606

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The formyl peptide receptor (FPR) family plays a crucial role in host defense against pathogens, but also in sensing internal molecules such as mitochondrial proteins that may be associated with cellular dysfunction. In humans, there are three types of receptors; hFPR1 (formerly hFPR), hFPR2/hFPR-like1 (hFPRL1), and hFPR3/hFPRL2. We demonstrated that HEK-293 cells stably expressing hFPR1 or hFPR2 exhibit a stimulus-specific increase in cytoplasmic free Ca^{2+} ($[Ca^{2+}]_i$) in response to various commonly used calpain inhibitors [previously published in: Fujita H et al. (2011) Arch Biochem Biophys, 51-60]. Peptide aldehyde type of calpain inhibitors, such as *N*-acetyl-Leu-Leu-Nle-CHO (ALLN) induced a transient increase in $[Ca^{2+}]_i$ in both HEK-293/hFPR1 and HEK-293/hFPR2 cells. In contrast, α-mercapto-acrylic acid derivative type of calpain inhibitors, such as PD150606, induced a transient increase in $[Ca^{2+}]_i$ only in HEK-293/hFPR2, but not in HEK-293/hFPR1 cells. To investigate the structural basis of the PD150606/ligand binding specificity, I constructed chimeric hFPRs and expressed them in HEK-293 cells, followed by calcium flux assays. Chimeric hFPR1/2 consisted of hFPR1 transmembrane (TM) domains 1 and 2, with hFPR2 TM domains 3~7, whereas chimeric hFPR2/1 consists of hFPR2 TM domains 1 and 2, with hFPR1 TM domain 3~7. W peptide (WKYMVm), a selective agonist for the formyl peptide receptors FPR1, FPR2, and FPR3, induced a transient increase in $[Ca^{2+}]_i$ in both HEK-293 cells expressing chimeric hFPR1/2 and chimeric hFPR2/1, indicating chimeric hFPRs were functional. On the other hand, PD150606 induced a transient increase in $[Ca^{2+}]_i$ only in HEK-293 cells expressing chimeric hFPR1/2, but not in HEK-293 cells expressing chimeric hFPR2/1. These findings indicate that hFPR2 TM domains 1 and 2 are dispensable for the PD150606-induced increase in $[Ca^{2+}]_i$.

LB-R-19-01
Targeting TRIB2 in melanoma: pathways to oncoprotein degradation

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Tribbles homolog 2 (TRIB2) is one of three members of the Tribbles family of pseudokinases (pseudo-serine/threonine kinases). Functioning as an oncogene, TRIB2 expression correlates with melanoma progression and response to therapy. While metastatic melanoma – the most aggressive form of skin cancer – now benefits from two major treatment strategies, namely MAPK pathway inhibitors and immune checkpoint blockade, the majority of patients either fail to respond initially or eventually develop resistance. Consequently, there is a critical need to identify novel, druggable targets that can overcome this therapeutic resistance. TRIB2 has been implicated in promoting resistance to various anti-cancer therapies, supporting its potential as a therapeutic target in refractory melanoma. This study investigates the pharmacological targeting of TRIB2 and reveals multiple independent strategies to downregulate its expression. Notably, TRIB2 is a short-lived protein stabilized by activation of the PI3K/AKT pathway. In contrast, inhibitors of BRAF, MEK, and ERK reduce TRIB2 expression at the transcriptional level. Furthermore, inhibition of polo-like kinases (PLKs) – particularly PLK2 – markedly decreases TRIB2 protein levels and stability. Strikingly, treatment with increasing concentrations of the kinase inhibitor PIK75 leads to near-complete elimination of TRIB2 in melanoma cells, exceeding its canonical PI3K-inhibitory activity. To elucidate the underlying mechanism, we performed phosphoproteomic profiling and identified hypophosphorylated peptides following PIK75 treatment, suggesting candidate kinases involved in TRIB2 regulation. Collectively, we identify three distinct classes of compounds that efficiently eliminate the oncogenic TRIB2 protein from melanoma cells based on different molecular

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mechanisms and exhibiting 40 to 200 times greater potency than the previously reported afatinib.

Glycobiology

P-20-001
Advanced glycan profiling: nanoRP-LC for high-sensitivity N- and O-glycan analysis

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This study aims to enhance the sensitivity and efficiency of N- and O-glycan analysis using nano-reversed-phase liquid chromatography (nanoRP-LC). Due to the low ionization efficiency and limited availability of glycans, optimizing ionization conditions and labeling strategies is crucial for accurate glycomic analysis. This study evaluates fluorescent labeling, mobile phase selection, and spray solvents to improve glycan detection. N- and O-glycans were analyzed using nanoRP-LC coupled with mass spectrometry. Fluorescent labels, including 2-aminobenzoic acid (2-AA), 2-aminobenzamide (2-AB), and procainamide (ProA), were tested to enhance ionization. The analytical column used was Acclaim PepMap 100 (3.0 μm particle size, 75 μm ID × 150 mm length, 100 Å, C18), with an Acclaim PepMap 100 trap column (100 μm × 2 cm, 5 μm particle size, 100 Å, C18). Various mobile phase compositions and spray solvents (acetonitrile, 2-propanol, and methanol) were tested to determine optimal conditions. Collision-induced dissociation parameters were optimized for improved glycan fragmentation and identification. N-glycans from fetuin glycoprotein were analyzed at a minimum concentration of 100 ng using different fluorescent labels. Among the tested labels, 2-AA was the most effective in enhancing ionization for both N- and O-glycans. Methanol significantly improved ionization efficiency compared to other spray solvents. The combination of 2-AA labeling and methanol as a spray solvent resulted in the highest sensitivity and most reliable glycan detection. These optimizations enabled improved glycomic analysis with minimal sample amounts. This study demonstrates that nanoRP-LC, combined with optimized fluorescent labeling and spray solvents, significantly enhances glycan analysis sensitivity. The findings highlight the potential of this approach for studying glycosylation in biomolecular research and clinical applications, with further research needed for complex biological samples.
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P-20-002
Machine learning-assisted glycan profiling of non-small cell lung cancer for supporting early diagnosis

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This study aims to investigate glycan abundance patterns in non-small cell lung cancer (NSCLC) and evaluate the feasibility of machine learning techniques for diagnostic classification. Glycan profiles were obtained through high-performance liquid chromatography (HPLC) combined with hydrophilic interaction liquid chromatography (HILIC) and fluorescence detection (FLD). The resulting glycosylation data underwent preprocessing and feature selection before being analyzed using machine learning algorithms. Supervised models, including support vector machines (SVM), random forest (RF), and gradient boosting (GB), were trained to differentiate NSCLC patients from healthy individuals based on their glycan signatures. Significant alterations in glycan abundances were observed in NSCLC patients compared to healthy controls. Machine learning-based classification demonstrated promising initial results in distinguishing between the two groups, indicating the potential of glycan profiling as a diagnostic tool. This preliminary study suggests that integrating glycan profiling with machine learning could enhance NSCLC diagnosis and patient stratification. The identification of specific glycan biomarkers may contribute to early detection strategies. However, further validation with larger and more diverse patient cohorts is necessary to confirm these findings and establish clinical applicability.

P-20-003
A comprehensive transcriptomic, proteomic, and glycophenotypic analysis of galactose supplementation in SLC35A2-deficient cells

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Nucleotide sugars, which serve as the key substrates for glycosylation, are primarily produced in the cytosol and act as essential building blocks for assembling complex glycoconjugates. For these molecules to participate effectively in glycosylation processes, they must be efficiently transported into the lumen of the Golgi apparatus or the endoplasmic reticulum (ER) by specialized transporter proteins belonging to the SLC35 family. Every year, an increasing number of congenital disorders of glycosylation (CDG) cases are diagnosed. Among these, some are caused by mutations in the gene encoding the UDP-galactose transporter SLC35A2. To date, one of the few therapies showing improvement in patient condition has been galactose supplementation. However, many questions remain unanswered – for instance, how does galactose reach the Golgi in the absence of a functional transporter? To address this, we investigated the

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impact of galactose feeding on the glycophenotype of cells lacking functional SLC35A2 protein, revealing a selective effect on specific glycan structures. Furthermore, we examined how galactose supplementation influences the transcriptome and proteome of these cells using RNA-seq and LC/MS techniques. Additionally, we evaluated the effects of galactose supplementation on dendritic cells to assess the safety of the therapy. This work was supported by the National Science Centre (Narodowe Centrum Nauki, NCN, grant no. 2023/51/B/NZ3/00810 & 2024/08/X/NZ3/00801), Krakow, Poland.

P-20-004
Production of sialylated glycoproteins in *Ixodes ricinus* ticks and tick cell lines

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Sialic acid is a negatively charged monosaccharide composed of nine carbons, typically attached to the ends of glycoproteins and glycolipids. It is present in many eukaryotes, including mammals and invertebrates, as well as in bacteria and viruses. However, it is primarily recognized as a typical glycan in mammals. The high levels of sialic acid on the cell surface and in secreted proteins indicate its significant role in cellular communication. It functions as a receptor for various signalling pathways, including cell adhesion, immune responses, neurogenesis, and processes related to pathogen invasion. For these reasons, sialic acid is considered a promising target for therapeutic interventions. The tick *Ixodes ricinus* is a key vector for pathogens that cause severe infections in humans and livestock. Evidence indicates that ticks contain sialylated glycoproteins, potentially acquired from the blood of their hosts. To confirm the potential of ticks to produce sialylated glycoproteins, we searched the *I. ricinus* genome to reveal enzymes in the metabolic pathway of sialic acid synthesis and sialyltransferases attaching sialic acid to glycans. Furthermore, we confirmed sialyltransferase expression in ticks and sialyltransferase activity in tick cells. Finally, using Click chemistry and an azide-modified sialic acid precursor, we detected sialylated glycoproteins of tick origin in tick cells and tick early life stages. Thus, we confirmed the ability of ticks to produce their own sialylated glycoproteins in addition to those originating from the host blood.

Protein chemistry

P-21-001
Abstract withdrawn

P-21-002
Manufacture and characterization of natural colorants from the sunflower press cake

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Due to their pronounced protein content, oilseed meals and cakes represent a sustainable by-product stream for manufacturing plant protein concentrates and isolates. Wet processing techniques in protein manufacture is generating an additional by-product stream. The aqueous phases are characterized by the presence of blue-green colorants in the case of sunflower press cake processing. In this study, these aqueous products were powdered by freeze- and spray-drying. Colorant powders were analyzed for their structural, physical, and antioxidative attributes. The performance of freeze-dried vs. spray-dried samples was compared, and the influence of carrier type (maltodextrin, gum arabic, pectin, and xanthan gum) was investigated. The total phenolic content (TPC), antioxidant capacity (TEAC ABTS and FRAP) and amino acid compositions were analyzed for the freeze-dried and spray-dried green color extract. The levels of fluorescent intermediate compounds (FICs) and browning index (BI) were examined, as markers of the Maillard reaction's development. The TPC of samples varied between 0.25 and 0.36 GAE g/100 g dw (dry weight). The antioxidant capacity of powders were in the range of 1.30 to 1.84 mmol Trolox/100 g dw and 1.20 to 1.50 mmol Trolox/100 g dw based on TEAC ABTS and FRAP assays, respectively. Spray-dried powder with gum arabic had the highest FICs level and tryptophan fluorescence compared to freeze-dried samples, possibly due to the high process temperature and chemical structure of the carrier. Non-essential and essential amino acids (lysine and leucine) were detected in trace quantities in the green color extract, obtained as by-products from the protein extraction processes.

P-21-003
Elucidating the oligomerization dynamics of hnRNPA2 and TDP-43 low-complexity domains: implications for neurodegenerative disease mechanisms

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The heterogeneous nuclear ribonucleoprotein A2 (hnRNPA2) and transactive response DNA-binding protein 43 kDa (TDP-43) are crucial RNA-binding proteins involved in RNA metabolism, stress granule formation, and neurodegenerative diseases such as amyotrophic lateral sclerosis (ALS) and frontotemporal dementia (FTD) [as published in Kim, H., Kim, N., Wang, YD. et al. (2013) Nature 495, 467–473 and Geuens, T., Bouhy, D. & Timmerman, V. (2016) Hum Genet 135, 851–867]. Their low-complexity domains (LCDs) mediate phase separation and aggregation, but their oligomerization mechanisms remain insufficiently characterized. In this study, we combined theoretical predictions and experimental techniques to determine key physicochemical properties of hnRNPA2 and TDP-43 LCDs in NaCl solutions. Theoretical calculations provided insights into monomer size, cross-section area, the dependence of nominal charge on pH, and the isoelectric point, forming a foundation for interpreting protein behavior. Using atomic force microscopy (AFM), laser Doppler velocimetry (LDV), and streaming potential measurements, we characterized the proteins' adsorption onto mica and polymer microparticles, enabling precise oligomer size determination. Our results revealed that both LCDs form oligomers, with the degree of oligomerization dependent on time and ionic strength. Furthermore, we developed a rapid and sensitive method to quantify oligomerization kinetics in unstable protein solutions, leveraging electrokinetic modeling to analyze protein-covered surfaces.

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These findings provide novel insights into the molecular interactions governing hnRNPA2 and TDP-43 LCDs aggregation and offer a robust approach for monitoring oligomerization in physiological and pathological conditions. Work partially based on Paulina Zeliszewska, et al. (2025) bioRxiv 2025.02.24.639801. Funded by the grant OPUS 2021/43/B/ST8/01900 (National Science Center, Poland) and the statutory activity of the Jerzy Haber ICSC PAS (ZA, AM). *The authors marked with an asterisk equally contributed to the work.

P-21-004
Comparison of *in vitro* stability of recombinant and native human cardiac troponin complexes

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Ternary troponin complex (ITC-complex), consisting of troponin I, troponin T and troponin C, plays a key role in the regulation of muscle contraction. In heart muscle cells, troponin I and troponin T are expressed as tissue-specific cardiac isoforms (cTnI and cTnT, respectively) that makes them essential biomarkers of myocardial injury. The choice and characterization of a standard for immunochemical determination of cardiac troponins is a crucial issue of their laboratory diagnostics. The aim of this work was to compare the stability of recombinant ITC-complex (recITC) and native ITC-complex (natITC) in different conditions. In our study, we incubated both complexes in various matrices at +4, +25, and +37°C for up to 48 hours and then measured troponin immunochemical activity at different time points using monoclonal antibody assays specific to individual troponins or to the whole ternary complex. Comparison of different buffer solutions showed that addition of BSA preserves immunochemical activity and increases stability of both ITC-complexes. At +4°C and +25°C, troponins were stable in the buffer solution, serum, citrate and heparin plasmas for up to 48 hours but partially dissociated in EDTA plasma. Incubation at +37°C led to fast dissociation of cTnT from both ITC-complexes in all matrices, which was most pronounced in heparin and EDTA plasmas. This was accompanied by degradation of cTnT and cTnI in serum and EDTA plasma. Immunochemical activity and stability of recITC was similar to those of natITC in all tested matrices and conditions, that makes it possible to use recITC as an immunochemical standard for the detection of troponins or troponin complexes.

P-21-005
Protein self-assemblies as a tool in water bioremediation from pesticide compounds

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The global increase in food production intensifies pesticide use, while the limited development of new active compounds and mechanisms of action contributes to growing pest resistance, requiring progressively higher doses of frequently used pesticides. These compounds can enter aquatic systems through surface runoff, subsurface drainage, and soil deposition. Their presence in water bodies could pose a risk to aquatic organisms and the environment, as well as to human health through the consumption of contaminated fish. To improve water remediation and the removal of harmful contaminants, the scientific community is exploring the use of biomaterials such as biochar, enzymes, and microorganisms. This study investigates the potential of protein self-assemblies as a base for bio-filter production. In particular, amyloid fibrils represent promising candidates due to their exceptional mechanical and chemical stability and the presence of versatile functional groups enabling broad adsorption selectivity. We have produced an amyloid-based adsorptive material, and it was tested in the removal of five fungicides from water solutions, both individually and in mixtures, indicating the material's potential to be used in the simultaneous removal of different contaminants. The removal percentage was calculated based on pesticide left-over concentrations measured using high-sensitivity HPLC-MS. The nature of pesticide adsorption was further examined by altering pH values of water solutions, which significantly influenced the adsorption of certain pesticides, implying that the process is primarily electrostatically driven. Our findings strongly support the use of amyloid-based adsorptive materials in water bioremediation from pesticide compounds, as they are emerging as a stable, broad-spectrum, and eco-friendly alternative to conventional methods.

P-21-006
Silk fibroin as a macromolecular crowder for inhibiting pathological amyloid formation

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Aggregation-prone proteins can form fibrillar amyloids demonstrating duality in functional or disordered-associated behavior in physiological conditions. This differentiation is traditionally assessed through biological responses – for instance, α-synuclein forms insoluble and stable misfolded amyloids fibrils which are the primary component of the Lewy bodies. Other proteins fold into ordered amyloids without disrupting cellular behavior or functionality – such as silk protein which is inherently used as a biomaterial due to its tunable and controllable structural conformation. While environmental factors and physicochemical parameters play a key role in the kinetic process, the presence of other assembly proteins has also been shown to either promote or inhibit the assembly of pathological amyloids. In this study, silk protein acts as a macromolecular crowder, preventing α-synuclein aggregation. Our findings reveal that silk modifies the structural transition of α-synuclein by non-specific interactions, as confirmed by protein–protein interaction techniques, whilst kinetic, structural, and morphological analyses supported these findings. Remarkably, amyloidogenic assembly in the presence of silk exhibited increased susceptibility to enzymatic degradation, whereas α-synuclein alone displayed enzymatic resistance in its aggregated form. These results further highlight the functional role of silk in inhibiting uncontrolled pathological aggregation while promoting the formation of ordered functional fibrillar amyloids. These findings have potential implications in therapeutic strategies against neurodegenerative diseases.

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LB-R-21-01
Characterization of D-allulose epimerase from *Leucobacter ruminantium*

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D-allulose is a low-calorie rare sugar with broad applications in the pharmaceutical, food and other industries. Currently D-allulose is extremely rare in nature and is mainly biosynthesized with enzymes called D-allulose epimerase (DAE). DAE catalyzes the C-3 epimerization reaction that converts D-fructose to D-allulose. Among the reported several DAEs, the highest conversion yield of 33% has been reported with the D-psicose epimerase from *Agrobacterium tumefaciens* (AtDPE). In order to find DAE for industrial usages, we performed a BLAST search and characterized the isomerization efficiency of more than 10 candidates. Among them, a DAE from *Leucobacter ruminantium* has exhibited 32.5% of conversion yield at pH 7.5 in the presence of Co metal ions. Its crystal structure was determined, whose overall structure is very similar to other reported DAE structures. The catalytic site of the enzyme and its structural features are now being analyzed, which may provide a molecular background for designing an engineering strategy.

LB-R-21-02
Biochemical characterization of three bacterial trehalose-6-phosphate phosphatases: kinetic properties and metal ion dependencies

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Trehalose-6-phosphate phosphatase (TPP) catalyzes the dephosphorylation of trehalose-6-phosphate (T6P) to trehalose, a key step in the trehalose biosynthesis pathway. In many pathogenic bacteria, T6P accumulation is toxic, making TPP a compelling target for antimicrobial intervention. Although several bacterial TPPs have been structurally and biochemically studied, the development of TPP inhibitors remains limited. Notably, no inhibitors with broad-spectrum activity against multiple bacterial TPPs have been reported, likely due to a lack of detailed comparative understanding of both conserved and divergent features across TPP homologs. In this study, we characterized three bacterial TPP homologs – St-TPP from *Stenotrophomonas maltophilia*, Xa-TPP from *Xanthomonas axonopodis*, and Dc-TPP from *Dermatophilus congolensis* – with an emphasis on enzymatic properties relevant to inhibitor design. Recombinant proteins were heterologously expressed, purified, and subjected to steady-state kinetic analysis. All three enzymes followed Michaelis-Menten kinetics, with *K*m and *k*cat values in line with previously reported bacterial TPPs. Metal ion dependency assays revealed that all enzymes require divalent cations for activity, with Mg^{2+} as the preferred cofactor. Notably, Xa-TPP retained substantial catalytic activity in the presence of Mn^{2+} and Co^{2+}, suggesting a more flexible metal-binding environment in its active site. These results provide mechanistic insights into both shared and unique features among bacterial TPPs and highlight the biochemical diversity that must be considered in the development of cross-species inhibitors. This comparative enzymatic framework establishes a foundation for rational design of broad-spectrum TPP inhibitors as potential antimicrobial agents.

Protein homeostasis

P-22-001
EGFR activates DNAJB1 to suppress -synuclein aggregation and mitigates Parkinson's disease

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Parkinson's disease (PD), marked by the accumulation of aggregated α-synuclein in dopaminergic neurons, is a common neurodegenerative disorder. DNAJB1 in known to serve as a vital component in disaggregating α-synuclein fibrils *in vitro*. However, the precise mechanism and the regulation of DNAJB1 in neuronal cells are largely unknown. Here, we demonstrated that DNAJB1 specifically facilitates the clearance of α-synuclein in neuronal cells through the Hsp70 folding system. DNAJB1 is phosphorylated by EGFR at tyrosine 5 which plays a crucial role in resolving α-synuclein aggregation. Furthermore, tyrosine 5 phosphorylation of DNAJB1 enhances its interaction with Hsp70. Dysregulation of this mechanism hampers the delivery of α-synuclein to Hsp70, consequently exacerbating α-synuclein aggregation in neuronal cells. Further investigation using human brain lysates derived from people with PD and unaffected controls showed reduced levels of EGFR and DNAJB1, while the phosphorylated DNAJB1 at Y5 was elevated. These findings not only illuminate the underlying mechanisms involved in PD pathology but also present DNAJB1 as a potential candidate for targeted treatment.

P-22-002
Neuronal membrane proteasomes (NMP) and NMP-derived peptides modulate neuronal signaling through NMDA receptor activation

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The novel neuroproteasome is localized to the neuronal plasma membrane to degrade intracellular proteins into peptides that are released to the extracellular space. Selective inhibition of this neuronal membrane proteasome (NMP) complex stopped the release of peptides and rapidly attenuated neuronal transmission. Based on these findings, we hypothesize that these neuron-specific peptides mediate a novel form of communication to promote intracellular signaling cascades relevant to neuronal development and function. Our work indicates that NMP peptides can rapidly induce N-methyl-D-aspartate receptor (NMDAR)-dependent calcium influx from dendrites to the soma, leading to rapid and sustained phosphorylation of the well-defined activity-dependent transcription factor CREB. We also determined that the gene expression program drastically changes upon NMP peptide treatment of neurons with an increase in expression of immediate early genes (e.g., *Fos*, *Npas4*) known to have critical neuroregulatory roles. These data suggest that NMP

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peptides are endogenous and selective activators of synaptic NMDA receptors and are critical for promoting activity-dependent gene expression. This pathway is orthogonal to the classic neurotransmitters previously described to activate NMDARs and points to NMP and its resulting peptides as key contributors to the development and function of the nervous system. However, the unique peptide sequences leading to neuronal activation are still poorly understood. We showed that the NMP peptides have tremendous sequence diversity through an unbiased peptidomics approach, and the current ongoing effort is to identify unique active peptide sequences with distinct receptor specificity. Elucidating the mechanism of the NMP and its active peptide products is crucial to understanding the role of this novel signaling process in the nervous system. Previously published in: Ramachandran KV et al. (2017) NSMB 24, 419-430 and Türker F et al. (2024) MBoC 35, ar6.

P-22-003
Effect of targeted cereblon proteolytic degradation on unfolded protein response and autophagy

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Ubiquitination maintains protein homeostasis by swiftly clearing misfolded or damaged proteins. This post-translational modification regulates critical cellular processes, including cell cycle, differentiation, immune responses, and apoptosis. Proteolysis-targeting chimeras (PROTACs) are innovative bifunctional molecules that exploit the ubiquitin-proteasome system to degrade specific proteins.1 PROTACs consist of two ligands: one targeting a protein of interest and the other recruiting E3 ubiquitin ligase, such as cereblon (CRBN), to induce protein ubiquitination and subsequent proteasomal degradation. By eliminating proteins entirely, PROTACs offer a catalytic and effective therapeutic approach beyond traditional inhibitors. In this work, we employ PROTAC to downregulate CRBN, a key component of the CRBN E3 ligase complex that plays an essential role in proteolysis. The effects of cereblon degradation on the unfolded protein response (UPR) and autophagy are evaluated in the A549 human lung carcinoma cell. At concentrations as low as 1–2 μM, significant CRBN degradation is observed, but higher doses lead to a loss of efficacy due to the Hook effect, where target saturation inhibits PROTAC-mediated degradation. Viability is preserved at these concentrations. Gene expression analysis reveals that eIF2A and IRE1, critical ER stress sensors, are downregulated 2-fold in the presence of 2 μM PROTAC, demonstrating UPR inhibition. Furthermore, ATG6 is significantly reduced at 2 and 4 μM doses, while FOXO is reduced only at 2 μM. These findings indicate that targeted CRBN degradation can modulate both UPR and autophagy, offering promising therapeutic applications for diseases where these processes are dysregulated, including certain cancers. EB, SEC acknowledge TUBI TAK 2209, SEC acknowledges NEU 25GAPMER03001, TU BI TAK Incentive/ TU BAGEBI P awards. 1. M. Békés, D.R. Langley, C.M. Crews, Nature Rev. Drug. Discov. 2022. 21, 181–200.

P-22-004
Development of inhibitory peptides to disrupt Bag-1S:p97/VCP complex

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Proteostasis encompasses all protein maintenance processes, including protein quality control (PQC) mechanisms such as molecular chaperones, endoplasmic reticulum-associated degradation (ERAD), and the ubiquitin/proteasome system (UPS). Bag-1 protein, upregulated in breast cancer cells, consist of two functional domains: BAG and ubiquitin-like (UbL) domain. Bag-1 interacts with various partner proteins, affecting proteostasis, protein folding, and protein degradation via Hsp70 and UPS. The AAA + ATPase p97/VCP separates substrate proteins from cellular structures through ATP hydrolysis, targeting them for proteasomal degradation. Studies conducted in our laboratory revealed that Bag-1S directly interacts with p97/VCP and plays a role in ubiquitin-dependent degradation by enhancing p97/VCP ATPase activity. We previously identified the interaction interface of the Bag-1S:p97 complex using hydrogen deuterium exchange mass spectrometry (HDX-MS), cross-linking mass spectrometry (XL-MS) and cryogenic electron microscopy (Cryo-EM). Based on our findings, we determined druggable sites within the complex. In this study, we present our designed peptides targeting Bag-1S:p97/VCP complex interface for therapeutic intervention. We designed nine peptides, five of which target Bag-1S and four of which target p97/VCP. We studied their binding affinity using *in silico* and experimental methods. The ability of our candidate peptides to modulate the ERAD mechanism was tested through ATPase activity measurement of p97/VCP. Also, after transient transfection of recombinant Bag-1S and p97/VCP to MCF-7 breast cancer cells, ERAD substrate accumulation was examined by treating cells with protein synthesis inhibitor, Emetine, in the presence and absence of peptides. As a result, we have two candidate peptides that have a propensity to block the interaction of Bag-1S:p97/VCP.

P-22-005
Amyloid-like transcription factors: is there any hint of interconnection with cancer?

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The failure of proteostasis mechanisms can result in the accumulation of misfolded proteins and the formation of amyloids. Amyloids are highly ordered, insoluble protein aggregates characterized by a cross-β-sheet structure. They are typically resistant to degradation, can disrupt cellular functions or perform diverse biological roles. While amyloids are mostly associated with neurodegenerative diseases, such as Alzheimer's and Parkinson's, they are also implicated in other conditions, including cancer. Some cancer-related proteins, such as p53, RAD51 and BRCA1, have been shown to form amyloid-like aggregates under certain conditions. These aggregates can impair the proper function of tumor suppressor proteins or promote oncogenic signaling. In a comprehensive screening initiative, we used bioinformatics tools to identify several potentially

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amyloidogenic transcription factors with potential amyloidogenic properties that may play a role in human cancer development. To test these bioinformatics predictions, we used a yeast assay, which allows for the evaluation of amyloidogenic potential based on phenotypic changes in *Saccharomyces cerevisiae* strains [previously published in: Chandramowlishwaran et al. (2018) J Biol Chem, 293, 3436-3450], supplemented by a detergent-resistance test. To further investigate amyloidogenicity *in vitro*, we employed a C-DAG approach [previously published in: Sivanathan et al. (2013) Nat Protoc, 8, 1381-1390] (bacterial production and export system), conducted experiments on HEK293T human cell culture, and used chromatographic techniques. We also intend to examine amyloidogenic candidates (transcription factors) in ThT assay – the "gold standard" for verifying amyloid-type protein polymerization. These findings seem to give more insight into the relationship between amyloid formation and cancer. The research was supported by St Petersburg State University project No 125021902561-6. *The authors marked with an asterisk equally contributed to the work.

P-22-006
Cytoplasmic pH as factor of protein phase separation

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Cytoplasmic pH (pH_{cyt}) is one of the fundamental parameters in living cells, influencing membrane transport, protein folding, enzyme activity and cells Red-Ox balance. In fungi, green algae and higher plants, hydrogen ions form the proton gradient and the membrane potential which serve as a driving force for nutrient uptake and ion homeostasis. Baker's yeast exhibits remarkable adaptability, thriving across a broad external pH range (3–7.5) while maintaining a neutral or slightly acidic cytoplasmic pH. However, cytoplasmic acidification may be part of cell response to environmental changes, such as glucose starvation, heat shock, exposure to weak acids and bases, hyperosmotic shock. On the other hand, there is growing evidence that environmental changes trigger formation of reversible assemblies by liquid–liquid phase separation (LLPS). This phenomenon is primarily driven by interactions of nucleic acids and multivalent proteins, containing folded interaction domains or intrinsically disordered regions (IDR). Despite ongoing research, the specific mechanisms of LLPS still remain elusive. The goal of this study was to investigate whether there is a correlation between cytoplasmic acidification and protein phase separation. To investigate this relationship, we compared protein phase separation under various stresses resulting in cytoplasmic acidification. To measure cytoplasmic pH, pH-sensitive fluorescent protein sfpHluorin was overproduced in *Saccharomyces cerevisiae* cells, subjected to various environmental stresses, i.e. hyperosmotic shock and starvation. Additionally, we overproduced IDR-containing proteins fused to fluorescent tags in *S. cerevisiae*. The formation of liquid condensates via phase separation was assessed by fluorescence microscopy. Our data show that protein phase separation during hyperosmotic shock is not triggered by cytoplasmic acidification. This work is supported by St Petersburg State University, project No 125021902561-6.

Proteomics

P-23-001
Exploring astringency in xerostomia: *ex-vivo* understanding of salivary dynamics

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Xerostomia is characterized by a dry mouth sensation that can be linked to hyposalivation. It affects overall well-being, causing difficulties in swallowing and food oral processing. Its etiology is autoimmune diseases, head and neck radiotherapy, polypharmacy or unknown factors. Saliva is composed of water, proteins [e.g. mucins, proline-rich proteins (PRPs)], enzymes (e.g. α-amylase) and plays a key role in oral health. Several of these proteins have been linked to astringency development, due to binding polyphenols, which is an oral sensation of dryness, roughness, and tightening, that at certain levels results in food rejection and disrupt the lubrication pellicle. Xerostomia patients have changes in saliva flow rate and proteome, although whether these patients sense astringency with higher or lower intensity is little known. This study aims to test the ability of saliva from xerostomia patients to bind tannins, and to infer about astringency perception in these patients., Samples were collected from 10 patients (6 female, 4 male) diagnosed with xerostomia at the Teknon Institute at diagnosis time and after at least 6 months of pilocarpine treatment. These samples were compared with those from 10 healthy individuals. Saliva samples underwent an *ex vivo* incubation protocol with enological tannins (1,5 g/L) (2:1) for 5 minutes at 37°C. Samples were then centrifuged at 10 000 g for 10 minutes to separate the supernatant and precipitate. Both were analyzed using SDS-PAGE to identify salivary protein profile. Although ongoing, the study hypothesizes that saliva from xerostomia patients will have differences, from controls, in salivary proteins able to bind tannins, and that pilocarpine treatment will produce variations in the amount of salivary proteins precipitated by these compounds, affecting astringency perception by these patients. Insights from this research may clarify the link between xerostomia and astringency, supporting the development of effective therapeutic strategies that increase patients' wellbeing.

P-23-002
Identification of proteins in the oncogenic FLT3-ITD signaling pathway using the APEX2 system

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Many patients with acute myeloid leukemia (AML) develop resistance following FLT3-targeted treatment. Identifying downstream effectors in the oncogenic FLT3-ITD signaling may help elucidate chemoresistance and potentially introduce new therapeutic targets. For this, we employed the engineered ascorbate peroxidase 2 (APEX2) system and searched for the

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FLT3-ITD interaction partners in murine cells. Given that quizartinib, an FLT3 inhibitor, alters the localization of FLT3-ITD protein to the cell surface, a quizartinib-treated group was used to mimic FLT3-WT. Liquid chromatography-mass spectrometry data were analyzed by state-of-the-art data independent acquisition (DIA), revealing differential associations between two groups. In total, we identified 506 protein interactors for FLT3-ITD. We clustered the identified FLT3-ITD partners according to the pathway enrichment analysis and domain search. Interestingly, the protein export group (mmu03060) was identified in only FLT3-ITD protein interactors. This set of proteins suggests a possible nuclear localization of FLT3. Besides, a significant number of FLT3-ITD interactor proteins were found to be involved in nuclear events, supporting the possible translocation of FLT3-ITD to the nucleus. Next, we selected some particular candidates, including CDK5, XPO1, CHEK1, and GSPT1, to validate by co-IP experiments. Of these, the CDK5/FLT3-ITD interaction was quite strong in tested HEK293T cells. Besides, CDK5 interaction of FLT3-ITD was stronger than that of wild-type FLT3. Hence, we surveyed the phosphorylation dependency of FLT3/CDK5 interaction by suppressing FLT3 phosphorylation in both FLT3-WT and FLT3-ITD. Inhibiting the kinase activity of FLT3 with quizartinib drastically reduced CDK5 interaction, revealing that CDK5/FLT3 interaction is dependent of FLT3 phosphorylation. These findings show that CDK5/FLT3 interaction might be a promising target to study for chemoresistance and to develop new treatment strategies for AML.

P-23-003
Phosphoproteomic analysis of IRE1 interactors in hypothalamic proopiomelanocortin (POMC) neurons

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The unfolded protein response (UPR) represents a process that is initiated by sensor proteins in the endoplasmic reticulum (ER) in response to disruption of ER homeostasis. The UPR serves as the primary adaptive signalling axis, facilitating communication among the ER lumen, the cytosol, and nucleus. The key sensor of UPR is IRE1α (IRE1), which possesses both cytoplasmic kinase and RNase activity, thereby initiating the cell survival or death, contingent on the severity. IRE1 is known to exert a regulatory role in neuroinflammation and protein abnormalities. Here, we aimed to investigate the additional non-canonical roles of IRE1 in hypothalamic neurons that regulate whole-body energy balance. We isolated the IRE1 interactome by immunoprecipitation from hypothalamic proopiomelanocortin neurons (POMCs) following pharmacologically ER stress induction. Quantitative label-free phosphoproteomic study was also conducted using LC-MS/MS. A total of 129 phosphosites on IRE1 interactome were identified. We revealed altered phosphorylation profiles upon ER stress. Gene ontology and functional enrichment analysis demonstrated that these peptides were involved in neuron projection, protein localization, synaptic formation, and autophagy. This study showed that IRE1 interacts with phosphorylated O-linked N-acetylglucosamine (O-GlcNAc) transferase (OGT) at residues Thr318 and Tyr316 in ER-stressed POMCs, while it interacts with phosphorylated O-GlcNAcase (OGA) at Thr806 in unstressed POMCs. The cycling of O-GlcNAcylation, which has been regulated by the activities of OGT and OGA, has been associated with synaptic plasticity, learning and memory, obesity, and neurodegenerative diseases. Deciphering upstream kinase identity, coupled with a mutational analysis of ER stress-responsive phosphorylation sites on OGT and OGA, followed by a systematic phenotype screening on POMC neurons, facilitates the clinical targeting of these critical regulatory enzymes in related diseases.

P-23-004
Serum proteomic signature of *Trypanosoma vivax*-infected sheep for identification of potential biomarkers

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Trypanosoma vivax, a hemoparasite, can infect a variety of domestic and wild ungulate species. The primary affected regions are Africa's tropical zones, Central America, South America, and Iran. The significant financial consequences of cow farming, as well as the spread of this protozoan to previously free areas, highlight the importance of investigating and understanding the various criteria associated with this illness. *T. vivax* is primarily an extracellular parasite that secretes metabolites into the host's circulation, potentially contributing to its pathogenic success. This study aims to identify proteins released by the parasite in the bloodstream of experimentally infected sheep, as well as to assess serum proteins in the animals that may be upregulated or downregulated, with the goal of using them as infection biomarkers. Following extensive protein depletion, serum samples were analysed using mass spectrometry and bioinformatics to identify proteins and potential molecular indicators of infection. This analysis identified 26 epitopes in 12 *T. vivax*-specific proteins with immunogenic properties, all of which were hypothetical, as well as 5 major host proteins whose levels were affected by trypanosomiasis. In addition to the LC-MS/MS findings, the current study sought to describe the immunohistochemistry technique as a diagnostic method for *T. vivax* infection, establishing itself as a novel experiment. Parasites were identified in various tissues with cherry-red and brown colouration, confirming the technique's effectiveness.

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P-23-005
Sculpting mitochondrial proteome in aged skeletal muscle: a sex-driven perspective

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The global demographic shift towards an aging population, marked by increased life expectancy, is accompanied by a rise in sarcopenia prevalence, a skeletal muscle wasting disease associated with higher risks of disability, prolonged hospital stays, postoperative complications and premature mortality. Several mechanisms have been proposed, including increased proteolysis, and neuromuscular junction (NMJ) impairment. However, the role of sex in age-related skeletal muscle remodeling remains poorly understood. To address this gap, markers of NMJ and mitochondria remodeling were evaluated in the gastrocnemius of young and old Wistar rats of both sexes. While no significant changes were observed in brain-derived neurotrophic factor (BDNF) or acetylcholine receptor alpha levels, sex-specific differences emerged in mitochondrial remodeling. LC-MS/MS analysis of mitochondria-enriched fractions identified 445 proteins, 44% of which are listed in MitoCarta. Bioinformatics revealed 41 proteins with significantly different abundances between groups, primarily associated with fatty acid metabolism, oxidative phosphorylation (OXPHOS), and ion transport. These age-related changes were predominantly female-specific. Immunoblot analyses confirmed an age-related decline in the mitochondrial DNA-encoded OXPHOS subunit MT-ATP6, which correlated with reduced ATP synthase activity in old females. Investigation into post-translational modifications (PTMs) demonstrated age-induced oxidation in females, emphasizing OXPHOS protein susceptibility to oxidation. Limited PTMs were observed in males, anew suggesting a sex-specific response to aging. Overall, this study unveils sex-specific mitochondrial proteome remodeling during aging, providing insights into the intricate interplay between sex, age, and mitochondrial function in skeletal muscle. These findings emphasize the importance of considering sex-specific nuances in mitochondrial biology. Previously published in: Moreira-Pais et al.(2024) Free Radic Biol Med218:68-81.

P-23-006
Universal pipeline for inter species proteome analysis: brain matrisome characterization across mouse, ferret and human

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Extracellular matrix proteins (ECMs or matrisome) play a pivotal role in neuronal development and function of the cerebral cortex (CC). In this context, evolutionary studies are interested in understanding the expansion of cortical parenchyma and the increased cellular complexity that the human CC has evolutionary achieved. To assess the role of ECM on these biological processes, we adapted a mass spectrometry-based profiling method to exert interspecies comparisons of CC ECM-enriched proteome among mammalians with convoluted and non-convoluted cortices, i.e. *Homo sapiens* (human) *vs Mus musculus* (mouse), respectively. The *Mustela furo* (ferret) serves as an intermediate animal model due to its greater cerebral complexity compared to mice, bridging the gap between rodents and humans. However, few studies have characterized ferret proteome and even fewer have provided a characterization of their matrisome. In addition, performing reliable interspecies differential abundance studies is complex and requires a think-out-of-the box approach. Here, we analysed the proteome of CC ECMs of mouse, ferret and human and propose a simple and general proteomics data analysis pipeline that enables reliable interspecies differential abundance comparisons. Shortly, for each detected precursor, we annotated all proteins where it could be found according to the three species-specific FASTA databases; and next, we kept for protein quantification only those precursors that are present in the three databases. This strategy ensures that each species' homolog protein is quantified with a set of peptides that could be potentially detected in all three species. We tested our approach within the specific context of the matrisome, aiming to analyze the change of ECM categories proportions across taxonomies to gain deeper insights into the role of matrisome in brain evolution. Finally, we compiled a preliminary matrisome list for ferret by inferring them from published human and mouse matrisome lists.

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P-23-007
Proteomic analysis of rheumatoid arthritis patients: identification of novel biomarkers

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Rheumatoid arthritis (RA) is a chronic inflammatory disease, causing joint destruction and disability. Current biomarkers, such as rheumatoid factor (RF) and anti-cyclic citrullinated peptide antibody (ACPA), are insufficient for distinguishing disease activity. Proteomic analysis offers a promising approach to identifying novel biomarkers for improved diagnosis and treatment. Serum samples from 13 RA patients and 16 healthy controls were analyzed using LC-MS/MS-based proteomics. Protein identification and quantification were performed using the ProteinLynx Global Server and Progenesis software. Differentially expressed proteins (DEPs) were identified based on statistical significance ($p < 0.05$) and fold-change (> 2). A total of 310 proteins were identified, with 57 showing significant differential expression. Among them, 10 candidate biomarkers were significantly upregulated in RA patients: fibrinogen gamma chain (FGG), clathrin heavy chain-1 (CLTCL1), MAPK/MAK/MRK overlapping kinase (MOK), fibronectin-1 (FN1), talin-1 (TLN1), sex hormone-binding globulin (SHBG), extracellular matrix protein-1 (ECM1), fibrinogen alpha chain (FGA), pre-B-cell leukemia transcription factor-interacting protein-1 (PBXIP1), and caspase-8 (CASP8). Functional analysis revealed their involvement in coagulation, immune response, and cell adhesion. Notably, FN1, TLN1, and ECM1 showed potential roles in RA pathogenesis, while CASP8 was linked to inflammatory signaling. This study identifies novel RA biomarkers, offering potential targets for early diagnosis and therapeutic strategies. Further validation in larger cohorts is necessary to confirm their clinical significance.

P-23-008
Comparative validation of a home-made ELISA and mass spectrometry method for the quantitation of histones in plasma from PICS patients: accuracy and applicability

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According to the Third International Consensus Definitions for Sepsis and Septic Shock (Sepsis-3), sepsis is defined as "life-threatening organ dysfunction caused by a dysregulated host response to infection". Sepsis is a major public health issue and one of the leading causes of death among critically ill patients in intensive care units. Additionally, it is a significant contributor to morbidity and mortality worldwide. However, in patients who survive severe cases of sepsis, certain associated complications persist. This progression to a chronic state is known as persistent inflammation, immunosuppression, and catabolism syndrome (PICS), leading to organ failure, inflammation and a high risk of new infections due to prolonged immunosuppression, among others. Timely initiation of treatment and early diagnosis improve the efficiency of patient management, reduce the length of hospital and intensive care unit stays and increase the likelihood of patient survival. To facilitate this, biomarkers are being sought to aid the prognosis of sepsis. After developing a diagnostic and prognostic method for sepsis through the quantification of extracellular histones in the bloodstream using mass spectrometry, we sought to use the same method to stratify patients likely to develop PICS after sepsis. The analysis of plasma was also performed using a nucleosome-specific home-made ELISA kit using a specific antibody recognizing human nucleosomes. The results obtained show a high concordance between the ELISA kit and mass spectrometry, indicating that both methods are suitable for the quantification of the studied analyte. The ELISA kit has the advantage of being a validated method, easy to implement and with reduced analysis times. Instead, mass spectrometry has the advantage of higher specificity and precision, making it a valuable tool for applications requiring a high degree of accuracy. *The authors marked with an asterisk equally contributed to the work.

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P-23-009
Protocol for enrichment of junctional sarcoplasmic reticulum vesicles from mouse heart for mass spectrometry analysis

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The junctional sarcoplasmic reticulum (jSR) is a specialized subdomain of the sarcoplasmic reticulum in cardiomyocytes, playing a pivotal role in calcium homeostasis and excitation-contraction coupling (ECC). Its function is tightly regulated by a network of membrane and luminal proteins, including the cardiac ryanodine receptor (RyR2), calsequestrin (CASQ2), junctin (JNT), and triadin (TRDN), whose quantitative characterization is essential for understanding cardiac physiology and disease mechanisms. However, due to its dynamic nature and strong membrane association, the isolation of intact jSR vesicles enriched in these proteins remains a major technical challenge. Here, we present an optimized protocol for the reproducible enrichment of jSR vesicles from a single murine heart, enabling the isolation of low-abundance membrane proteins while preserving their native interactions. By leveraging this strategy, we achieve a comprehensive and qualitative proteomic characterization of the jSR using high-resolution mass spectrometry (Dionex Ultimate 3000 nano-LC system connected to an Orbitrap Exploris™ 240 Mass Spectrometer). This approach overcomes previous limitations in detecting challenging jSR components and provides unprecedented insights into the molecular organization of the jSR. Among ECC-related cardiac disorders, catecholaminergic polymorphic ventricular tachycardia (CPVT) is a life-threatening arrhythmogenic disease characterized by adrenergically induced ventricular tachycardia, leading to syncope and sudden cardiac death. CPVT is primarily caused by mutations in key calcium-handling proteins, such as RyR2 (CPVT1) and CASQ2 (CPVT2), which disrupt intracellular calcium dynamics and impair ECC. A detailed characterization of the jSR proteome is crucial for understanding the molecular mechanisms underlying CPVT and related disorders, providing a foundation for future therapeutic strategies targeting jSR dysfunction.

P-23-010
Combining proximity labeling and SILAC based proteomic approaches to identify proteins at the interface of homotypic and heterotypic cancer cell interactions

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Cell–cell interactions are crucial for the growth of organisms and maintaining homeostasis. In the tumor microenvironment, different types of cell interactions promote cancer progression. Given their significance in both healthy and diseased states, we developed a method that integrates proximity labelling with quantitative proteomics to profile the cell-cell interactome using co-culture system. This study combined horseradish peroxidase (HRP)-dependent proximity biotinylation with stable isotope labeling by amino acids in cell culture (SILAC) to identify proteins involved in homotypic and heterotypic cancer cell interactions. To model these interactions, we co-cultured epithelial (MCF7) and mesenchymal (MDA-MB-231) breast cancer cells. HRP was targeted to the extracellular leaflet of the plasma membrane in 'bait' cells, facilitating the labeling of proximal proteins in 'prey' cells. Prey cells were metabolically labeled with heavy isotopes to distinguish their proteins from self-biotinylated bait cell proteins. This approach enabled the mapping of both homotypic and heterotypic interactomes of epithelial and mesenchymal breast cancer cells. The enrichment of cell surface and extracellular proteins validated the specificity of our methodology. Additionally, selected hits from different cell–cell interactomes were verified through microscopy in co-cultures. Our findings identified signaling pathways governing homotypic and heterotypic interactions of epithelial and mesenchymal cells and highlighted the importance of exosomes in these interactions. The efficient proximity biotinylation in spheroids suggested that the methodology can be adapted for 3D co-culture models. Overall, this study presents a new pipeline of experimental and bioinformatic strategies to comprehensively examine the proteome of diverse cell-cell interactions.

P-23-011
A comparison of peptide- and protein-based calibration strategies for LC-MS/MS quantification of cardiac troponin I

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The selection of calibrators and isotopically labelled internal standards plays a critical role in ensuring the reliability and accuracy of LC-MS-based protein quantification. This study presents a comparative evaluation of two calibration approaches – protein-based and peptide-based – within an isotope dilution LC-MS/MS (ID-LC-MS/MS) framework for the quantification of cardiac troponin I (cTnI), a low-abundance protein in human serum. A peptide-based calibration strategy was developed and evaluated, and its performance was compared with a previously established protein-based calibration method. The peptide-based calibration approach utilized SI-traceable synthetic peptides as primary calibrants with isotopically labeled peptide standards, while the protein-based method relied on recombinant cTnI and isotopically labeled protein standards. Immunoaffinity enrichment was employed to isolate cTnI from serum, followed by targeted quantification using parallel reaction monitoring. The analytical performance of the peptide-based calibration method was assessed in terms of selectivity, specificity, precision, accuracy, and recovery, and its results were compared with those obtained using the protein-based approach. While both strategies demonstrated similar limits of quantification (LOQ), the peptide-based method introduced a quantitative bias in recovery rates, indicating potential limitations in its application for low-abundance protein quantification. Measurement uncertainty and

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calibration strategy selection were also evaluated. These findings contribute to the development of metrologically robust methodologies for biomarker quantification and support the advancement of SI-traceable peptide or protein primary calibrators. Previously published in: Asicioglu M et al. (2025) Clin Chem Lab Med, 1–15. & Asicioglu M et al. (2023) Clin Proteom 20, 40.

P-23-012
Quantification of glial fibrillary acidic protein in serum using ID-LC-MS/MS

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Glial fibrillary acidic protein (GFAP) is an emerging biomarker involved in various pathological conditions, including traumatic brain injuries and neurodegenerative disorders such as dementia. This study aims to quantify GFAP levels in human serum using an isotope dilution liquid chromatography-tandem mass spectrometry (ID-LC-MS/MS) approach. Both natural and isotopically labeled recombinant GFAP proteins were produced in-house, with SI-traceable value assignment performed through amino acid analysis. The natural GFAP protein was used as a calibrant for the calibration curve, while isotopically labeled GFAP served as an internal standard. An antibody specific to GFAP was immobilized onto magnetic dextran nanoparticles and GFAP was selectively isolated from human serum using immunoaffinity enrichment. Following to the trypsin digestion, three GFAP-specific signature peptides (LEAENNLAAYR, ALAAELNQLR, and LADVYQAELR) were subsequently monitored via parallel reaction monitoring mass spectrometry (PRM-MS). The developed method successfully quantified GFAP in human serum. Further optimization will extend the detection range to 0.3–54 ng/mL, followed by comprehensive validation. These advancements will contribute to the early-phase standardization of GFAP measurement, supporting its integration into clinical practice.

P-23-013
Expression, purification, and characterization of recombinant glial fibrillary acidic protein (GFAP) for ID-LC-MS/MS quantification in cerebrospinal fluid

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Glial fibrillary acidic protein (GFAP) is a key intermediate filament protein of astrocytes and has gained significant attention as a potential biomarker for neurodegenerative diseases and traumatic brain or spinal cord injuries. Cerebrospinal fluid (CSF) analysis plays a crucial role in the diagnosis, prognosis, and management of various neurological disorders. The aim of this study was to develop a highly purified recombinant GFAP protein to be used as a calibrant for isotope dilution liquid chromatography-tandem mass spectrometry (ID-LC-MS/MS), a gold-standard technique for absolute protein quantification. Recombinant GFAP was expressed in *Escherichia coli* BL21 (DE3) using a pET32a expression system. Following sonication-based lysis, the protein was purified via immobilized metal affinity chromatography (IMAC) using a HiTrap Talon Crude column. To ensure its suitability as a calibrant, an optimized expression and purification strategy was employed to achieve the highest possible purity. Optimization of expression and purification conditions enabled the production of 2 mg of highly purified GFAP from a 1-liter bacterial culture, ensuring its suitability as a calibrant. The purified GFAP protein was further characterized by bottom-up proteomics, confirming its sequence integrity. The development of a robust ID-LC-MS/MS method for GFAP quantification in CSF is crucial for its clinical application as a diagnostic biomarker. The availability of a highly pure recombinant GFAP calibrant is expected to enhance assay precision, improve inter-laboratory reproducibility, and facilitate the standardization of GFAP measurements in clinical and research settings.

P-23-014
Beyond the internal cargo: examining the surface proteome and immunopeptidome of exosomes

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Exosomes (exos) are nanometer-sized extracellular vesicles secreted by diverse cell types and playing vital roles in intercellular communication. Although exos have primarily been studied as vehicles for molecular cargo, questions remain regarding the composition and functional significance of proteins on their surface, as well as the repertoire of peptides presented by their human leukocyte antigen (HLA) molecules. In this study, we investigated how an inflammatory microenvironment influences the exosomal protein corona and sought to characterize the HLA class I immunopeptidome of exos. Exos were isolated from different cell lines and either incubated with an inflammatory stimulus for proteomic analysis of the corona or subjected to immunoprecipitation of HLA/peptide complexes for immunopeptidomic analysis. Proteomic profiling involved sample pre-digestion, filtration, and liquid chromatography-tandem mass spectrometry (LC-MS/MS) analysis, revealing that inflammation-related proteins became associated with exos, potentially enhancing their capacity to propagate inflammatory signals. In parallel, comparative analysis showed that exos substantially mirrored the cellular immunopeptidome, displaying canonical HLA-I ligand lengths and high predicted binding affinities, while also featuring subsets of unique peptides not detected in parental cells. Collectively, our results suggest that exos, beyond functioning as simple cargo carriers, may act as specialized vehicles for disseminating immune-related signals, especially under inflammatory conditions. These findings open new avenues for exploring exos-based therapeutics, especially in

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cancer and chronic inflammatory disorders, where exos have emerged as central players.

P-23-015
Bone-enhancing effects of equisetum: from traditional use to scientific validation

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Equisetum species have been used in traditional medicine for various purposes such as wound healing, kidney stone reduction, and healing of bone fractures. There are no studies to clarify the effects of different *Equisetum* species on bone metabolism and structure. In this study, we aimed to investigate the effects of different *Equisetum* species on bone cell proliferation and molecular changes in bone tissue, bone turnover biomarkers, and vitamin D metabolism using *in vivo* models. According to the first part of the study, the most effective species [*E. ramosissimum* (ER) and *E. telmateia* (ET)] on bone cell proliferation were determined *in vitro*, and then the molecular effects of these species on rat bone tissues (femur and tibia) were examined *in vivo* by attenuated total reflectance-Fourier transform infrared (ATR-FTIR) spectroscopy. Moreover, biomarkers of bone turnover were determined by ELISA and tissue levels of enzymes involved in vitamin D metabolism were examined by the western blot technique. When the molecular changes in bone structure were analyzed, it was observed that ER plant extract strengthened both the mineral and collagen content of femur and tibia bone tissues, while ET plant extract had a significant effect on the mineral phase in only femur tissues. It was observed that both *Equisetum* species significantly decreased bone resorption biomarker levels implying a decrease in the rate of bone resorption. Moreover, both ER and ET plant extracts decreased the CYP24A1 enzyme levels that inactivate vitamin D in the kidney. In addition, we observed that 25(OH)D production, which is the first step of vitamin D activation in the liver, accelerated by increasing the protein amounts of the enzymes that perform this reaction. Considering the effects on bone molecular structure, bone turnover biomarkers, and vitamin D metabolism, it was found that *E. ramosissmum* was the most active plant species for bone development by showing its effects mostly at high doses.

LB-R-23-01
Unravelling the TR3-56 cell proteome in healthy and T1D pediatric patients by tandem mass tag-based high-resolution mass spectrometry

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Type 1 diabetes (T1D) is an autoimmune disease characterized by the destruction of pancreatic β cells by autoreactive T lymphocytes, with $CD8^+$ T cells playing a central role in this process. Although the pathogenic mechanisms underlying T1D onset have been extensively investigated, the complex interplay between the various immune cell populations has not been completely understood. Recently, TR3-56 cells have been proposed as a novel regulatory immune population involved in T1D pathogenesis. Published data revealed that while in healthy subjects TR3-56 cells suppress the function of T cell receptor (TCR)-activated $CD8^+$ T lymphocytes, in recent-onset T1D children TR3-56 cells are defective in number and suppressive capability [1]. The biological role of these cells has been explored, but studies addressing the comprehensive molecular asset under physiological and pathological conditions of this novel regulatory T cell subset are still missing. Using an advanced quantitative proteomic approach based on tandem mass tag (TMT) isobaric labelling and nano-liquid chromatography coupled with high-resolution mass spectrometry (nanoLC MS/MS), we investigated the proteomic signature of TR3-56 cells in both healthy and recent-onset T1D children. Our findings reveal that proteins related to cytotoxic functions and activation pathways are differentially modulated in recent-onset T1D children TR3-56 cells compared to healthy individuals. Specifically, TR3-56 cells in pediatric T1D patients exhibit a molecular program more characteristic of cytotoxic effector cells, than that of immunoregulatory subsets. Understanding the mechanisms driving this phenotypic alteration also during disease progression may provide new insights into T1D pathogenesis. Reference: 1. Terrazzano G et al. (2020) Nat Metab 2(2), 142-152.

LB-R-23-02
Untargeted proteomic analysis reveals an oxidative stress-response pattern in radiotherapy-treated glioblastoma cells

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Glioblastoma multiforme (GBM) is a primary brain tumor with limited therapeutic options. Conventional Radiotherapy (CONV-

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RT) remains a cornerstone treatment but its molecular consequences are not fully elucidated. This study investigates the impact of CONV-RT on GMB cells focusing on oxidative stress, mitochondrial function and proteomic alterations, to elucidate the mechanisms underlying radiation-induced damage. GBM cell lines U87 were exposed to 15 Gy dose of CONV-RT; 0 Gy cells were used as controls. Cell viability was assessed using Annexin V/PI staining. Intracellular reactive oxygen species (ROS) levels were measured using the DCF-DA and mitochondrial membrane potential (MMP) alterations were evaluated via DiOC2(3). Untargeted proteomic profiling was performed using LC-MS/MS, followed by ingenuity pathway analysis (IPA). Irradiated U87 cells exhibited reduced viability and increased apoptosis coupled with a concomitant rise in intracellular ROS levels, at 24 hours post-irradiation. Irradiation induced mitochondrial hyperpolarization, suggesting early stress responses linked to apoptosis. Proteins singly expressed in irradiated cells were associated with oxidative stress (GLRX), translational regulation (EIF3H, RPL31), and cytoskeletal reorganization. In contrast, control-specific proteins were primarily involved in DNA replication and cell cycle regulation (MCM2, MCM4, TOP1, RPA1), with enrichment in G2/M checkpoint pathways. IPA showed a significant impact on cell cycle checkpoints and autophagic processes involving misfolded protein aggregates (Aggrephagy). 15 Gy dose of CONV-RT induces oxidative stress, MMP changes, and proteomic remodeling in GBM cells. These data suggest that irradiation impairs cell cycle progression and activates adaptive stress response pathways, enhancing a deeper understanding of the molecular impact of radiotherapy in GBM to support future improvement of treatment efficacy.

AI in molecular life sciences

P-24-001
Deep learning-based image analysis for cell determination from microscopic images via biochemical properties

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Immunohistochemical examinations via biochemical biomarkers are challenging factors for the diagnosis of diseases. The specific and selective binding to the target biomolecule is possible, however, assessment of this binding needs a professional person and time. Although precision maybe depends on the fluorescent labels that interacted with target biomolecules, a combination of the multiple parameters can be missed. Therefore, more accurate techniques are needed to assess microscopic images. Thanks to the advancing AI based techniques such as deep learning (DL) convolutional neural networks (CNN), DL can be trained to analyze those pictures with lower error margins. This study introduces a novel approach to biochemical marker based immunobiological testing using colorimetric analysis of microscopy images, enhanced by YOLOv8 (You Only Look Once) object detection algorithm. This colorimetric detection leverages smartphone imaging with no need for any professional person with histochemistry experience, making it both accessible and scalable. Initial experiments demonstrate that YOLOv8's precision and efficiency, when combined with synthetic data, significantly enhance the system's ability to recognize and analyze colorimetric signals, positioning it as a promising tool for diagnostics in liver diseases. The study can recognize the genetic and proteomic labeling and processed with deep learning algorithms to determination of liver diseases. Even if the limited number of images, our trained YOLOv8 model achieved an accuracy of 0.84, highlighting its potential to improve the reliability of colorimetric analysis without a professional.

P-24-002
Predicting critically short telomeres and DNA methylation status from MARK-AGE data

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Specific genomic markers are among the most important hallmarks of ageing, as previously identified within the European study of biomarkers of human ageing - MARK-AGE. However, research aiming at predicting such outcomes is scarce. The aim of the current study was to develop and validate machine learning models for estimating relevant DNA damage and gene methylation markers from MARK-AGE data. Several algorithms based on regression (both boosting and classical models) were built in Python Programming Language on randomly recruited age- and sex-stratified individuals from the general population (RASIG). The three continuous outcomes were the percentage (%) of telomeres shorter than 3 kb (critically short telomeres, CST) (2012 subjects), the cumulative DNA methylation status of 13-15 CpG islands in FHL2 gene and the cumulative DNA methylation status of 11-14 CpG islands in ELOVL2 gene (2287 subjects). The validation strategy implied automatically selecting the predictive variables and implementing a repeated 10-fold cross-validation with hyperparameter tuning. For % of CST, the best results were obtained for 20 predictive variables with a 1:1 voting regressor combining CatBoost and Random Forest (RF) (a mean absolute error (MAE) of 9.243%), while for the DNA methylation of FHL2 and ELOVL2 the optimum results were obtained for 15 variables with CatBoost (a MAE of 3.584% for FHL2) or CatBoost and RF voting (a MAE of 3.709% for ELOVL2); in all cases, the MAE was significantly lower than the range of outcome values. To our knowledge, the current study is the first to implement machine learning prediction of CST and gene methylation markers based on simple clinical and blood parameters and lifestyle factors, such as systolic blood pressure, LDL oxidation, nutritional profile and fitness levels. Future studies must aim at optimizing the models and developing personalized genomic markers estimation platforms for use by clinicians, researchers and the general population.

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P-24-003
Iterative modeling via structural diffusion (IMSD): algorithm for prediction of metamorphic proteins fold-switching pathways using AlphaFold2-based generative diffusion model UFConf

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Metamorphic proteins (MPs), which are also called fold-switching proteins, can fold into two or more distinct spatial structures. Increasing interest in MPs has spurred search for computational tools to predict proteins fold-switching potential and model their refolding pathways. In this work, we address this problem by using the recently reported generative diffusion predictor UFConf, based on the specially adapted AlphaFold2 network. We have developed a new UFConf-driven algorithm dubbed IMSD (iterative modeling via structural diffusion) to trace the MP's path from one conformational state to another. In brief, we begin with the experimental structure of state A, perturb this structure through the "noising" process, and then infer a number of models (replicas) through the reverse diffusion or "denoising" process. From this set of models, we choose the one that is closest to the alternative-state structure B. Then we use this particular model (termed iteration minimum, or IM) as a starting point, perform noising-denoising routine and thus generate the next batch of models, from which we again select the one that is closest to state B. Repeating this process in an iterative fashion, we have been able to map the entire path from state A to state B for several metamorphic proteins, including GA98, SA1 and C-terminal domain of RfaH. Comparison of IMSD predictions with experimental data available in the literature suggests that UFConf-based IMSD scheme offers a sound and reasonable model of fold-switching transition in these metamorphic proteins. We believe that due to their mastery of protein architecture and high computational efficiency deep learning algorithms can greatly contribute not only to the field of protein structure, but also to the field of protein folding. This work was supported by SPbU grant 127400313.

P-24-004
SymProFold: predicting symmetrical biological assemblies and uncovering novel structural features

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Symmetry in nature often arises through self-assembly processes and serves diverse biological functions. Surface layers (S-layers) form symmetrical lattices on bacterial and archaeal cells, contributing to adhesion, immune evasion, and protection against environmental stress. However, their structural characterization remains challenging due to 2D self-assembly dynamics and high sequence variability. Here we present SymProFold (1,2), a computational pipeline that leverages the accuracy of AlphaFold-Multimer to predict symmetrical protein assemblies, focusing on two-dimensional S-layer arrays and spherical viral capsids. The pipeline begins by providing the sequence, dividing the proteins into domains, systematically testing all relevant symmetry operations (p1, p2, p3, p4, p6), and identifying the most probable symmetries for each assembly. This is followed by applying translation by superposition of different symmetry complexes and generating the final assembly. More than 20 S-layer protein assembly predictions are reported, where no atomic resolution data were available to date. Predicted models were validated against available experimental data at the cellular level, with additional crystal structures confirming symmetry and intermolecular interfaces in select cases. This led to the discovery and further experimental exploration of a novel anchoring mechanism in Archaea, demonstrating the power of SymProFold in revealing unique structural features. In conclusion, SymProFold facilitates the structural determination of symmetric protein assemblies with key biological functions, opening avenues for applications in nanotechnology, biotechnology, medicine and materials science. (1) C. Buhlheller, T. Sagmeister, C. Grininger, N. Gubensäk, U.B. Sleytr, I. Usón, T. Pavkov-Keller*. SymProFold: Structural prediction of symmetrical biological assemblies. Nat Commun, 2024, 15, 8152. DOI: https://doi.org/10.1038/s41467-024-52138-3 (2) https://github.com/symprofold. *The authors marked with an asterisk equally contributed to the work.

P-24-005
Structural characterization and machine learning based prediction of PD-1/PD-L1 inhibitors using ChEMBL bioactivity data

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The inhibition of the PD-1/PD-L1 axis is a critical target in cancer immunotherapy, necessitating a deeper understanding of the structural features associated with bioactivity. In this study, 1112 assay records for 849 compounds from the ChEMBL bioactivity database were analyzed. Molecular fingerprints were generated from SMILES representations using RDKit, and Principal Component Analysis (PCA) was applied for dimensionality reduction. Hierarchical clustering (HCL) was employed to group compounds based on structural similarities, and scaffold analysis using Bemis-Murcko frameworks was integrated with biological activity data (pChEMBL values) to identify key structural motifs associated with inhibitory activity. A total of 493 active compounds (IC_{50}) were identified, of which 232 were orally active. HCL analysis revealed 83 clusters, indicating limited common structural features among the compounds. The most frequently observed Murcko fragments associated with PD-1/PD-L1 inhibition were 1-(phenoxy)-3-phenylbenzene and its derivatives. Among the machine learning models applied, the RandomForestClassifier achieved the highest performance in classifying active inhibitors, with an accuracy of 0.81. These findings provide critical insights into the structural

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determinants of PD-1/PD-L1 inhibition, emphasizing the role of specific Murcko fragments and demonstrating the utility of machine learning in predicting bioactivity. This work supports the rational design of more effective immunotherapeutic agents targeting the PD-1/PD-L1 axis.

LB-R-24-01
Machine learning-based microRNA expression analysis to discover new targets for vascular calcification diagnostics

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Vascular calcification is a common age-related disease which plays a significant role in the development of adverse cardiovascular events. Unfortunately, current diagnostic approaches struggle to identify the early stages of vascular calcification development in a cost-effective and non-invasive way. The aim of this study was to bridge this gap by developing models capable of predicting vascular calcification based on microRNA expression in tissue and whole blood microRNA expression. We utilized the GTEx database data, including small RNA sequencing data (called Small RNA-seq) and histology data. The data included 184 tissue samples (120 samples of patients with calcinosis and 64 from patients without it labeled "clean specimens" in the database) and 155 whole blood samples. In our analysis, we used histology samples of different tissues: Artery–Aorta, Artery – Coronary, Artery – Tibial. To develop our model, we utilized a two-stage feature selection process and trained three classifiers: SVC, CatBoostClassifier and LGBMClassifier. The SVC outperformed other models, demonstrating the best performance in predicting calcification based on arterial tissue miRNA data achieving an accuracy: 0.870 and F1-score: 0.906. For predicting arterial calcification using blood miRNA data, the CatBoostClassifier model achieved superior results with an accuracy: 0.795 F1-score: 0.826. For prediction based on tissue microRNa data 21 microRNA were selected (hsa-miR-374a-3p, hsa-miR-551b-3p, etc.), whereas for prediction based on blood microRNA 13 microRNA and age (hsa-Mir-3140-3p, hsa-miR-432-5p, etc.) were selected. Given the high prevalence of calcinosis ($\geq 50\% +$) in risk groups (those above 40 years old, those with CKD, diabetics, e.g.), the identified potential markers could serve as a basis for screening in the early stages of calcification. Our results can also be used to develop future drugs targeting microRNA expression in tissues. *The authors marked with an asterisk equally contributed to the work.

LB-R-24-02
Artificial intelligence in oncology: exploring machine learning and deep learning approaches for breast cancer prediction

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Breast cancer is a significant health concern, being the leading cause of cancer among women in Morocco. With the increasing incidence, it is crucial to better understand patient profiles to optimize diagnostic and therapeutic strategies. Although early screening is essential, it requires considerable resources. The integration of artificial intelligence (AI), particularly machine learning and deep learning, enables the analysis of large datasets and the creation of predictive models, accelerating cancer detection and improving treatment accuracy, especially through medical image analysis. For instance, the use of lightweight convolutional neural networks (LWCNN) to analyze mammograms from datasets like Kaggle and BreakHis achieved detection accuracy rates of 93% to 99% for breast cancer. These technologies offer more precise and personalized patient monitoring, although their adoption is hindered by limited access to clinical data and insufficient training for healthcare professionals. AI shows great potential in oncology, as evidenced by the analysis of clinical and histological data from 1794 patients. Among eight tested algorithms, the Random Forest model achieved the highest accuracy of 97.7%. Furthermore, to predict metastasis in 509 patients, the AdaBoost algorithm demonstrated optimal performance with an AUC of 78%, followed by XGBoost with an AUC of 75%. These results highlight the importance of AI in better understanding patient characteristics and predicting the progression of breast cancer. The integration of predictive models could enhance clinical management and hospital care organization. In the future, incorporating genomic, biological, and real-time data could further improve predictive model accuracy, allowing for better anticipation of phenomena such as treatment resistance and more personalized treatment cycles.

Computational biology

P-25-001
Performance comparison of the machine learning algorithms in relation with the differential expression analysis in cancer transcriptomics data

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Metastasis accounts for the majority of cancer deaths, and understanding its genetic mechanisms are essential for the development of effective treatments. Moreover, the tumor microenvironment, especially T-cell infiltration, is one of the most important drivers of tumor progression and immune response effectiveness. Here, we used several machine learning (ML) approaches to find gene expression signatures discriminating metastatic and primary melanoma and revealing genes linked with T-cell infiltration. We systematically compared the predictive potential of various ML classifiers, including random forest, extreme gradient boosting, artificial neural

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networks, support vector machines, and regularized generalized linear models, on transcriptomic profiles of publicly available TCGA melanoma data. Feature selection was performed to identify the most discriminative genes governing classification performance, and the biological relevance of the selected genes was assessed using functional enrichment analysis. The performance of the models was assessed with a range of metrics, such as accuracy, sensitivity, specificity, and area under the receiver operating characteristic curve (AUC-ROC), the latter providing an overall measure of classification performance. We also compared ML-derived gene signatures with differentially expressed genes detected by DESeq2, a popular statistical approach to RNA sequencing data analysis. Concordance with DESeq2 results was explored for ML-selected genes, with genes being reproducibly associated with metastatic potential and immune infiltration across approaches. While these ML approaches are widespread in cancer genomics, comparative investigations for metastasis and immune infiltration scenarios are sparse. Our findings offer a systematic evaluation of ML-based classification in melanoma, revealing novel insights into molecular drivers of tumor progression and immunity. *The authors marked with an asterisk equally contributed to the work.

P-25-002
Genome-wide discovery and characterization of intronic RNAs in human

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Transcriptional activity in intronic regions has often been overlooked in RNAseq analyses, particularly with short-read data. While long-read sequencing advancements have facilitated the discovery of novel lincRNAs and transcript variants, efforts to specifically uncover independent transcription units within introns remain limited. To address this gap, we developed a Nextflow pipeline to identify previously unnoticed intronic RNAs from short-read RNAseq data. Our method combines a k-mer-based strategy with direct read alignments to the reference genome, utilizing a k-mer abundance matrix independent of the genome reference. This matrix is processed into contigs through the KaMRaT tool and aligned to the reference genome to filter intronic regions. Strand-specific alignment data is used to eliminate intron retention artifacts and alternative splicing, focusing on independent transcription units. We applied this pipeline to public datasets, using unitigs from the Logan project, analyzing five cancer cell lines with high-quality (paired-end, ≥30M reads per replicate, ≥triplicates) RNAseq data, including polyA+ and ribosomal RNA-depleted libraries, and conducted comparative analyses of their polyA tail abundance, position within genes and introns, sequence motifs, and conservations across 100 vertebrates. Despite the expansion of the GENCODE catalog to over 385 000 transcripts in version 47, no new intronic transcript annotations have been reported, highlighting the significance of our study to uncover hidden intronic transcripts that have gone unnoticed by current annotations. Our findings demonstrate the potential for mining existing short-read datasets to uncover novel, independent lncRNAs within intronic regions, advancing our understanding of transcriptional complexity and gene discovery in unexplored genomic territories.

P-25-003
In silico analysis of triamterene as a potential dual inhibitor of VEGFR-2 and c-Met receptors

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The vascular endothelial growth factor receptor 2 (VEGFR2) and the hepatocyte growth factor receptor (C-Met) are critical receptors for signaling pathways controlling crucial cellular processes such as cell growth, angiogenesis and tissue regeneration. However, dysregulation of these proteins has been reported in different diseases, particularly cancer, where these proteins promote tumour growth, invasiveness, metastasis and resistance to conventional therapies. The identification of dual inhibitors targeting both VEGFR-2 and c-Met has emerged as a strategic therapeutic approach to overcome the limitations and resistance mechanisms associated with single-target therapies in clinical settings. Through molecular dynamics simulations and comparative docking analysis, we tested the inhibitory potential of 2016 Food and Drug Administration (FDA)-approved drugs targeting VEGFR-2 and/or c-Met receptors. The results revealed that entacapone and telmisartan are potent and selective inhibitors for c-Met and VEGFR-2, respectively. Interestingly, triamterene was identified as a promising dual inhibitor, demonstrating specific and significant binding affinity to both proteins. Molecular dynamics simulations revealed key interactions between the identified compounds and critical residues in the catalytic domains of both VEGFR-2 (e.g., Lys868, Asp1028, Asp1046) and c-Met (e.g., Asp1204, His1202, Asp1222), providing insights into their mechanism of action. These findings underscore the therapeutic potential of triamterene in targeting multiple signaling pathways involved in cancer progression, metastasis and poor prognosis in patients. Our study provides a foundational framework for the development of novel anticancer compounds able to target multiple pathways in cancer. Further preclinical and clinical investigations are needed to validate the efficacy of these compounds in clinical settings and to test their ability to overcome resistance and improve patient outcome. *The authors marked with an asterisk equally contributed to the work.

P-25-004
Machine learning based analysis of hyperspectral images of diatoms

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Diatoms are one of the ecologically most important groups of eukaryotic phytoplankton, contributing almost a quarter of the Earth's global primary production but accounting for only 1% of the Earth's photosynthetic mass. Diatoms contain specific fucoxanthin (Fx)-chlorophyll (Chl) proteins (FCPs) that belong to the photosynthetic light-harvesting complexes responsible for collecting solar energy for photochemical reactions. The spatial

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distribution of photosystems I and II (PSI, PSII) in the thylakoid membranes of diatoms favors the optimization of light capture. There is little data on the PS distribution in the membranes. Coherent anti-Stokes Raman (CARS) microscopy enables the collection of spectral data applicable for hyperspectral analysis of the diatom and imaging of various components of the photosynthetic machinery in the cell. CARS microscopy is molecular specific, rapid, and non-destructive, allowing Fx studies without damaging the cell. CARS can be easily combined with two-photon excitation fluorescence (TPEF) microscopy, so that the spectral dataset can be supplemented with TPEF images of the Chl distribution. In the present study, information on the spatial distribution of Fx and Chl was extracted from CARS/TPEF hyperspectral images by machine learning (ML) algorithms, aiming at the identification of segments in images connected by feature similarities. The application of ML techniques on the CARS/TPEF data enabled revealing the specificity of the distribution of carotenoids with respect to Chl in the thylakoid membranes of a diatom *Cyclotella meneghiniana*. The reconstruction of the spatial distribution of the photosynthetic machinery of diatoms at the whole-cell level can provide insight into the evolutionary success of diatoms' efficiency in light harvesting and conversion.

P-25-005
Aggregation-prone region mapping in olfactomedin domain of myocilin through classical and enhanced sampling molecular dynamics simulations

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Myocilin, a relatively recent addition to the amyloidogenic proteins, is implicated in primary open-angle glaucoma (POAG), a disease characterized by optic nerve damage. It features an N-terminal coiled-coil domain, a C-terminal olfactomedin (OLF) domain, and a linker region. Proteolytic cleavage of myocilin releases its OLF domain into the ECM, and mutations within it are strongly associated with POAG. Understanding the OLF domain's unfolding is crucial for developing therapeutic strategies against POAG. However, previous studies employing molecular dynamics (MD) simulations struggle to capture relevant misfolded conformations even in long timescales. This study aimed to overcome this limitation by employing various enhanced sampling techniques, including Gaussian accelerated MD (GaMD) with varying boost energies, simulations with mutations, chemical denaturants (urea), and increased kinetic energy (temperature). Fifteen simulations, each lasting at least 1 microsecond, were performed. Our key findings revealed that (i) WT and I499F, a severe POAG mutant, OLF domains exhibited highly rigid backbones; (ii) urea solvation and accelerated simulations produced limited structural changes, while elevated temperatures significantly increased fluctuations and drove unfolding; (iii) increased temperature primarily affected the blade interfaces, particularly blades A and E, rather than internal blade structures; (4) the disulfide bond between C245-C433 had a stabilizing effect on the overall OLF fold. Utilizing two structure-based aggregation propensity prediction tools, we have identified seven aggregation-prone regions (APRs) through high-temperature and accelerated simulations. Three of these seven APRs were previously identified through bioinformatics analysis. Overall, we reported that elevated temperatures and enhanced sampling techniques proved effective in unraveling the APRs in OLF, whereas urea solvation and I499F mutation did not yield unfolding-related dynamics.

P-25-006
Design of proteins by parallel tempering in the sequence space

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Protein design is a new strategy to obtain interesting functional proteins, in addition to protein prospecting and protein engineering. Many protein design programs sample different sequences and score them by an energy score to find a sequence with the best potential to fold into a compact protein with predefined properties. General optimization algorithms such as Monte Carlo simulated annealing have been used. Here we show an application of the parallel tempering algorithm. Initially, a series of random amino acid sequences was generated. They were then sampled in parallel replicas, each at different temperatures. Occasionally, pairs of replicas swapped their sequences based on a predefined criterion. By this method, we successfully designed a series of 100- or 200-residue proteins and computationally verified them using ESMfold and Alphafold. Both, helical and β-sheet rich proteins were designed. The method is especially suitable for applications requiring a continuous flow of designed proteins. In future, we plan to extend our method for better exploration of protein designs and to enforce design of proteins with desired properties, in order to design new protein scaffold and binding proteins.

P-25-007
An *in silico* pipeline to identify small molecule inhibitors of the mitochondrial fission protein Fis1

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Mitochondrial fission, i.e. the division of the organelle into two daughter organelles, depends on the cytosolic protein dynamin-related protein 1 (DRP1). DRP1 binds to its adaptors on the outer mitochondrial membrane including Fission 1 (FIS1) which also participates in mitophagy and contact sites with lysosomes by interacting with TBC1D15, a Rab-GTPase domain protein. Fis1 modulates leukemic stem cells differentiation in acute myeloid leukemia (AML), calling for the identification of small molecules that modulate Fis1 function with potential applications in AML. To this end, we established a computational pipeline mimicking the recognition pattern of a peptide (8U1Z PDB) that engages on the concave surface of Fis1, formed by its two tetratricopeptide-like motifs (TRP). After filtering a virtual library of 6 million

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commercially available small molecules in Oprea Lead Like, we virtually screened 2 million compounds in Plants 1.2 coupled to chemPLP scoring function, generating 20 million conformations. By using the Molecular Operating Environment (MOE) suite we filtered out compounds with unfavorable poses, leading to 500 candidates with promising binding affinities in Plants PLP. These compounds where further subjected to molecular dynamics simulation to assess the pose stability and rescored by MM-GBSA procedure (three replicates of 10 ns for each compound; total simulated time 15 μs). The most promising compounds resulted from our computation pipeline, including triazines, amides, and haloacetic acids, had chemPLP docking scores ranging from -131 to -123, favorable MM-GBSA, good pose stability (ligand RMSD, RMSF), suggesting strong binding affinities and favorable interactions with their target protein, Fis1. We will present the preliminary characterization of the most promising Fis1 inhibitors that may offer potential for leukemia treatment.

P-25-008
Potential allosteric sites investigated in hexokinase of *Plasmodium vivax* and humans, to identify possible species-specific drugs

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Development of species-specific drugs to treat diseases caused by antimalarial-resistant parasites infecting millions of people, has found emphasis in literature. Since the glycolytic pathway is the cell's most essential energy source, enzymes in this pathway are potential drug targets. However, the proposed drug candidates should not inhibit the same enzymes in human species. Accordingly, allosteric inhibitors are an alternative as they target specific areas in the enzymes belonging to infecting species. Our project's focus is the identification and analysis of alternative potential and specific allosteric sites in hexokinase of *Plasmodium vivax*. In the first step of our computational approach, a serious of effective tools were used to identify potential binding sites on the surface of the enzyme. A total of twelve binding sites were identified and they were all located at the interface of subunits which makes them critical hot spots for allosteric regulation. In the second stage, the allosteric potential of each binding site was evaluated by various computational tools including elastic network modeling such as CorrSite, Ohm, AlloSigma, and ESSA, and the machine learning algorithm PASSER. Half of these binding sites were identified as having allosteric potentials. Finally, the evolutionary conservation of these allosteric sites was determined through sequence alignment between the parasite and human.

P-25-009
Analysis of gene expression to explore the contribution of cancer-associated fibroblasts to breast cancer progression: a computational analysis

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Cancer-associated fibroblasts (CAFs) are critical components of the tumor microenvironment and play a significant role in breast cancer progression. This study aims to identify potential pathways and genes associated with CAFs in breast cancer using *in silico* analysis. Gene expression data from breast cancer patients, specifically GSE119253, GSE29270, and GSE228582, were retrieved from the Gene Expression Omnibus (GEO) database. The workflow of this study consists of three main steps: (1) Differentially expressed gene (DEG) analysis of RNA-seq data from breast cancer patients, followed by Cytoscape Network Analysis on protein–protein interaction (PPI) enrichment data; (2) Gene Ontology (GO) analysis to investigate the biological significance of upregulated and downregulated genes; and (3) pathway enrichment analysis using the Kyoto Encyclopedia of Genes and Genomes (KEGG) database in RStudio performed using the clusterProfiler 3.8 package. In CAFs, 940 genes were upregulated, while 561 genes were downregulated. These genes were significantly linked to key pathways, including MAPK signaling, estrogen signaling, NF-κB signaling, IL-17 signaling, and TGF-β signaling. These pathways are known to be involved in various aspects of cancer progression, such as cell proliferation, survival, and immune response modulation, thereby highlighting the involvement of CAFs in breast cancer progression. Further, hub gene analysis identified ESR1, MET, IL1B, SMAD3, and FOS as key regulators, all of which are enriched in critical pathways and associated with anti-tumor drugs currently in clinical trials or approved for use. Our findings suggest that ESR1, MET, IL1B, SMAD3, and FOS are crucial mediators of CAF-induced breast cancer progression. Importantly, these key regulators also present potential therapeutic targets, offering hope and optimism for the development of new treatments in the fight against breast cancer.

P-25-010
Analysis of protein–protein interaction networks of actin-binding proteins

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Actin is one of the most abundant proteins in eukaryotic cells. It is present in all cell types but was first identified in muscle cells, where it participates in the contraction-relaxation system. To date, six actin isoforms have been identified: four belong to muscle α-actin, while the remaining two are non-muscle isoforms (β- and γ-actin). Despite their high structural similarity, actin isoforms are not functionally interchangeable [1]. All isoforms are involved in various intracellular processes through interactions with a large pool of actin-binding proteins (ABPs), with each isoform exhibiting a unique set of these proteins. The aim of this study is to analyze protein–protein interaction (PPI) networks of ABPs for all actin isoforms to identify potential protein complexes and compare their functional roles. Data on experimentally confirmed interactions for 1150 α-actin interactors, 1148 β-actin interactors, and 553 γ-actin interactors were obtained from open PPI databases. The interaction networks for all actin isoforms met the criteria of scale-free networks, enabling the application of social graph analysis methods and advanced enrichment analysis techniques. As a result, potential protein complexes determining the biological roles of actin isoforms were identified. These included complexes with similar functions as well as those responsible for functional differences between isoforms. Additionally, hub proteins, which play a key role in organizing connections within the PPI networks of all actin types, were identified. During the analysis,

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the Python-based package ProteinNetworks [2] was developed, enabling the replication of the analysis protocol for actin and other proteins. The work was supported by the Russian Science Foundation project No. 23-15-00494. References: 1) Otey CA et al. (1987) J Cellular Biochemistry 34, 113-124. 2) ProteinNetworks. GitHub repository. Available at: https://github.com/skewer33/ProteinNetworks (Accessed: 10.03.2025).

P-25-011
In silico drug repurposing to identify potential allosteric regulators of CD73: a promising target for PDAC

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Pancreatic ductal adenocarcinoma (PDAC) remains a lethal malignancy with no effective therapeutic strategy, necessitating urgent identification of novel molecular targets and the development of effective treatments. TCGA PDAC cohort, we found that the tumoral mRNA expression of the NT5E gene, which encodes CD73 (ecto-5-nucleotidase), was significantly higher in tumor tissues (n = 179) compared to non-tumor tissues (TCGA normal and GTEx normal; n = 171) ($p < 0.01$, -Log2FC- > 3). Subsequent analyses revealed that higher tumoral NT5E mRNA levels were associated with decreased overall survival (n = 177, HR = 1.53 (1.01-2.33), logrank p = 0.04) and relapse-free survival (n = 69, HR = 2.32 (0.98-5.98), logrank p = 0.04) in TCGA PDAC patients. The tumoral protein levels of CD73 were consistent with mRNA data, showing higher levels in the Clinical Proteomic Tumor Analysis Consortium (CPTAC) PDAC cohort (n = 137) compared to non-tumor tissues (n = 74) (p = 2.83e-08). These preliminary bioinformatics findings suggest that CD73 could serve as a potential molecular target for the treatment of PDAC. Utilizing computational approaches, including Essential Site Scanning Analysis (ESSA), FTMap, ClustENMD, and virtual screening (VS) of FDA-approved drugs, CD73, analyzed in both open and closed conformations, revealed potential allosteric sites at the dimer interface. VS identified four potential modulators (docking score range -11,6, -10,2 kcal/mol) for both conformations of CD73. These findings also conform to the blind docking analysis using CBDock2. Molecular docking and ClustENMD unbiased sampling simulations indicated that these compounds exhibit favorable interactions within the allosteric sites, suggesting promising allosteric modulation of CD73. Our study is one of the first suggestions for a binding site different from that of the substrate. Further MD simulations and experimental validation are crucial to confirm these findings and explore the therapeutic potential of these compounds in PDAC.

P-25-012
Optimal Gibbs blocks selection in biomolecular sampling: a bridge between probability and continuous mechanics

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Hamiltonian Monte Carlo coupled with Gibbs sampling (GCHMC) [published by Spiridon et al. (2020), Biochim. Biophys. Acta Gen. Subj., 1864(8)] proved to be an efficient sampling method for high-dimensional and highly multimodal probability distributions specific to biomolecular free energy surfaces. Still, its greatest challenge lies in optimal block selection, due to the deep, and often unintuitive, interplay between the molecular degrees of freedom. This work aims to find the optimal clustering methods that enhance blocked Gibbs sampling efficiency in chemical systems. To this end, we performed GCHMC simulations on a diverse dataset of NMR-resolved protein structures and analyzed correlations in internal coordinates. Our findings show that dihedral angles fall into two categories: (1) a single block of largely uncorrelated dihedrals, primarily χ angles, and (2) multiple blocks with high intra-block correlation. Backbone phi and psi angles generally form continuous, well-defined clusters, however, there are also highly correlated clusters of dihedral angles from spatially distant regions, reflecting global protein mobility rather than local flexibility. In contrast, χ angles show minimal correlation with each other or with backbone conformations, with only a few exhibiting dependencies on the latter. To bridge probabilistic sampling with molecular mechanics, we introduce a framework that interprets block choices through continuum mechanics principles, employing strain-stress analysis to rationalize dihedral correlations. Although Gibbs sampling blocks are purely probabilistic constructs, their underlying force-driven nature explains the observed dependencies in molecular flexibility. This interdisciplinary approach offers a physically grounded and computationally efficient strategy for optimizing biomolecular sampling.

P-25-013
Unrevealing the interaction sites of neuropeptide Y5 receptor/G-protein coupling and elucidating the intrinsic signaling mechanism of the NPY5-GPCR complex

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The neuropeptide Y receptor family, which belongs to the Class A (Rhodopsin-like) group of the G-protein coupled receptor (GPCR) family, plays a significant role in regulating neurological and psychiatric disorders, pain management, anxiety, depression, migraines, and certain cardiovascular diseases.[1,2] So far, 4 types of receptors from this family (Y1, Y2, Y4, Y5) have been cloned from humans[2], and only the 3D structure of the Y5 receptor is not available in databases. However, although the Neuropeptide Y receptor family members are generally similar to each other, there is a difference of 64 amino acids in NPY5R, some of which are in the intracellular region and others in the extracellular region. The impact of this difference on the $G_{i,o}$ protein coupling domain and the mechanisms of agonistic/antagonistic binding remains unknown, and research in this area is limited. Therefore, in this study, we performed molecular dynamics simulations by embedding the Y5 receptor-GPCR complex into the cell membrane to better understand these aspects. To determine the 3D structure of the NPY5 receptor, we also applied homology modeling methodology. Based on preliminary results from MD simulations, the loop structure (IL3), which contains a helix and connecting TM5 to TM6 of the receptor, as well as the NPxxY motif in TM7 of the receptor, were found to interact with the alpha subunit of the $G_{i,o}$ protein. While this interaction is maintained in the presence of an agonist, a counterclockwise

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movement of the beta and gamma subunits occurs in the absence of an agonist. References 1. Nelson TY et al. (2024) ACS Pharmacol Transl Sci. 7 (12), 3718-3728 2. Pedragosa-Badia X et al. (2013). Front Endocrinol (Lausanne). 4(FEB):1-13.

P-25-014
Next-generation PRMT5:MEP50 inhibitors: a multi-tiered hybrid virtual screening pipeline for the discovery of allosteric and PPI disruptors in precision cancer therapy

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Protein arginine methyltransferase 5 (PRMT5) is a pivotal epigenetic regulator implicated in transcription, DNA repair, and RNA metabolism. Its aberrant overexpression is tightly linked to oncogenesis and poor clinical outcomes, positioning PRMT5 as an attractive therapeutic target. While catalytic inhibition has been extensively explored, selectively disrupting allosteric sites or PRMT5:MEP50 protein–protein interactions (PPIs) offers a novel and potentially more precise therapeutic avenue. In this study, we employed an integrative structure- and computation-guided approach to identify allosteric and PPI inhibitors of PRMT5:MEP50. Structural mapping of the PRMT5:MEP50 complex (PDB ID: 4GQB) uncovered previously uncharacterized druggable pockets enriched with key hotspot residues. A multi-tiered hybrid virtual screening pipeline was implemented, combining structure-based dynamic pharmacophore modeling, molecular docking, and ligand-based screening of the ZINC20 ultra-large chemical library. Top-ranking candidates were subjected to long-timescale molecular dynamics (MD) simulations and MM/GBSA free energy calculations to refine binding predictions. Promising inhibitors underwent additional in silico assessment of their anticancer activity via binary QSAR models. Our computational framework revealed a new class of high-affinity PRMT5:MEP50 inhibitors, offering mechanistic insights into their potential anticancer efficacy. These findings lay the groundwork for future biochemical and cellular validation, particularly in targeting PRMT5-driven malignancies such as brain tumors. Acknowledgement: This research is supported by TÜSEB under Project Number: 33626.

P-25-015
The acidic hinge of RAG2: insights into RAG recombinase function and evolution

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The proteins RAG1 and RAG2 form the RAG recombinase tetramer, essential for V(D)J recombination and antigen receptor diversity in T and B lymphocytes. RAG1 recognizes and cleaves DNA, while RAG2 acts as a regulatory cofactor with an acidic hinge, a 50–70 amino acid linker connecting its two main domains. According to current models, the RAG recombinase originates from a domesticated transposon. Related mobile elements share structural similarities with RAG, except for a few components, including the acidic hinge of RAG2, which plays vital regulatory roles in maintaining genomic integrity by suppressing transposition [Martin et al. (2020) Mob DNA 11,17; Martin et al. (2023), Mol Biol Evol 40(11):msad232; Zhang et al. (2012), Nature 569:79-84]. However, due to its flexible nature, its coordinates have not been mapped in resolved structures. Utilizing advanced molecular sampling techniques, we seek to investigate the conformational space adopted by this flexible linker during the various stages of the enzymatic reaction. Our objective is to provide critical insights into its structural and functional roles in recombinase activity, as well as to identify key factors that inhibit the transposition step, while not affecting the cleavage process. Utilizing Robosample, a molecular simulation package founded on robotics algorithms, we constructed a conformational map showing that acidic linkers predominantly localize on either side of the tetramer's upper surface, aligning along the edges of the groove where DNA typically binds. This suggests the acidic linkers might affect DNA accessibility and the stability of the protein DNA complex which aligns with experimental observations. Investigating structural determinants that suppress transposition will provide insights into the evolutionary transition of RAG from a transposon to a recombinase and may reveal additional regulatory elements that are critical in preventing aberrant transpositional activity, linked to genomic instability and lymphoid malignancies.

LB-R-25-01
Investigation of inhibition capacities of several anthocyanins on AGEs-RAGE complex by molecular docking

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Advanced glycation end products (AGEs) play a role in various chronic diseases due to their proinflammatory and oxidative properties. AGEs bind to human receptor for AGEs (RAGE, PDB ID: 3O3U) that is found in most of the cells such as vascular cells, neurons, immune cells, cardiomyocytes, adipocytes etc. (López-Díez et al., 2016, Biochim Biophys Acta. 1862(12):2244–2252. DOI: 10.1016/j.bbadis.2016.05.00). Development of the diseases are induced through binding of AGEs with the receptor that leads activation of NF- κB and so the secretion of pro-inflammatory cytokines (Cheng et al., 2017, Clin Diabetes Res. 1(1):16–34. DOI: 10.36959/647/490). Anthocyanins, natural pigments found in fruits and vegetables, exhibit potent antioxidant and anti-inflammatory activities, making them potential candidates for therapeutic interventions targeting AGEs. Therefore, these compounds have ability to inhibit activation of NF-κB by binding to RAGE instead of AGEs. In this study, it is aimed to determine inhibition abilities of anthocyanins on AGEs-RAGE interaction by molecular docking studies. For this purpose, we identified binding scores and key binding interactions between the AGEs-RAGE complex with and without the presence of several anthocyanins using Autodock Tools-1.5.6 and Autodock Vina programs. Our results suggest that anthocyanins can reduce the deleterious effects of AGEs by binding its receptor with high affinities. The results revealed physico-chemical interactions between functional groups of studied anthocyanins and AGEs, and the binding affinities were associated with specific structural features of anthocyanins. These findings highlight the potential of anthocyanin compounds in AGE-related disease management and pave the way for further *in vitro* and *in vivo* validation studies. (Cheng et al., 2017)

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LB-R-25-02
Computational approaches RAB-GTAPases

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Rab proteins, in the active (GTP-bound) state promote downstream signaling through the interaction of effector proteins. Based on membrane trafficking function, Rab GTPases are important to cancer progression. Targeting Rab GTPases provides possibilities for cancer treatment strategies. 16 000 molecules were downloaded from Chemdiv Kras-Targeted Library. For this purpose, the LigPrep module in the Schrödinger Maestro program was used. For RAB10, the PDB ID 5SJZ structure was used. We performed docking using Schrödinger Glide, where small molecules were placed separately into previously identified docking pockets then calculated the docking score RMSD. According to the reaction results, MM/GBSA, Prime energy and docking score values were obtained. According to these results, compounds with high docking scores were separated to be included in molecular dynamics (MD) simulations. The Desmond program was used for this purpose. Simulations are conducted at 310 K and 1 bar. Length of MD simulations are conducted (500 ns). While 100 trajectory frames are collected throughout the simulations, 1000 frames are collected in long MD simulations. We used sitemap protein preparation to predict the binding pockets. SiteMap identifies potential binding sites by linking together 'sitepoints' that are most likely to contribute to tight protein–ligand binding. Units of 500 ns and 100 ns md simulations in each protein region with the best 10 molecules according to the docking list. P950-0240 molecule was found to bind to the protein molecule in simulations at 500 ns and 100 ns. The development of small GTPase inhibitors could be a useful new treatment strategy for both non-carcinogenic and carcinogenic diseases. However, the generation of these inhibitors is a challenging issue owing to the fine regulatory roles assigned to each of the members of the small GTPases protein family.

LB-R-25-03
Web-based deep learning framework for classifying stages of periodontal disease

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Periodontal disease is a condition affecting the gums and the tissues surrounding the teeth. Within periodontal disease, periodontitis is a common gum infection that can lead to tooth loss or damage to the tooth structure. Regular dental checkups and scaling can prevent the progression of the disease. However, detecting and classifying periodontitis is a challenging task that requires specialized technical knowledge. To address this issue, this study designed a user-friendly web-based application that allows non-expert users to perform the detection and stage classification of periodontitis with just a few clicks. The proposed application can serve as a bridge between the deep learning model and end-users without coding experience, thereby potentially improving the accessibility and efficiency of periodontal disease diagnosis.

LB-R-25-04
In silico reading of programmed cell death: integrative analysis of the effects of L-carnitine and cisplatin and 5-FU combinations on apoptosis

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L-carnitine is an amino acid derivative that facilitates the conversion of fat into energy. L-carnitine is synthesized endogenously in the liver, kidneys, and brain from the amino acids lysine and methionine. Chemotherapy drugs can reduce L-carnitine levels due to mitochondrial damage, renal dysfunction and nutritional deficiency. In the treatment of breast and colon cancer, treatment is unsuccessful due to developing chemo-resistance, so its combined use with new drug candidate molecules is the main research topic. The drug candidate profile of L-carnitine, 5-FU and cisplatin and protein–ligand interaction in pathways will be carried out *in silico*. AutoDock Vina, PyMOL-3, CB-DOCK-2, ADMElab 3.0 and ProTox II were used as database programmes. The cytotoxicity of different concentrations of L-carnitine combined with 5-FU and Cisplatin in MCF-7 and Caco-2 cell lines was calculated (IC_{50} value using MTT test) and its effects on other apoptotic pathways were analyzed. These pathways, Caspas-3, Caspas-8, Bcl-2 and p53 values for apoptosis were evaluated using the ELISA method with the cell lysate obtained. In this study, antiproliferative effects were observed in 24-h and 48-h application of different concentrations of L-carnitine to MCF-7 and Caco-2 cells. In drug combinations, Cisplatin and 5-FU with L-carnitine showed a synergistic effect according to Chou-Talalay method (CI curve). Molecular docking for bioinformatics analysis showed a vina score of –6.4 kj/mol and vina score of –8.7 kj/mol in the interaction with Caspase-3 and Caspase-8, which play an important role in the evaluation of apoptotic pathways, respectively. ADMET (Absorption, Distribution, Metabolism, Excretion, Toxicity) analysis of L-carnitine showed that it exhibited strong pharmacokinetic properties and low toxicity profiles. *The authors marked with an asterisk equally contributed to the work.

LB-R-25-05
Computational investigation of protein-ligand interactions in heat shock protein (HSPA5)

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This project aims to elucidate the molecular mechanism of heat shock protein HSPA5 inhibition by small ligands and to use the rational design of drugs for HSPA5-related diseases. HSPA5 belongs to a chaperone machinery within the endoplasmic reticulum and can recognize and bind to misfolded proteins assisting their proper folding. Despite its vital role in human cells, its high expression is linked with numerous diseases. Especially, intense proliferation of cancer cells requires highly efficient chaperon machinery. HSPA5 as a potential target to

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impair tumor growth. Performed studies showed the impact of known HSPA5 inhibitors on the viability of tested cancer cell lines. The activity of HSPA5 is controlled by ATP binding and hydrolysis to ADP, hence their analogs were the first candidates to modulate HSPA5. Indeed, adenosine derivatives have been proved to deactivate this protein. However, the detailed mechanism is still not fully understood. Here, we elucidate the advantage of the molecular dynamics calculations and take closer insight into the inhibition process at the molecular level. Protein-ligand interactions were investigated with atomistic precision to get more precise information on the most critical contacts which stabilize HSPA5 complexes. Microsecond trajectories reveal how the conformational protein dynamics is affected by presence of small ligands at nucleotide-binding center. Tracking the changes in HSPA5 behavior upon coupling with its known inhibitors, we get the tool to test *in silico* new HSPA5 inhibitors. The potential drug candidates were obtained from screening of the commercially available compounds using a multi-techniques approach, which includes a popular molecular-docking method. The common feature of selected molecules is the presence of adenosine moiety; however, they differ significantly in chemical properties. Coupling bio-informatics studies with further experimental validation gives us a chance to find more efficient candidates.

Synthetic biology

P-26-001
Synthetic cells as a model to study TNFα signaling in pancreatic ductal adenocarcinoma (PDAC)

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Pancreatic ductal adenocarcinoma (PDAC) is among the most aggressive malignancies, characterized by poor survival rates, late-stage diagnosis, and a heterogeneous tumor immune microenvironment (TIME). TIME consists of immune cells, stromal components and signaling molecules such as cytokines. Among these, tumor necrosis factor-α (TNFα) plays a critical role in cancer progression and immune regulation. TNFα exists in two isoforms, soluble (sTNFα) and membrane-bound (mTNFα), each engaging a preferential receptor (TNFR1 or TNFR2) and eliciting different responses, which might mediate a switch between basal-like (BL) and classical (CLA) PDAC subtypes. While sTNFα has been extensively studied, mTNFα remains less characterized, particularly in relation to TNFR2 signaling, due to the lack of a model that accurately mimics mTNFα presentation and quantification on a membrane. To address this gap, we employed droplet-supported lipid bilayers (dsLBs) as a synthetic cell platform to present mTNFα to cancer cells within PDAC spheroids. We cultured PANC-1 (PDAC cell line) and MCF-7 (TNFR2-negative breast cancer cell line) spheroids to investigate the different effects of TNFα isoforms. Following mTNFα and sTNFα exposure, cytotoxicity assays were conducted, and molecular signaling patterns were examined via automated immunoassays. Additionally, histological analyses were performed to evaluate morphological changes in tumor cells under different TNFα conditions. Cytotoxicity assays revealed an interplay between mTNFα and sTNFα, with effects differing from those observed when each isoform was tested individually. Histological analysis showed structural changes upon mTNFα addition, emphasizing its distinct effects from its soluble counterpart. Our controlled and tunable dsLBs model not only successfully mimics mTNFα signaling but also unveils TNFα's dual role in the PDAC environment, offering a powerful and versatile tool for further exploration of this complex signaling pathway.

P-26-002
A novel biotechnological pathway for the upcycling of post-consumer PET into valuable compounds

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In today's world, the widespread reliance on plastics has led to a growing environmental challenge, since most of them are disposed in incinerators and landfills. Polyethylene terephthalate (PET), with 413.8 million tons, is the third most abundantly produced plastic, thus its recycling is necessary for the transition to circular economy. Enzymatic PET depolymerization into its monomers, terephthalic acid (TPA) and ethylene glycol, is now a reality thanks to the discovery of PET-hydrolysing enzymes (PHEs) which offer several advantages compared to traditional chemical recycling. To 'close the loop' and generate value-added compounds from PET waste streams, we developed a novel bioprocess based on engineered enzymes and *Escherichia coli* strains to depolymerize PET (1 $/kg) and convert TPA into L-DOPA (600 $/g), used in the treatment of Parkinson's disease. A novel biosynthetic pathway involving twelve enzymes from three different microorganisms has been developed by our laboratory, and it has been optimised on recombinant proteins and whole-cell *E. coli* strains. Following PET depolymerization into TPA through an engineered variant of leaf branch compost cutinase (LCC) active at 55°C [Previously published in: Pirillo et al. (2023) FEBS J. 290(12):3185-3202], TPA was converted to pyruvate by an engineered *E. coli* RARE strain and recombinant enzymes, and to catechol by another engineered *E. coli* strain. Finally, a novel tyrosine phenol-lyase (TPL) from *Fusobacterium nucleatum* was successfully overexpressed in *E. coli* cells and evaluated for L-DOPA synthesis starting from commercial catechol and pyruvate, yielding 80 mM L-DOPA in 2 h with a catechol conversion greater than 99%. Our findings demonstrate the feasibility of biomolecules production from plastics by a sustainable process and lay the groundwork for future improvements, especially for developing a single engineered strain as a more sustainable one-pot system. *The authors marked with an asterisk equally contributed to the work.

P-26-003
Artificial tumor immune microenvironments for hybrid 3D cancer cultures

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While great progress has been made in the field of cancer immunotherapy, a variety of cancers are able to evade detection by the immune system by developing a suppressive tumor immune microenvironment (TIME), a dense structure of extracellular matrix proteins and anti-inflammatory immune cells.

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Research into the influence of the various components of TIME on treatment efficacy is hampered by both its inherent complexity and the lack of suitable *in vitro* models. To meet this challenge, we developed a bottom-up assembled synthetic cell system, referred to as droplet-supported lipid bilayers (dsLBs). DsLBs are soft mimetic structures designed to imitate the surface properties of immune cells by displaying immune cell-associated proteins on their surface. We are able to decorate dsLBs with defined quantities of surface receptors and regulatory proteins, allowing us to reproduce the cell–cell interfaces that naturally occur between tumor and TIME in a highly controlled manner. We show that cancer cells incorporate the dispersed synthetic dsLBs into 3D spheroids during their self-assembly formation process, creating a hybrid material that we call the artificial tumor immune microenvironment (ART-TIME). Using this approach, we demonstrated via transcriptome analysis, phosphoprotein signaling pathway assays and bispecific T cell engager therapies that incorporation of dsLBs decorated with different immune cell-derived receptors results in distinct ART-TIMEs that affect gene expression of pancreatic ductal adenocarcinomas (PDAC) cancer cell lines, including several proteins involved in the outcome of cancer therapy and relevant for immune evasion. We envision ART-TIMEs to be applied as controllable *in vitro* models for research into cancers-immune evasion and for systematic analysis of receptor co-signalling during phenotypic cancer.

Systems biology

P-27-001
Investigating the effects of age-related genes on tissue specific damage markers

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Aging is a complex process characterised by the interaction of genetic and environmental factors, leading to comprehensive changes in biological systems. This study analyses the effects of age-related genes on damage markers in different tissue types. The research aims to understand the effects of aging processes on genetic regulation, especially in liver tissues. Firstly, differential gene expression analysis of aging-related genes was performed using RNA sequencing data of liver tissues obtained from different age groups. As a result of this analysis, gene regulatory networks (GRNs) were constructed and the correlation between these genes and damage markers in the aging process was analysed. In addition, it is also planned to calculate a 'damage score' to quantitatively assess the level of damage markers. The damage score will be used to better understand the effects of genetic changes in the aging process on biological pathways. Analyses so far have shown that some genes have big effects on damage markers in aging. In the next step of the study, the impact score will be calculated to understand the effects of damage markers on molecular pathways in more detail, and the relationship of these scores with tissue-specific biological mechanisms will be evaluated. The preliminary findings of the study promise to provide new insights into genetic regulation in the aging process and will contribute to the identification of potential therapeutic targets in the prevention and treatment of age-related diseases. In the next step of this study, the findings obtained from liver tissue will be extended to other tissue types and the gene regulatory networks obtained will be comprehensively compared. Further modelling studies will be performed to evaluate tissue-specific biological pathways in more detail. This research aims to shed light on the molecular basis of the aging biology and contribute to the identification of new targets for the prevention of aging-related diseases.

P-27-002
Implications of machine learning approaches in acute myeloid leukemia for biomarker discovery

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Acute myeloid leukemia (AML) is an aggressively differentiated and heterogeneous sub-type of blood cancer that is characterized by the involvement of bone marrow, blood and other tissues. While some progress has been made in developing strategies for AML treatment in recent years, the outcome of patients with AML remains unsatisfactory, especially following relapse or refractoriness. This study aimed to analyze the AML cancer transcriptome, integrating machine learning approaches to identify novel molecular signatures. Transcriptome data associated with AML were comparatively analyzed and differentially expressed genes (DEGs) were identified. Machine learning algorithms, such as least absolute shrinkage and selection operator (LASSO), Relief, random forest (RF) and support vector machines (SVM), were applied to eliminate irrelevant features. Following feature selection, differentially expressed features were considered key genes of AML. The expression levels of key genes were used to construct machine learning models using SVM, RF, diagonal linear discriminant analysis (DLDA), and Poisson linear discriminant analysis (PLDA) algorithms. The accuracy values were compared to assess key gene performances in discriminating diseased patient from healthy controls. Proteins encoded by key genes with high accuracy were used as drug targets and repurposed drugs were discovered as drug candidates for AML. The study provided a different perspective by implementing machine learning with algorithms on transcriptome data to investigate the complex molecular nature of AML. In addition, our findings contributed to the advancement of novel strategies for personalized medicine in AML management.

LB-R-27-01
Harnessing *Pycnoporus sanguineus* for lignocellulosic waste valorization: a systems biology approach

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By-products of agricultural and food industries can be valuable raw materials for the production of high-value compounds. Lignocellulosic waste, which is resistant to degradation due to its lignin content, can be converted into valuable resources, promoting a circular bioeconomy approach. Microbial cell factories are one of the main production "facilities", fostering this approach. The white rot fungus *Pyncoporus sanguineus* (*P.*

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sanguineus) is highlighted for its ability to degrade lignin and produce bioactive compounds with antimicrobial, antioxidant, and anticancer properties. However, the metabolic potential of *P. sanguineus* remains largely untapped. Genome-scale metabolic models (GEMs) offer a computable overview of the metabolic potential of an organism. GEMs have been used to better understand and develop microbial cell factories to valorize lignocellulosic waste. GEM models, particularly using flux balance analysis (FBA) and dynamic FBA (dFBA), are used to simulate microbial growth, product production, and other metabolic activities. These models offer strategies for strain engineering and metabolic pathway optimization, accelerating the development of microbial cell factories. The research aims to create a genome-scale model of *P. sanguineus*, which could lead to new bioprocesses for waste valorization and uncover new metabolic pathways with potential pharmaceutical and industrial applications. *The authors marked with an asterisk equally contributed to the work.

Biotechnology

P-28-001
Enhancing nicotine bioconversion in *Paenarthrobacter nicotinovorans* ATCC 49919 using CRISPR system

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Paenarthrobacter nicotinovorans ATCC 49919 is a nicotine-degrading microorganism with biotechnological potential in converting the alkaloid into compounds of industrial and pharmaceutical importance such as 6-hydroxy-L-nicotine (6-HLN), methylamine and γ-amino-butyric-acid. A CRISPR-based genetic engineering tool that would allow fast and easy editing of the *P. nicotinovorans* genome is key for increasing its applications. Hence, our aim is to develop such a tool by focusing on reducing the expression of 6-HLN oxidase (*6hlnO*), a key enzyme that catabolizes the conversion of 6-HLN to 6-hydroxy-methylmyosmine. A 20 bp spacer targeting the gene of interest was obtained by annealing four 5' phosphorylated synthetic oligonucleotides. The spacer was cloned using Golden-Gate assembly into pCasiART. Positive colonies were selected by blue-white screening. To evaluate the efficiency of the resulting construct, pCasiART-Δ6hlnO was grown in citrate medium (28°C, 190 rpm) with 0.3, 1.5, 2.25 and 3 mM nicotine. Cell density (OD_{600}) and nicotine degradation (nicotine blue, OD_{580}) were monitored over 24 hours, with hourly supernatant sampled for HPLC analysis. No significant delays in cell growth were observed for S3(6), S2(1) and S2(2) strains across the tested conditions. However, higher nicotine concentrations led to delayed degradation, with a lag phase extending to 7 hours for 0.3 mM nicotine and to 9–10 hours for 3 mM nicotine. The HPLC analysis used to quantify the conversion rate of nicotine to 6-HLN reveals a correlation with the extension of the lag phase in nicotine degradation. At 0.3 mM, nicotine is fully degraded after 11 hours, whereas at 3 mM, the process extends to 17–19 hours after inoculation. Compared to the wild-type, pCasiART-Δ6hlnO_S3(6), pCasiART-Δ6hlnO_S2(1) and pCasiART-Δ6hlnO_S2(2) demonstrated improved efficiency in the bioconversion of nicotine to 6-HLN, highlighting their potential for further biotechnological applications.

P-28-002
Extreme enzymes: unlocking biomass energy

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Lignocellulosic biomass (LCB), comprising cellulose, hemicellulose, and lignin, is a renewable resource essential for the sustainable production of high-value products such as biofuels, biomaterials, and supplements(1). However, due to its resistant nature, LCB requires innovative and cost-efficient deconstruction strategies, which often leverage the synergistic activity of microbial enzymes(2). Geothermal environments, distinguished by elevated temperatures and unique geochemical properties, host diverse microbial communities adept at thriving in these extreme conditions. These consortia are a promising source of novel thermostable enzymes, presenting significant opportunities in industrial biorefineries and medical fields. This study focuses on the microbial diversity and lignocellulolytic potential at the geothermal site Maronti Bay (Italy, 60-100°C, pH 8.8). Through a combination of metagenomic sequencing and culture-dependent enrichments, we determined the composition of the microbial community and its inherent capacity for LCB degradation. After enrichments, Archaea decreased significantly (5.4% in the metagenome vs 0.1% in enrichments). Also, GH repertoires shifted: the metagenome favored biofilm-related sequences, while enrichments selected lignocellulose-hydrolyzing enzymes such as cellulase, glucanase, xylanase, and arabinosidase. The activity of these enzymes leads to the production of xylooligosaccharides (XOS), which present considerable potential for biotechnological applications, mainly as ingredients in food supplements and nutraceutical compositions with high added value and significant relevance to human health. 1. Vuong, P., Chong, S., & Kaur, P. (2022) NPJ Biodivers 1, 4 https://doi.org/10.1038/s44185-022-00006-y. 2. Dumorné, K., Córdova, D. C., Astorga-Eló, M., & Renganathan, P. (2017) J Microbiol Biotechnol. 27(4):649-659. doi: https://doi.org/10.4014/jmb.1611.11006.

P-28-003
Surface modification of biocompatible rifaximin-loaded PLGA nanoparticles with P6.2 targeting peptide: synergetic combination at fighting *Staphylococcus aureus* infections

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Rifaximin (RFX) is a semi-synthetic antibiotic known for its broad-spectrum activity; however, its therapeutic potential is limited by low aqueous solubility (Kumar et al. (2021) Int. J. Biol. Macromol., 183: 962-71) and restricted permeability. Enhancing the solubility of hydrophobic drugs is often achieved through the use of carrier systems, and among these, nanoparticle (NP)-based delivery platforms have recently

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garnered significant interest. The aim of this study is to develop an NP-based carrier system to improve solubility, bioavailability, and targeted delivery of RFX, thereby providing a promising alternative for the treatment of *Staphylococcus aureus*-associated infections. For this purpose, RFX-loaded poly (lactic-co-glycolic acid) (PLGA) NPs were synthesized using the single emulsion solvent evaporation method. Subsequently, Peptide 6.2 (P6.2) was conjugated to the NP surface to facilitate targeted delivery to the intended microorganism. The resulting P6.2-conjugated, RFX-loaded PLGA NPs (TR-NP) demonstrated robust antibacterial activity against both *S. aureus* and methicillin-resistant *S. aureus* (MRSA). Compared to free RFX, the non-targeted RFX-loaded PLGA NPs (R-NP) achieved an 8-fold and 16-fold increase in antibacterial efficacy against *S. aureus* and MRSA, respectively. Moreover, the TR-NP formulation further enhanced antibacterial performance by up to 32-fold or 16-fold, reaching a minimum bactericidal concentration as low as 0.0078 μg/mL. All NP formulations exhibited biocompatibility at the effective antimicrobial doses. Notably, the TR-NP preferentially accumulated in *S. aureus* over *E. coli*, underscoring their specificity and potential clinical relevance. Overall, these findings highlight the potential of TR-NP as a powerful platform for improving the therapeutic effectiveness of RFX against the challenging bacterial pathogen *S. aureus*. This work was supported by YTU Scientific Research Projects Coordination Unit (Project Number: FKD-2021-4737).

P-28-004
A new immunotoxin design against allergy based on a single chain variable fragment targeted against FcεRI and the ribotoxin α-sarcin

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Allergy constitutes one of the main health problems nowadays, affecting the lifespan and quality of life of many people. The main treatments include allergen avoidance, pharmacotherapy and immunotherapy. Besides, the use of antibodies and immunoconjugates against FcεRI, the receptor that binds IgE on the surface of basophils and mastocytes and is responsible for the allergic symptoms, has arisen as a promising therapeutic tool. In this sense, immunotoxins are chimeric proteins, that consist of a target domain (an antibody or a derived fragment, such as a single chain variable fragment, scFv) that directs the action of a toxic domain (a protein derived from plants or fungi) towards the target cell, leading to its death. Among the toxins employed in immunotoxin design, fungal ribotoxins family, and α-sarcin as its most studied member, stand as promising therapeutic candidates, due to their high ribonucleolytic activity and low immunogenicity. We have designed, produced and characterized both the scFv againt FcεRI (scFv) and the corresponding immunotoxin combining the target domain scFv-FcεRI and a non-immunogenic variant of the ribotoxin α-sarcin (IMTX). Both proteins were produced as recombinant proteins using the methylotrophic yeast *Pichia pastoris* and purified to homogeneity through affinity chromatography. Both proteins (scFv and IMTX) were correctly folded, exhibiting mostly a β-sheet secondary structure. Besides, both proteins were able to bind specifically to FcεRI on the RBL-2H3 cell line, and IMTX kept the ribonucleolytic activity of the toxic domain. The functionality of both domains was assayed through viability assays against the RBL-2H3 cells, showing that IMTX was cytotoxic to these cells, whereas scFv wasn´t toxic. Finally, the incubation of both proteins with LAD2 cells (a mastocyte-like cell line) prevented the allergic degranulation. These results suggest the therapeutic efficiency of immunotoxins in the treatment and prevention of allergy.

P-28-005
Comparative analysis of microbiome and biotechnological potential of saxicolous and corticolous lichens distributed in Armenia

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Due to their wide range of secondary metabolites, lichens were used from antiquity as sources of colorants, perfumes and medicaments. Our research focuses on exploring the antioxidant, antimicrobial and cytotoxic activities of methanol, ethanol, acetone extracts and aqueous infusions of corticolous and saxicolous lichens sampled from Armenia, as well as comparative microbiome of two representatives of *Ramalina* genus. Methanol, ethanol and acetone extracts from all tested lichens were active against Gram-positive bacterial strains. The most effective solvent to retrieve antimicrobial compounds was methanol. Aqueous infusions of tested lichens didn't show any significant antibacterial and antifungal activity. The highest antimicrobial activity was observed for methanol extract of *Ramalina sinensis* and *Ramalina polymorpha*, inhibition zone, respectively 25 ± 0.5 and 50 ± 0.5 mm on *Bacillus subtilis*. Methanol extract of *Parmelia sulcata* demonstrated the largest 1,1-diphenyl-2-picryl-hydrazil (DPPH) radical scavenging activity (71%) and second most potent sample was methanolic extract of *Xanthoparmelia stenophylla* with 70% scavenging activity. The cytotoxicity was measured on human HeLa (cervical carcinoma) cell lines using microculture tetrazolium test assay. The IC50 values estimated for methanol extracts of *Peltigera praetextata*, *Evernia p*runastri, *Ramalina sinensis* and *Ramalina farinacea* species in HeLa cell line were within 1.8–2.8 mg mL^{-1} and considered as non-cytotoxic. Taxonomic analysis showed that *R. sinensis* and *R. polymorpha* share common phyla of Acidobacteriota, Baccilota and Actimoycetota with some differences. For these lichen species, the core microbiome was also identified, which shows that *R. sinesis* and *R. polymorpha* contain heap number of shared members. *The authors marked with an asterisk equally contributed to the work.

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P-28-006
Characterization of a novel hyperthermophilic α-L-rhamnosidase from Archaea for biotechnological applications

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α-L-Rhamnosidases, a class of glycosyl hydrolases (GHs), catalyze the removal of α-L-rhamnose residues from the non-reducing ends of glycans. Based on their regiospecificity for α-1,2, 1,3, 1,4, and 1,6 linkages, these enzymes have diverse applications in the food, beverage, and pharmaceutical industries [1]. Microbial α-L-rhamnosidases are classified into GH13, GH78, and GH106 families within the CAZy database (www.cazy.org). Among them, GH78 enzymes play a crucial role in degrading rhamnose-containing polysaccharides and glycoconjugates such as naringin and rutin [1]. Although bacterial and fungal α-L-rhamnosidases have been extensively studied and characterized, no α-L-rhamnosidases from archaea have been reported, and the study of thermostable enzymes from thermophilic microorganisms remains limited. In this work, we identified, from a metagenomic dataset derived from the solfataric hot spring of Pisciarelli in Agnano (Naples, Italy) [2], a novel archaeal thermostable α-L-rhamnosidase (SaRham) capable of hydrolyzing naringin, a key flavonoid with different biological activities, including anticancer, anti-inflammatory, and antioxidant. The enzyme showed optimal activity at 95°C and pH 6.5, with a specific activity of approximately ~30 U/mg on 4NP-α-rhamnopyranoside. It efficiently hydrolyzed naringin, cleaving the α-1,2-glycosidic linkage between L-rhamnose and β-D-glucoside, producing rhamnose and prunin. SaRham holds significant potential for biotechnological applications, including the production of L-rhamnose, isoquercitrin, and prunin, which are known for their antioxidant, antiarrhythmic, and anticomplementary activities [3]. This is the first report of an archaeal hyperthermophilic α-L-rhamnosidase and the first enzyme characterized to date within GH78 family. References 1. Pan L et al. (2023) World J Microbiol Biotechnol. 10;39(7):191. 2. Strazzulli A et al. (2020) FEBS J. 1;287(6):1116–37. 3. Jung HA et al. (2017) Bioorg Med Chem Lett. 1;27(11):2274–80

P-28-007
Exploring a novel recombinant laccase for advanced textile dye decolorization and sustainable wastewater management in simulated effluents

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Laccases, versatile multicopper oxidase enzymes, are widely studied for their ability to oxidize diverse substrates while reducing molecular oxygen to water. These enzymes have gained prominence in biotechnological applications due to their eco-friendly properties and broad substrate specificity. The textile industry is a leading source of water pollution, as dye-bath effluents contain high color intensity, chemical oxygen demand (COD), and toxic synthetic dyes resistant to biodegradation. Conventional treatments often produce secondary pollutants and excessive sludge, highlighting the need for sustainable alternatives. The aim of this study was to investigate the potential of a novel laccase from *Coriolopsis polyzona* MUCL 38443 for sustainable textile dye decolorization under simulated industrial conditions. The coding sequence of the enzyme was subcloned into the *Pichia pastoris* expression vector pPICZ B under the control of the inducible *AOX1* promoter, and expression conditions were optimized for maximal production. The purified enzyme demonstrated a 68.39-fold increase in specific activity (274.24 U/mg) and exhibited substrate-dependent pH optima,an optimal temperature of 70°C and a Tm of 50.77°C. Decolorization studies were conducted using azo and triarylmethane dyes,initially in citrate buffer systems to assess enzymatic performance under controlled conditions. Subsequent experiments utilized simulated dye-bath effluents to evaluate the enzyme's efficacy in the presence of industrially relevant contaminants. These investigations addressed critical challenges such as the complexity of dye mixtures, variable chemical compositions, and potential enzyme inhibitors, providing valuable insights into the applicability of laccase-based treatments for industrial wastewater. The findings highlight the importance of bridging laboratory-scale research with real-world applications to advance sustainable solutions for textile wastewater management. (ITU-BAP Project ID: 42925)

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P-28-008
Identification of plastic binding peptides with phage display, next generation sequencing and binding of nanoparticles

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Sub-micron plastic fragments can be found ubiquitously in nature, but we have limited data on the extent and toxic effect of this nano-pollution. There is a need for improved tools for detection and identification of plastic in environmental samples. A way to tackle this problem is to develop specific plastic binding peptides to be used in environmental probes. We have used phage display to identify peptides that bind a range of the most common plastic types. Phages displaying short peptides have usually been used to identify protein binding partners via specific biomolecular recognition interactions, but this selection system must be modified to be applied to material surfaces. We have altered the panning setup, especially the method of elution, as well as exploiting next generation sequencing (NGS) to remove phage replication bias. Examining the diversity of the NGS data also gives an indication whether the panning has been successful. By comparing phage libraries panned against different plastics, we have elucidated whether a peptide binds many types of plastic or whether it may be specific to a single type of plastic. Following identification of peptides of interest, we have measured their binding to plastic using flow-induced dispersion analysis (FIDA) (binding to nanoparticles) and a pull-down plate-assay (binding to bulk plastic). We believe our phage display pipeline could be a useful step towards developing environmental plastic probes.

P-28-009
Probing the potential of C-phycocyanin to replace serum albumin in cell media for meat cultivation

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Cultured meat has the potential to reduce the environmental impact of traditional farming by using less land and water and creating less pollution. However, its high production cost, mainly due to fetal bovine serum (FBS) use, remains a significant challenge. FBS is widely used in cell cultivation because it provides essential growth factors, proteins, and nutrients. However, it is expensive, ethically controversial, and suffers from batch-to-batch variability, making it unsuitable for large-scale applications like cultured meat production. Albumin, the most abundant protein in FBS, is essential for cell growth, so finding cheaper, more sustainable alternatives is a priority. C-phycocyanin (C-PC), a major protein in cyanobacteria Spirulina, shows promise because it has antioxidant and metal-binding properties, is in high abundance, and is produced in an eco-friendly way. This study aims to probe the potential of C-PC to replace albumin for growing quail muscle cells. We tested how C-PC binds fatty acids (FA) using fluorescence quenching, focusing on saturated (palmitic, stearic), monounsaturated (oleic), and polyunsaturated (linoleic, linolenic) fatty acids. The results showed that C-PC better binds unsaturated fatty acids, with linolenic acid having the strongest affinity with K_d at low micromolar range. Cell growth experiments, including Neutral Red assay and direct cell counting, showed that C-PC improved cell growth significantly compared to control. Additionally, multi-passage cultivation demonstrated that C-PC increases cell viability over multiple growth cycles, proving it can support long-term cell cultivation. We showed that C-PC is a promising and sustainable alternative to albumin, with good fatty acid-binding abilities and strong support for cell growth. This makes it a valuable opportunity to produce cultured meat more affordable.

P-28-010
Development of a yeast-based platform for glucosyl glycerol production

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2-O-(α-D-glucopyranosyl)-sn-glycerol (glucosyl glycerol, α-GG) is a natural osmolyte with diverse applications, ranging from its use as a moisturizing agent in cosmetics and a low-calorie sweetener in food products to its potential use as a prebiotic. The production of α-GG is based on biocatalytic selective transglucosylation, where glucose from sucrose is transferred to glycerol in a reaction catalyzed by sucrose phosphorylase (SPase). To date, sucrose phosphorylase has been successfully chemically immobilized on various carriers; however, this method of α-GG production is accompanied by costly and time-consuming purification processes due to the challenging separation of α-GG from fructose, a byproduct of the enzymatic reaction. To address this challenge, we first developed and optimized the Ccw12-tag, a modified native yeast cell wall protein that is an ideal anchor for immobilizing enzymes on the cell wall. Next, we created a fusion protein combining the novel Ccw12-tag with SPase from *Leuconostoc mesenteroides* and overexpressed it in a *Saccharomyces cerevisiae* strain that can metabolize the byproduct fructose but not the substrate sucrose, thus significantly simplifying the downstream processing. The localization of SPase on the yeast cell surface was confirmed with western blot, while enzyme characterization and kinetic parameters were determined with UPLC analysis. Using this approach, we successfully produced up to 37.5 mg/mL of α-GG, demonstrating the efficiency of the process. Furthermore, this immobilization technique significantly enhanced the enzyme's longevity and stability, making it a reliable option for long-term and continuous production. This approach establishes the first eukaryotic microbial factory capable of producing α-GG in a single step from readily available substrates, sucrose and glycerol.

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P-28-011
Solving the mystery: algaenan biosynthetic pathway in unicellular green algae as revealed by comparative genomics

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Unicellular green algae express considerable differences in the structure and chemical composition of their cell walls. Algaenans are non-hydrolyzable, acetolysis-resistant materials found in cell walls of Chlorophyta, and are often described as analogues of plant sporopollenin. Algaenans consist long fatty acids and their derivatives, cross-linked via ester and ether bonds into a rigid polymeric network. Although algaenans are essential components of algal cell walls, the current knowledge about their chemical nature, biological function, and strain diversity is filled with gaps and ambiguities. The strains accumulating algaenans (referred to A+ strains) were previously found to have tri-laminar cell walls. Consequently, it was postulated that plant-like, bilaminar cell walls are present in strains of non-accumulating algaenans (A- strains). In this study, 10 Chlorophyta strains isolated from natural habitats and previously assigned as A+ and A- based on their resistance to acetolysis has been sequenced. The obtained genome was evaluated and, on this basis, gene prediction and annotation were performed. Proteomes derived from genomes showed that all studied strains contain homologues of six of the eight basic proteins involved in the plant (*Arabidopsis*, rice) sporopollenin biosynthetic pathway. Significantly, homologues of two genes of this pathway, encoding type III polyketide synthases A and B, were found to be functional only in strains accumulating algaenan in trilaminar cell wall structures. These results support a hypothesis that type III polyketide synthases are crucial for algaenan synthesis. Since there is a little overlap between the algal species whose genomes have been sequenced and those species for which algaenans have been reported, these results shed new light on the functioning of biosynthetic pathway of this polymer in unicellular green algae.

P-28-012
Modeling of BBB with self-assembled microvascular network in different ECM compositions by comprising mouse brain tissue in a novel BBB-on-chip demonstrates distict MVN patterns

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Organ-on-chip technologies are a promising strategy to study diseases and pathologies in a more realistic modelling by containing cell–ECM interaction, recapitulating the tissue architecture and functional units. In the studies of blood–brain–barrier (BBB) models, construction of self-assembled microvascular networks (MVNs) in the 3D environments is a functional model for drug screening and functional/structural BBB assays. In this study, we aimed to design a 3D self-assembled MVN formed by mouse brain microvascular endothelial cells (bEn.d3) with comprising living mouse brain tissue by evaluating the different ECM compositions and surface chemistry modifications. Matrigel, Collagen type-I and fibrin gel compositions and various surface chemical modifications by APTES and gluteraldehyde coating were evaluated in a novel BBB-on-chip model. CAD software and 3D printing was used in the design of chip molds. BBB-on-chip model was fabricated using polydimethylsiloxane (PDMS) and glass. MVN formation of GFP-labeled bEnd.3 cells was imaged via confocal microscopy. The combination of matrigel and fibrin gel created a highly vascularized network but resulted in gel retraction. Surface coating with 2% APTES and 3% gluteraldehyde prevented this gel retraction. In conclusion, combinations of tissue hydrogels and surface coating was evaluated for realistic microvascular network formation in a novel BBB-on- chip.

P-28-013
The role of human prostatic stem cell antigen PSCA in the brain function

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Prostate stem cell antigen (PSCA) belongs to the Ly6/uPAR protein family. Increased expression of PSCA (by ~70%) was found in the cortex of patients with Alzheimer disease (AD) and in the samples from patients with glioblastoma. Presently, role of PSCA in the body is unknown. Herein, we developed the expression system for bacterial production of PSCA in the form of inclusion bodies with subsequent solubilization and refolding and studied its functional and structural properties. The spatial structure of recombinant PSCA was studied by solution NMR using unlabeled and $^{13}C^{15}N$-labeled protein samples. In contrast to predicted by Alphafold structure, NMR analysis revealed that PSCA structure contains large, disordered regions at the tips of the loops I and III and an unusual for Ly6/uPAR proteins gap of the β-sheet formed by the central loop II. Through electrophysiological experiments, we demonstrated that PSCA is the negative allosteric modulator of α4β2- and α3β2-nAChRs. Computer modeling revealed the interface of PSCA/nAChR interaction. To understand possible effects from increased PSCA expression in the AD brain, we tested morphological changes of the primary neurons incubated with PSCA. Significant decreased dendritic spine density was revealed upon incubation with PSCA. Data obtained point to PSCA as an important regulatory protein in the human body. Moreover, we found that increased PSCA expression in glioblastomas relative to normal brain tissue corresponds to better survival prognosis. We studied the PSCA effect on proliferation, migration and invasion of U251 MG glioma and metastatic melanoma mel Kor cells derived from patients. It was revealed that recombinant PSCA down-regulates growth and invasion of U251 MG cells and shows no effect on mel Kor cells. Perhaps, the effect of PSCA is tissue-specific and depends on the repertoire of target receptors expressed on the surface of tumor cells.

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P-28-014
A human cell-based 3D glioblastoma-BBB-on-a-chip model for studying tumor microenvironment

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Glioblastoma (GBM), the most aggressive and lethal brain tumor, remains challenging despite advancements in surgery and chemotherapy. Survival improvements are modest, hindered by tumor heterogeneity, drug resistance, and the restrictive nature of the blood–brain barrier (BBB) and blood–tumor barrier, which limit therapeutic delivery to the brain. To address these challenges, we have developed a human cell-based 3D glioblastoma model combining organ-on-a-chip technology with human-induced pluripotent stem cell (hiPSC) methodologies. This *in vitro* model replicates key aspects of the GBM microenvironment, including the BBB, to facilitate the study of tumor biology and the evaluation of therapeutic strategies. The system employs a custom-designed two-channel microfluidic device, where the two channels are separated by a transparent porous membrane. One channel mimics the brain capillary with hiPSC-derived brain microvascular endothelial cells, while the other represents the brain parenchyma with astrocytes, pericytes, and U87MG GBM cells. The device was characterized using dextran permeability assays, immunostaining, transcytosis assays, and evaluations of drug efflux transporters, confirming its ability to replicate key BBB properties. To study intercellular communication, extracellular vesicles (EVs) were isolated using healthy and GBM chips and characterized via nanoparticle tracking analysis and scanning electron microscopy. Ongoing studies aim to identify the molecular content of the EVs through western blotting and RNA sequencing, shedding light on their contributions to GBM pathology. This 3D humanized GBM-BBB-on-a-chip model provides a robust platform for exploring glioblastoma biology, studying tumor–BBB interactions, and testing innovative drug delivery approaches. By bridging the gap between *in vitro* and *in vivo* systems, this model represents a significant step forward in advancing research toward improved GBM treatment. (EMBO Installation grant: IG-5352-2023)

P-28-015
Identification of transcytosis proteins to traverse the blood–brain barrier

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The blood–brain barrier (BBB) is a dynamic interphase that provides physical and biochemical protection to the brain, shielding it from potentially harmful substances. However, this protective function presents a significant drawback; it restricts the entry of most pharmaceutical agents, creating a substantial challenge in the treatment of brain diseases. To address this problem, we sought to identify new targets for transcytosis that are highly specific to brain endothelial cells, which are the main component of the BBB. These proteins can be used as a non-invasive delivery system for therapeutics, offering a promising strategy to overcome the barrier's selective permeability. Candidate proteins were identified through integrating two comprehensive computational approaches: analysis of published proteomics and transcriptomics datasets to determine proteins highly expressed in brain endothelial cells, and identification of transmembrane proteins with unique motifs potentially associated with transcytosis. To experimentally validate the transcytosis capabilities of the identified candidates, a functional assay was performed using a transwell system constructed with induced pluripotent stem cell-derived brain endothelial cells. The antibodies targeting the candidate proteins expressed on the brain endothelial cells were introduced into the system, and their transcytosis activity was quantitatively assessed using ELISA, providing evidence for their potential application in therapeutic delivery. Acknowledgement: This work is supported by TUBITAK 1001 – Project Number: 122S347.

P-28-016
Development of microenvironment responsive G4 ligands for the regulation of cancer metabolism and immune response

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The G-quadruplex (G4) structure in the guanine-rich DNA and RNA nucleic acid chain is effective in important biological processes such as regulation of gene expression at transcriptional and translational level and control of telomere activity (1). The effect of G4 structure has exposed a new potential avenue for anticancer drug development (2). Although many ligands that can bind to the G4 structure have been designed, very few of them are able to be activated or sensitive to their environment. A novel BODPIY-based ligand designed by our research group has shown to downregulate oncogene transcription in hypoxic environments (3). In this study, the novel BODIPY-based G4 ligands have been synthesized with different charge or molecular groups and their binding and selectivity properties were optimised. Ligands distinct properties such that L1 ligand is able to be activated in the hypoxic tumour microenvironment and induce singlet oxygen dependent oxidation, L2, L3 and L4 ligands have different charges, and L5 ligand has a unit that can be activated by histone deacetylase (HDAC) enzyme. FRET DNA melting assay and DNA titration assays were performed to determine binding affinities of the designed ligands to the G4 structure. It was found that they bind with high affinity at a concentration of 5 μM. The toxicity analysis showed that in MFC-7, A549, B16-F10 cancer cells, the ligands did not have a direct toxic effect, but oncogenes such as HIF1α, BCL2, cMYC expression levels were suppressed. In addition, immune system-regulated genes such as PDL1, Notch3, Jag1, Hes have been regulated in the hypoxia environment specific to cancer. This research demonstrates the potential use of G4 ligands selectively and for the regulation of many cellular processes such as metabolic programing and immune response. This Project is supported by TÜBITAK Project No: 120C125. References Bochman ML et. al. (2012) Nat Rev Genet. (11):770-80. Neidle S. (2017) Nat. Rev. Chem. 1:0041. Ozsamur NG et al. (2023) Chem. Commun. 59, 2247-2250.

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P-28-017
Enzymatic production of cyclodextrins in *Escherichia coli*

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Cyclodextrin glycosyltransferases (CGTases) are enzymes that catalyse the cyclization of polysaccharides, primarily starch and its derivatives, to produce cyclodextrins (CDs). Despite belonging to the α-amylase family of glycoside hydrolases, CGTases exhibit a distinct catalytic function. CDs, oligosaccharides linked by α-(1→4) glycosidic bonds, have unique structures and properties that render them versatile molecules with a wide range of applications in various industries. Despite the undeniable significance of CGTases, there is no thermostable one on the European market. Thus, our research focuses on expressing a thermostable CGTase capable of producing α-CD and β-CD in varying concentrations. Based on our previous results, we have successfully expressed the target enzyme in *Escherichia coli* from pET28a_CGTase with a yield of 30% of all proteins and demonstrated its activity in β-CD production. The purification methods used so far, IMAC and ANEX, are insufficient, so we aim to optimize the expression of this enzyme in *E. coli* using different expression vectors and adjust purification protocols to obtain a highly purified enzyme. The future steps of our research include improving conditions for cyclization activity and enhancing α-cyclodextrins production via bacterial CGTases. This work aims to provide optimal producing methods of thermostable CGTases, offering new possibilities for industrial applications. The Slovak Research and Development Agency supported the research through grants APVV-19-0196, APVV-20-0284, APVV-21-0215, and APVV-22-0161. *The authors marked with an asterisk equally contributed to the work.

P-28-018
Enhancing the solubility and stability of reverse transcriptase through rational mutagenesis

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Reverse transcriptase (RT) is an enzyme used in RT-qPCR for RNA virus detection, transcriptomics, alternative splicing studies, and other molecular biology applications. However, the solubility of RT often limits its yield, posing challenges for efficient large-scale production. In this study, we applied a rational mutagenesis approach to enhance the solubility and stability of RT. Using bioinformatic analysis with Aggrescan3D 2.0, we identified amino acid substitutions predicted to enhance protein solubility and stability. Two variants were selected for experimental validation. Mutant constructs were generated through overlap extension PCR with mutagenic primers, and the successful incorporation of mutations was confirmed by sequencing. Recombinant RT variants were expressed in *E. coli* BL21(DE3) under the control of the T7 promoter in 50 mL LB medium for 4 hours. Western blot analysis of soluble fractions demonstrated a twofold increase in solubility for one variant compared to the wild-type enzyme. Following purification using immobilized metal affinity chromatography (IMAC), chromatography fractions were analyzed by SDS-PAGE, and the activity of purified enzymes was confirmed. The yield of purified proteins was quantified using the Bradford method. Future work includes evaluating the production levels of these RT mutants in *Vibrio natriegens*, a host known for enhanced recombinant protein expression. The Slovak Research and Development Agency supported the research through grants APVV-19-0196, APVV-20-0284, APVV-21-0215, and APVV-22-0161.

P-28-019
Engineering nano-enabled biopesticides for plant disease management in sustainable agriculture

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The growing challenges of pesticide resistance and their environmental impact underscore the urgent need for greener approaches to plant disease management. In this context, nano-biopesticides (nBioPs) – innovative formulations combining biocontrol agents with nanotechnology – emerge as a promising solution. Here, a one-step ultrasound-laccase-assisted process was used to synthesise lignin-based nanoparticles functionalised with tannic acid, rutin, or stinging nettle (*Urtica dioica*) extract. The resulting nBioPs were characterised for size, stability, and surface properties using dynamic light scattering (DLS) and Fourier-transform infrared spectroscopy (FTIR), confirming the grafting of phenolic compounds. The antifungal and antibacterial activity of the nBioPs was evaluated against two common phytopathogens, *Penicillium expansum* and *Rhizobium vitis*. Among the tested formulations, nBioPs grafted with tannic acid demonstrated the highest antifungal activity, reducing *P. expansum* growth by 28% at 0.2 mg/mL (from 6.3×10 to 4.6×10 log10 spores/mL) and achieving complete inhibition at 1 mg/mL. Similar results were observed for *R. vitis*. In contrast, nBioPs grafted with rutin and nettle extract showed limited antifungal efficacy, likely due to their lower phenolic content (18.4 mg/g and 15.1 mg/g, respectively, compared to 36.2 mg/g in tannic acid-grafted nBioPs). These findings highlight the potential of eco-friendly lignin-based nBioPs as effective tools for controlling plant pathogens and advancing sustainable agriculture.

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P-28-020
A novel protein-based therapeutic approach to radiation-induced fibrosis: an *in vivo* experimental study

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Radiotherapy (RT)-induced chronic inflammation of the capsular tissue leads to an increased expression of profibrotic mediators, contributing to the formation of capsular contracture (CC). This study evaluates the preventive effect of transforming growth factor (TGF)-Beta3 nanoparticles (NP)-loaded chitosan films on CC formation through *in vivo* studies. Rats were randomly assigned to six groups (n = 8): Group 1– Sham; Group 2 – Implant (I); Group 3 – I + RT; Group 4 – I + RT + blank NP-loaded film; Group 5 – I + RT + free TGF-Beta3-loaded film; and Group 6 – I + RT + TGF-Beta3 NP-loaded film. Group 1 served as the control. A pocket was created in the dorsal skin at the fascial attachment on the chest wall. In Groups 4, 5, and 6, films were placed on the outer surface of the implants. Groups 3, 4, 5, and 6 received a single dose of 10 Gy radiation. After 12 weeks, animals were euthanized and the surrounding tissues, along with the implants, were excised for biochemical and histological analyses. Biochemical results showed that the levels of TGF-Beta1, TGF-Beta3, and collagen type III, which were decreased in the I + RT group, were restored to sham levels in Groups 5 and 6. The increase in TGF-Beta1 levels, which were reduced after RT, upon TGF-Beta3 treatment suggests that TGF-Beta3 restores the impaired TGF-Beta3/Beta1 balance, promoting scarless healing. The collagen type 1/type 3 ratio, which was elevated in the I + RT group, decreased in Groups 5 and 6. Vimentin levels were significantly higher in Group 6 compared to both the positive and negative control groups. Furthermore, the increased ACTA2 levels in Group 6 confirmed the prolonged activity of TGF-Beta3 through controlled release. Histological analysis revealed that the thickened collagen and fibrosis induced by RT were significantly reduced in Groups 5 and 6. These results demonstrate the potential of TGF-Beta3-loaded nanoparticle films in preventing CC by promoting scarless healing and modulating the fibrotic response after RT.

P-28-021
Protein-loaded PLGA-b-PEG nanoparticle film formulations: their efficacy in preventing fibrosis

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Fibrosis, characterized by excessive accumulation of extracellular matrix (ECM) proteins, results in structural and functional tissue disturbances. The transforming growth factor-beta (TGF-B) family plays a crucial role in regulating cell differentiation, migration, and proliferation during both tissue repair and fibrosis. While TGF-B1 contributes to disorganized healing, TGF-B3 promotes scarless healing and reverses fibrosis. However, the short half-life of TGF-B3 limits its therapeutic potential when administered parenterally. To overcome this limitation, TGF-B3-loaded nanoparticle (NP) film formulations were developed to enhance bioavailability and prolong the therapeutic effect through controlled release. The study focused on the preparation and characterization of TGF-B3-loaded NPs and chitosan films, including optimization of loading efficiency and encapsulation. *In vitro* release studies showed that the optimal NP formulation released 67% of TGF-B3 over 144 hours, while chitosan films released 40% of the protein over 4 days. The MTT assay revealed no significant cytotoxicity between loaded and unloaded NP films ($p > 0.05$). NP formulations, with sizes ranging from 120 nm to 170 nm and zeta potentials between -20 mV and -25 mV, were selected for passive targeting of chronic inflammation. Therapeutic efficacy was further evaluated in dermal fibroblast cells through gene/protein expression analysis and cell migration assays using RT-PCR, ELISA, and Transwell assays. The results highlight the potential of TGF-B3-loaded PLGA-b-PEG NP films as an effective strategy for promoting scarless tissue healing and reversing fibrosis, without significant cytotoxicity, while offering prolonged therapeutic action. This work provides insight into the optimization of TGF-B3 delivery systems and lays the groundwork for future clinical applications in fibrosis and tissue repair.

P-28-022
Argan seedlings biochemical alterations following the application of subsurface water retention technology, compost, and arbuscular mycorrhizal fungi under open field conditions

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The argan (*Argania spinosa* L. Skeels) ecosystem is severely degrading in arid and semi-arid lands due to climate change, particularly in terms of density loss and reforestation failure. Thus, it is important to adopt innovative effective sustainable practices to optimize the densification and reforestation success

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of the argan tree. The purpose of the present research was to investigate the combined effect of subsurface water retention technology (SWRT) and the use of compost and native arbuscular mycorrhizal fungi (AMF) on the biochemical parameters of field-grown argan seedlings in the Essaouira region, Morocco. In this experiment, one-year-old argan seedlings were transplanted in the absence and presence of SWRT, compost, and AMF. Our findings revealed that the application of these technologies, separately or in combination, induced significant alterations in stress markers and organic osmolytes content and the antioxidant defense of the argan seedlings after one year of transplantation. The combination of the three treatments recorded the lowest values of malondialdehyde (MDA) and H_2O_2 content in the argan leaves with the greatest decline (55%) recorded for MDA content in comparison to the control. This combination also induced the highest values of proteins and soluble sugars (29 and 55% respectively) content and the activities of the antioxidant enzymes including catalase (37% increase), polyphenol oxidase (166% increase), and peroxidase (100% increase). In summary, the combined application of SWRT, compost, and AMF may be used as a valuable strategy to promote the success of argan reforestation and to limit soil erosion and desertification in arid and semi-arid climates.

P-28-023
Advanced biocontained microbial chassis: multicopy genomic integration of orthogonal translation system

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The use of bacterial cells for biotherapeutic manufacturing raises significant concerns regarding biosafety and the potential for lab leakage, particularly when producing harmful compounds. To address these challenges, we engineered a synthetic auxotrophic strain of *Escherichia coli* as a model system by integrating an orthogonal translation system (OTS) into its genome using CRISPR-associated transposase technology. This OTS consists of an evolved bioorthogonal aminoacyl-tRNA synthetase and its cognate tRNA from *Methanocaldococcus jannaschii*, enabling site-specific incorporation of a synthetic amino acid. The functionality of the engineered strain was assessed by expressing superfolder green fluorescent protein (sfGFP) containing three amber (UAG) codons in its open reading frame, which allowed the incorporation of meta-(ortho-(2-nitrobenzyl))-3,4-dihydroxy-phenylalanine (*m*-oNB-Dopa). Strains with varying copy numbers of the OTS cassette were evaluated, and the strain BNH24, containing four copies, exhibited optimal performance. To establish auxotrophy, two essential genes, SerS and MurG, were further modified by introducing amber stop codons in place of tyrosine residues using CRISPR-Cas9-assisted genome editing. The resulting synthetic auxotrophic strain, MS47, exhibited no growth in rich media such as Luria-Bertani (LB) broth. However, supplementation with 50 μM oNB-Dopa fully restored growth, demonstrating a growth curve pattern similar to that of the wild-type *E. coli* strain. The newly developed synthetic *E. coli* strain exhibited an escape frequency of 10^{-8}, making it a promising candidate for biotechnological applications requiring enhanced biosafety.

P-28-024
Fabrication and *in vitro* evaluation of a multifunctional PLA/gelatin/cinnamon membrane for wound healing applications

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Designing an ideal wound dressing is still problematic in achieving effective wound healing. To address this issue, electrospun membranes (EM) containing different ratios of *Cinnamomum zeylanicum* extract, polylactic acid (PLA), and gelatin were manufactured with electrospinning to produce antibacterial wound dressing. The extract was characterized by FT-IR and gas GC-MS. EM morphology was analyzed with SEM. Hydrophilic properties were studied by contact angle measurement, with the increase in extract concentration, diminished contact angle values were determined. Mechanical properties were evaluated and the CE7.5 displayed better UTS and E values than others. Also, biodegradability, swelling, and release properties were checked and found as 29.60 ± 7.20 and $542.1 \pm 48.3\%$, 66.9% for CE7.5, respectively. Antibacterial activity was observed in CE7.5 and CE10 against *E. coli* and *S. aureus*. EMs were shown to allow adhesion and proliferation of the fibroblast cells. At the highest concentration (CE10), $111.7 \pm 5.6\%$ and $96 \pm 12.375\%$ cell viability was detected in fibroblasts and differentiated/LPS-induced THP-1 cells, respectively. Annexin-V/PI staining demonstrated that $97.95 \pm 1.63\%$ of cells were viable in the CE7.5 and only $1.85 \pm 1.49\%$ necrotic cells were detected in the same population. Enhanced wound closure (42% compared to non-treated cells) was observed with CE7.5. The anti-inflammatory properties were investigated at the transcription and protein level via qRT-PCR and immunofluorescent staining, respectively. Although any significant change was not detected at the transcription level of pro-inflammatory TNF-α, NF-κB, and IL-6 genes in fibroblast cells, treatment of differentiated/LPS-induced THP-1 cells with conditioned CE7.5 membrane downregulated Iba1 and tau proteins up to 45.1 and 51.1%, respectively. The overall results indicate that the "CE7.5" electrospun membrane might be a suitable wound dressing material to prevent problems related to wound healing.

P-28-025
Effect of CML30-type gene knockout on anthocyanin formation in *Solanum tuberosum*

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Calcium (Ca^{2+}) serves as a vital and dynamic component, acting as one of the key messengers in plant cell signaling. Four key protein families that possess EF domains are calmodulins (CaMs), calmodulin-like proteins (CMLs), calcium-dependent protein kinases (CDPKs), and calcineurin B-like proteins (CBLs). CMLs are specialized Ca^{2+} sensors unique to plants, characterized by a plant-specific EF-Hand domain. Taking into account the important influence of CML family genes on plant metabolism, a gene of this family was chosen for the study, the sequence of which was identified as CML30-type according to

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the *Arabidopsis thaliana* database. The genetic transformation of Desiree variety *Solanum tuberosum* potato plants was achieved using an *Agrobacterium tumefaciens*-mediated approach with a vector derived from the plasmid pKSE401, targeting the aforementioned genes through the CRISPR/Cas9 system. Currently, knockout of the CML30-type gene has been verified in eight lines. Molecular analysis utilizing T7 endonuclease-I was performed, allowing for the selection of lines for further investigation, with sequencing confirming the genetic editing of the intended gene regions. Sometimes, CML genes act as negative regulators in the formation of plant immune responses to various pathogens, so it is planned to study the obtained mutant plants to the known potato pathogen *Ralstonia solanacearum*. Further plant studies revealed marked differences in stem morphology and reduced anthocyanin content in the leaves and peel of potato tubers in one of the resulting mutant lines. Biochemical and molecular analyses are being carried out to further elucidate the effect of this gene expression on the metabolism of potato plants. The work was supported by National Laboratory project No. RRF-2.3.1-21-2022-00007, ECOST STSM grant and EMBO Solidarity Grant No. 5422-2023.

P-28-026
Functionalized nanostructured lipid carriers: physicochemical characterization and cellular analysis for enhanced uptake and binding

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The composition of nanostructured lipid carriers (NLCs) plays a pivotal role in modulating their physicochemical, mechanical, and surface properties, including shape, size, hardness, and surface charge. These properties are critical in overcoming various biological barriers – such as cell membrane, tumor interstitial matrix, and blood–brain barrier – and facilitating cellular internalization. This study aims to investigate the optimal physicochemical properties and lipid-surfactant compositions of NLCs formulations to enhance cellular uptake to be able use in drug delivery purposes. NLCs were prepared by using the melt-emulsification technique, and the stearic acid and two distinct liquid lipids, oleic acid and docosahexaenoic acid (DHA), were selected as the solid lipid matrix and the liquid lipid, respectively. A comprehensive analysis of the crystallinity, physicochemical, and morphological properties of NLCs were performed by using dynamic light scattering and atomic force microscopy. NLCs exhibited homogeneous dispersion with hydrodynamic sizes ranging from 100 to 200 nm. To assess their biological interactions, cellular response analyses, including cytotoxicity, cellular uptake, and cellular binding, were conducted in two different cell lines: HUVEC-derived carcinoma, ECV304, and glioblastoma, U87. Cellular viability assays demonstrated no significant reduction in cell viability at NLC concentrations up to 5 μg/ml. Notably, DHA-formulated NLCs exhibited superior cellular binding and uptake in both cell lines compared to other formulations. Consequently, this study offers significant insights into the design of NLC formulations for enhanced drug delivery across biological barriers and enhanced cellular internalization, emphasizing the critical role of lipid and surfactant selection in modulating cellular responses.

P-28-027
Activity of mucus of the gastropod *Arion vulgaris* against yeast-like fungi and bacteria

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A land gastropod mollusk of the species *Arion vulgaris* (AV), nicknamed the Spanish slug, has spread widely in Russia in the last decade, causing significant damage to many agricultural crops. For the first time, such a slug was reliably registered in Crimea in 2013 and was then registered in the southern territories of our country. The highest fertility, a wide range of feed and low popularity among predators due to thick and viscous mucus, allow these mollusks to achieve a high population density. A promising direction in biotechnology is the use of mollusk mucus as antimicrobial agents. Mucus also contains proteins: collagen, elastin, glycolic acid, allantoin. AV mucus was collected by students of the agronomy specialty of the Agrarian and Technological Institute, diluted in phosphate-buffered saline (PBS), and the supernatant was tested against yeast-like fungi and bacteria using the Bauer-Kirby method. The mucus was passed through a series of columns to separate the protein size, and these experiments proved that the antimicrobial substances were in the range of 50 to 100 kDa. The results showed that AV mucus had a strong antifungal effect against several strains of the genus *Candida* (*C. albicans*, *C. parapsilosis*, *C. dubliniensis*) and a weak effect against *Cryptococcus neoformans* and *Nakaseomyces g*labratus. AV mucus also inhibited the growth of *Pseudomonas aeruginosa* and *Staphylococcus aureus* well, but poorly against *Escherichia coli*. Museum cultures of microorganisms were used in the experiment. This mucus can be used as a bioconservant for long-term storage of food and seeds. Perhaps, with clinical strains there will be a different activity. This study has been supported by the RUDN University Strategic Academic Leadership Program.

P-28-028
Investigation of molecularly imprinted nanoparticles for transdermal drug release

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Fluticasone propionate (FP) is an inhaled drug commonly used for lung diseases and is also a corticosteroid drug with strong anti-inflammatory effects. It is used as an anti-inflammatory drug in the treatment of many dermatological diseases such as atopic dermatitis. When applied through the skin, its dermal absorption and bioavailability are low. The aim of this study was to prepare temperature-sensitive MIP nanoparticles for the controlled release of FP. For this purpose, MIP nanoparticles were synthesized by surfactant-free emulsion polymerization method using temperature sensitive N-isopropylacrylamide (NIPAM) and 2-hydroxyethylmethacrylate (HEMA) monomers with high biocompatibility. The synthesized MIP nanoparticles were

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characterized using attenuated total reflectance-Fourier transform infrared spectrometry (ATR-FITR), Scanning Electron Microscopy (SEM), zeta sizer analysis, and their specific surface areas were calculated. After investigating MIP nanoparticles' drug adsorption capacity and determining the optimum drug loading conditions, the MIP nanoparticles' selectivity was investigated. Finally, drug release studies were carried out from MIP nanoparticles. The molecular imprinting technique provided a slower, more controlled release. The data showed that the synthesized temperature-sensitive MIP nanoparticles have proven to be suitable for use in the controlled release of FP by transdermal route. Acknowledgement: This study was supported by TUBITAK-2209-A University Students Research Projects Support Program with project number 1919B012202229.

P-28-029
Engineering of novel alginate-based gels with modified endolysin and bacterial alginates with enhanced antibacterial activity against ESKAPE pathogens

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New approaches and the development of innovative antimicrobial agents are urgently required to overcome bacterial resistance worldwide. Here we describe the results of the development of a gel formulated using bacterial alginate, incorporating a modified endolysin as the active component. For this, a recombinant modified endolysin with a broadened activity spectrum was constructed using genetic hybridization of LysSi3 endolysin with antimicrobial peptide (AMP) as an agent capable of permeabilizing the outer membrane of Gram-negative bacteria. For the modification a series of previously researched AMPs were assessed, and dose dependence, the spectrum of antimicrobial activity against clinical isolates of ESKAPE bacteria and their biofilms, the rate of action, and potential toxic effects were determined. Based on the obtained data, we selected a modification with an amphiphilic alpha-helical peptide consisting of leucine and lysine residues resulted in engineered LysSi3-LK. As carriers, we considered several bacterial alginates in the form of sodium salts, obtained during the cultivation of *Azotobacter vinelandii* 12. As a result, two alginates with high molecular weights were chosen for research: ALG2 and ALG3 (279 and 418 kDa, respectively), differing in acetylation and M/G monomer blocks ratios. Gels based on these biopolymers demonstrated significant differences in dissolving rate and viscosity. Both experimental antimicrobial compositions containing the endolysin in concentration 0.5 mg/ml were effective against MDR *Acinetobacter baumannii* strains. Stability studies of the resulting formulations showed that lysin did not degrade in the gel and retained 100% efficacy against growing bacterial culture within 3 months. Thus, the developed gels represent a promising form for further research as a potential ready-to-use topical antibacterial formulations. This research was funded by the Russian Science Foundation (RSF), grant no. 23-74-10027, https://rscf.ru/project/23-74-10027/.

P-28-030
An innovative biocatalytic process for converting PET waste into amino acids

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The breakdown of single-use plastic items, primarily composed of polyethylene terephthalate (PET), through enzymatic processes has become a significant achievement, paving the way for the production of valuable compounds. In this study, we initiated the degradation of post-consumer PET using an engineered variant of the leaf-branch compost cutinase (LCC, a thermostable enzyme isolated by a metagenomic approach), namely the S101N/F243T-ΔLCC variant [previously published in: Pirillo et al., 2023, FEBS J 290(12) 3185–3202]. This treatment resulted in the efficient depolymerization of PET to terephthalic acid (TPA) and ethylene glycol at a temperature of 37°C, a condition that is compatible with the growth of *E. coli* cells in the subsequent bioconversion steps. TPA was subsequently utilized by an engineered *E. coli* RARE strain allowing its conversion into 2-pyrone-4,6-dicarboxylic acid. This compound was further transformed into pyruvate, a process carried out by recombinant enzymes to prevent the depletion of pyruvate due to cellular metabolic activities. Ultimately, the pyruvate underwent an enzymatic amination to produce enantiopure alanine and other relevant biomolecules. This novel biosynthetic pathway comprising twelve enzymes derived from four distinct microorganisms was optimized using recombinant proteins and whole-cell systems that harbour different modules of the process. As a result, post-consumer PET waste was converted into alanine at gram-per-liter concentrations, achieving an overall yield of approx. 0.3 g alanine per g of PET. This study demonstrates the feasibility of bio-converting plastic waste into valuable biomolecules through a sustainable biotechnological approach. *The authors marked with an asterisk equally contributed to the work.

P-28-031
Diversification of a fluorescent RNA aptamer for intracellular RNA imaging via a CRISPR/Cas9-based directed evolution platform

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Functionality of RNAs in cells is organized by the dynamic processes such as folding, translocation and degradation. Genetically encoded fluorescent RNA aptamers are unique tools for real-time tracking of the dynamics of an RNA-of-interest in live cells. However, despite being promising, aptamers developed with current cell-free *in vitro* technologies have major shortcomings such as low intracellular stability and brightness, due to their incompatibility with the harsh cellular environment causing short intracellular lifetime. Our study aims to develop a mammalian cell-based directed evolution platform to engineer a fluorescent RNA aptamer of superior intracellular stability with enhanced brightness. Our strategy is based on creating double-strand breaks (DSBs) with CRISPR/Cas9 on the target gene encoding the aptamer. These DSBs lead to diversification of the aptamer gene

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through the DSB-activated DNA repair mechanisms. We then apply a high-throughput directed evolution scheme to enrich the variant cells that express improved variants. We perform our diversification experiments on the recently developed Pepper aptamer. We cloned Pepper gene sequence at the 3' end of the mRuby3, a red fluorescent protein used as an expression marker, in the pcDNA5/FRT mammalian expression vector. The pcDNA5/FRT/mRuby3-Pepper construct is then stably integrated into the genome of Flp-In-293 cells using Flp/FRT recombination. CRISPR/Cas9-mediated diversification of the Pepper sequence is performed in the stable mRuby3-Pepper Flp-In-293 cell line. DSB-induced mutations are initially examined by T7 Endonuclease I assay using the genomic DNA library. Next, the mutated regions are cloned into plasmid vectors for Sanger sequencing to inspect the mutational spectrum. Overall, we have developed a cell-based directed evolution platform and diversified the fluorescent Pepper RNA aptamer via CRISPR/Cas9 to engineer an improved variant. This work is supported by TUBITAK 1001 (project ID: 123Z765).

P-28-032
Development of highly active enzyme complexes produced by *Penicillium verruculosum* recombinant strains

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Annually, 1–1.5 billion tons of wood waste are generated worldwide, with most (up to 70%) remaining unprocessed, remaining in logging areas or being burned. Lignocellulosic biomass consists of cellulose (30–60%), hemicelluloses (20–40%), and lignin (10–25%), forming a heterogeneous matrix. This makes it a multifunctional resource for the bioeconomy, enabling the production of a wide range of products and energy. The most environmentally friendly method for extracting technical sugars from cellulose and hemicelluloses is enzymatic hydrolysis. Specific enzymes convert them into C5 and C6 sugars (glucose, xylose, arabinose, etc.), which serve as the basis for producing biofuels, organic acids, bioplastics, biogas, vitamins, and more. The primary sources of industrial enzymes are filamentous fungi such as *Penicillium*, *Trichoderma*, and *Aspergillus*. The fungus *P. verruculosum*, isolated at the Enzymes Biotechnology Lab of the Federal Research Center of Biotechnology of the RAS, has a powerful hydrolytic complex. Due to its high secretory capacity (up to 60 g/L of total protein) and optimized fermentation schemes, *P. verruculosum* is a leader in Russia and is used to produce enzyme preparations (EPs) at the Agroferment LLC plant (Tambov Region). EPs based on *P. verruculosum* completely hydrolyze MCC within 48 hours. However, for more complex substrates (aspen and pine wood, bagasse, miscanthus, rice husk, etc.), new strains with an optimized enzyme complex have been developed using genetic engineering. For example, xylan hydrolysis requires the synergistic action of not only cellulases but also hemicellulases, including β-xylosidase (XLD), which converts xylobiose into xylose, increasing hydrolysis efficiency. The new EP PV-β-XLD, with increased XLD content, yielded nearly 70 g/L of reducing sugars (RS) during bagasse hydrolysis, which is 20% more than with the original strain's EP (55 g/L RS). Thus, adding XLD to the enzyme complex improved bagasse hydrolysis efficiency.

P-28-033
Assessing the potential of *Trametes versicolor* and *Cunninghamella* species to biodegrade amitriptyline and fluoxetine: *in vitro* toxicity evaluation of mycological transformation products

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A rising incidence of depression and anxiety disorders is resulting in the rapid expansion of antidepressant consumption worldwide. The leakage of these agents into the aquatic environment and the difficulty of removing them by conventional methods have led to antidepressants being identified as newly emerging pollutants. The use of selective microorganisms to degrade xenobiotics may provide an environmentally beneficial method of removing drug contaminants. The aim of the present study was to verify the ability of fungi *Trametes versicolor* and *Cunninghamella* spp. (*C. echinulata*, *C. blakesleeana* and *C. elegans*) to degrade the antidepressants amitriptyline and fluoxetine. In addition, *in vitro* ecotoxicity (Microtox system) and mutagenicity (the Ames microplate assay) of the obtained fungal biotransformation products were evaluated. Mycological biotransformation was carried out for 7 days, followed by analysis of the obtained samples using UHPLC/MS. *T. versicolor* showed a negligible ability to degrade amitriptyline and fluoxetine, metabolizing only 1% and 2% of the parent compounds, respectively. The biotransformation capacity of *Cunninghamella* was diversified but promising. *C. echinulata* transformed amitriptyline with 100% efficiency, and producing three derivatives, including the main metabolite generated by dehydrogenation, while fluoxetine was most efficiently degraded by O-dearylation by *C. elegans*, resulting in one main derivative. *In vitro* toxicity studies showed no mutagenicity of the obtained products and their lower ecotoxicity compared to their parent compounds. The results confirm the ability of *Cunninghamella* fungi to remove amitriptyline and fluoxetine from the aquatic environment. Acknowledgements The project was supported by the National Science Center Grant No 2020/37/B/NZ7/02546.

P-28-034
Abstract withdrawn

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P-28-035
Study of *Lactobacillus leichmannii* NDT II properties under transglycosylation reaction conditions: influence of pH value on reaction progress and target nucleosides yield

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Enzymes belong to the class of glycosyltransferases used to produce biologically active compounds by enzymatic transglycosylation. Enzymatic transglycosylation is a stereo- and regiospecific reaction that allows obtaining target nucleosides of high purity, following the principles of 'green chemistry'. Transglycosylation catalysed by *Lactobacillus leichmannii* nucleoside deoxyribosyltransferase type II (*L. leichmannii* NDT II) in different value pH media were studied. Some natural 2′-deoxynucleosides (dIno, Thd) and modified purine nucleoside 7-methyl-2′-deoxyguanosine (7-mdGuo) were used as the donors of sugar moiety, various purine and pyrimidine heterocyclic bases were used as the acceptors. Both dIno, Thd, and 7-mdGuo have been shown to be suitable substrates, whereas modifications in the deoxyribose ring (4′-N_3-Thd) result in a loss of substrate properties of the latter. It was revealed that the pH value of the reaction mixture can affect the reaction rate if the heterocyclic base contains a carbonyl group 6 in the case of purine bases or 4 position in the case of pyrimidine bases. The highest rate of transglycosylation is observed at pH 5.5 and the lowest at pH 8.5. It was also found that in the case of the presence of a substituent in the 5-position of uracil, the pH value also influences the yield of the reaction of pyrimidine nucleosides. Maximum yields are observed at pH 7.0, at pH 5.5 the yield decreases by about 10%, and at pH 8.5 there is a significant decrease in yields. The obtained results can be used to develop environmentally friendly biotechnological methods for synthesis of practically important nucleosides. This work was supported by the Russian Science Foundation (project No. 24-24-00542).

P-28-036
Biochemical composition and functional application of enzyme-assisted extracts from leaves of *Cannabis sativa* L.

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Enzyme-assisted extraction (EAE) has recently gained attention for its effectiveness and eco-friendly manner of extracting valuable compounds, enhancing their nutritional value at a lower cost. *Cannabis sativa* L. is a widely distributed and one of the oldest plant species in the Cannabaceae family. However, the plant leaves have not been widely studied for their biochemical and functional applications. This study aimed to investigate the biochemical composition, antioxidant activity, and prebiotic potential of EAE from *C. sativa* leaves. Extracts were obtained under optimal conditions (3:15 h of extraction, temperature 45°C, pH 4.9, and 1% enzyme (v/w of dry matter)). The extractions were performed using Viscozyme L, Cellulase enzymes, and control without enzyme. The antioxidant activity of *C. sativa* leaf extracts was assessed using $ABTS^{\bullet+}$, FRAP, and ORAC assays, while the total phenolic content was obtained using the Folin-Ciocalteu method. The prebiotic potential of *C. sativa* leaf extracts was evaluated in an *in vitro* screening model using probiotic strains *Lactobacillus casei* 01 and *Bifidobacterium animalis* BLC. Extracts were tested at 5% (v/v) and 50% (v/v) concentrations. The results indicated that the yield after EAE with Viscozyme L enzyme mixture increased by up to 44% compared with EAE with Cellulase enzyme and control. Moreover, the highest antioxidant activity was established in EAE with Viscozyme L enzyme extract within all tested assays. Furthermore, all tested extracts were established to possess prebiotic potential with tested probiotic strains, but the greatest results were obtained in EAE with Viscozyme L enzyme mixture extract. In conclusion, EAE may be an effective and sustainable method for biorefining bioactive compounds, potentially applicable in high-value functional foods and nutraceuticals. Acknowledgment: This work was supported by the Research Council of Lithuania research and experimental development project No. S-MIP-24-75.

P-28-037
Characterization of a recombinant multifunctional enzyme CBM2a-GH3 with enhanced catalytic activity towards cellulosic substrates

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Cellulose, a major component of plant cell walls, is a promising renewable biomass for the production of biofuels and chemicals. However, its hydrolysis into simple sugars remains a significant bottleneck. In this study, a recombinant multifunctional cellulase CBM2a-GH3 was cloned, expressed, and characterized for its biochemical properties and substrate specificity. The enzyme consists of a carbohydrate-binding module (CBM2a) fused to a glycoside hydrolase family 3 (GH3) catalytic domain. Our results show that CBM2a-GH3 exhibits high catalytic efficiency towards various cellulosic substrates, including crystalline cellulose, carboxymethyl cellulose, L-arabinose, D-galactose, and α-lactose monohydrate. The enzyme displayed optimal activity at pH 7 and 50°C, with a high stability across a wide range of pH and temperature conditions. Furthermore, the kinetic parameters of CBM2a-GH3 were evaluated, revealing a higher catalytic efficiency towards L-arabinose and D-galactose. The enzyme activity was also enhanced by the presence of Na^+ ions and was relatively unaffected by other metal ions and additives. Thin-layer chromatography analysis confirmed the hydrolysis of various cellulosic substrates into simple sugars. Overall, our findings suggest that CBM2a-GH3 is a promising candidate for

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the saccharification of cellulosic biomass, with potential applications in the production of biofuels and chemicals.

P-28-038
Hot enzymes, cool solutions: sustainable PET hydrolysis catalysed by a novel thermophilic enzyme

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Plastic waste accumulation is one of the most overwhelming environmental issues of this century[1]. Particularly, single-use products made from polyethylene terephthalate (PET) have heavily contributed to this problem, considering that PET alone accounts for about 6.2% of global plastic production[2]. The discovery of enzymes capable of hydrolysing PET (PHEs) has sparked research into more environmentally friendly methods for managing PET waste[3]. This study proposes a one-step process for the depolymerization of PET at mid-high temperatures (60°C) within 4 days, exploiting the enzymatic activity of a novel thermophilic PHE, called PP_EG. PP_EG has been identified from geothermal metagenomic samples and produced in recombinant way in its active form. The characterization of PP_EG demonstrated its activity on high-purity substrates such as bis(2-hydroxyethyl)-TPA (BHET) and nanoPET, as well as PET waste from food packaging materials like food trays. The depolymerization process was set up and optimized on PET waste by incubating it with different concentrations of PP_EG, by monitoring its degradation over time, in terms of macro and microscopic modification of the solid substrate and by analysing products formation through HPLC. These findings suggest that PP_EG could serve as a key enzyme for PET recycling, offering a sustainable approach to PET waste management. The study advances the understanding of PHEs and opens new possibilities for more efficient and environmentally friendly recycling processes. This work is dedicated to the memory of our young colleague Prof. Giuseppe "Pino" Perugino References [1] Odigbo C., Adenipekun C., Oladosu I., Ogunjobi A. (2023). Environmental Monitoring and Assessment, 195, 585. [2] Guo W., Duan J., Shi Z., Yu X., Shao Z. (2023). Communications Biology, 6, 1-13. [3] Benavides Fernández C.D., Guzmán Castillo M.P., Quijano Pérez S.A., Carvajal Rodríguez L.V. (2022). SN Applied Science, 4, 263.

P-28-039
Bioactivities in fungal biomass grown on buckwheat husk

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Buckwheat husk, the main byproduct of buckwheat processing, is rich in fiber and has good potential as a source of bioactive peptides and polyphenols. However, recovery of these compounds is hindered by the husk's complex supramolecular structure, which also impairs its use in staple food. Nevertheless, husk can be a substrate for edible mushrooms (*Ganoderma annularis* and *Pleurotus ostreatus*). The resulting fungal/husk biomass may help to overcome matrix effects while retaining bioactives or improving their bioavailability. This study addresses the overall macromolecular organization and bioactive properties of a mixed fungal/husk biomass. Structural changes were relevant, regarding the α-/β-glucan ratio and the soluble/insoluble fiber ratio. Bioactive compound profiling highlighted increased ergosterol levels and a marked peptide release. Polyphenol profiling showed a decrease in total phenolics, but an increase in free polyphenols, leading to an increase in their activity as α-amylase inhibitors and in full retention of their anti-inflammatory ability in a Caco 2 cell model. Thus, the physicochemical and bioactivity properties of fungal/buckwheat husk biomass suggest a possible use of these sustainable materials as ingredients in functional food formulations. Acknowledgement. This investigation is partially supported by National Recovery and Resilience Plan (NRRP), Mission4 Component 2 Investment 1.3 -Call for tender No. 341 of 15/03/2022 of Italian Ministry of University and Research funded by the European Union–NextGenerationEU, in the frame of the project: Research and innovation network on food and nutrition Sustainability, Safety and Security (ON Foods). *The authors marked with an asterisk equally contributed to the work.

P-28-040
Eliciting sustainability: biochemical strategy for the valorization of the agrifood by-products

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Valorizing bioactive compounds from biomass, by-products, and waste is crucial for the circular economy and nutrition sustainability. Agri-food by-products retain valuable bioactive molecules capable of eliciting beneficial biological responses, particularly biodefense and biostimulation, with promising applications in agriculture and food technology. However, their complex matrix hinders efficient extraction and bioavailability, as supramolecular structures influence molecular interactions during biological and technological processes. Biochemical strategies, including enzymatic and chemical treatments, are essential to optimize bioactive recovery and enhance their functional potential. This study explores enzymatic approaches to improve bioactive extraction within a circular economy framework applied to the agri-food system. Proteins from okara and tomato seeds were enzymatically digested using trypsin-like enzymes and cysteine endopeptidases, generating a peptide mixture characterized to evaluate its bioactivity through *in vitro* assays. The peptide mixtures have been shown to trigger a biodefense response against the agriculturally significant fungus *Fusarium graminearum*, as demonstrated through *in vivo* assays. Further investigations aim to elucidate the molecular mechanisms underlying this bioactivity and assess its broader applicability in sustainable agriculture. These findings demonstrate the potential of enzymatic strategies to enhance bioactive compound extraction from agri-food by-products, supporting the development of sustainable solutions for agriculture and food technology while reducing waste and environmental impact. This

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study was conducted within the Agritech National Research Center, funded by the EU Next-GenerationEU (PNRR – M4C2, Investment 1.4 – D.D. 1032 17/06/2022, CN00000022). The authors are solely responsible for its content; the EU and the European Commission have no liability.

P-28-041
PET degradation by co-culture of recombinant *Escherichia coli* cells with PETase and MHETase

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The persistence of plastic waste in nature is a significant environmental challenge. Although PET (polyethylene terephthalate) can be mechanically recycled, its biodegradability remains low. PETase and MHETase enzymes isolated from the bacterium *Ideonella sakaiensis* offer a biotechnological recycling strategy by decomposing PET into its monomers. In this work, PETase and MHETase genes were separately cloned into the pETDuet-1 vector, expressed in *E. coli* BL21, and purified via Ni-NTA and S-tag affinity chromatography. SDS-PAGE and western blot analyses confirmed protein expression. Enzymatic activity was confirmed using p-nitrophenyl butyrate (pNPB) at 405 nm. PET degradation studies were performed in a minimal medium at pH 7.0 and 37°C for a duration of one week. In weight loss measurements, the cells with PETase achieved 1.5% PET degradation, the cells with MHETase achieved 0.7% degradation, and the PETase-harboring and MHETase-harboring cells together degraded 2.6% of PET. The cells having pETDuet-1 without PETase and MHETase genes was used as control group. HPLC analysis (Cosmosil 5C18-AR-II column, UV 254 nm) revealed that PETase produces intermediate products bis(2-hydroxyethyl) terephthalate (BHET) in the medium, whereas MHETase promotes the degradation of these products and enhances the quantity of the final product (terephthalic acid: TPA). Structural and chemical changes on the PET surface detected through FTIR and SEM analyses prove PET degradation by recombinant E. coli cells. The results show that PETase and MHETase enzymes show their synergistic effect and enhance PET biodegradation. This study is supported by TUBITAK (Project no: 123Y405). *The authors marked with an asterisk equally contributed to the work.

P-28-042
In vitro assessment of biofermentative unsulfated chondroitin, hyaluronic acid and platelet rich plasma on osteoarthritic chondrocytes

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Glycosaminoglycans (GAGs), usually hyaluronic acid (HA) and chondroitin sulfate (CS), are employed for osteoarthritis (OA) symptoms treatment by intra-articular injections. Recently, a pharma-grade biofermentative unsulfated chondroitin (BC), was obtained by biotechnological process. Also, platelet-rich plasma (PRP) showed positive effects, but many clinical studies lack proper control groups. In this study, the viscoelastic features and the relation with specific biochemical pathways activation of BC coupled with high molecular weight HA (HHA/BC) and also to PRP (HHA/BC + PRP) were explored. Flow curves and mechanical spectra were obtained to assess the properties of these formulations in relation to synovial fluid characteristics. Chondrocytes were isolated from OA patients joints and cultivated *in vitro* for 24 or 48 h in the presence of HHA/BC (40 mg/mL) diluted 4-fold or HHA/BC + PRP diluted 4-fold and 20-fold respectively. The affection of selected biomarkers was evaluated by quantitative reverse transcription PCR (qRT-PCR), western blotting (WB), immunofluorescence (IF) and multiple ELISA assay. Additionally, chondrocyte monolayer scratch assay was performed to test the formulations repair potential using time-lapse video-microscopy. HHA/BC + PRP exhibited a consistent rheological profile, providing support for the potential application for intra-articular injections. Furthermore, the maintenance of cell phenotype was confirmed by the analyses of collagen type 2A1 (COLII) and aggrecan (AGN) expression. In addition, PRP addition enhanced GAGs ability to reduce specific pro-inflammatory and degradative OA related players (interleukin-6 (IL-6), NF-kB and metalloprotease -13 (MMP-13)). Both tested solutions similarly increased the rate of wound repair. These outcomes allowed us to better evaluate the biochemical and biological properties of these innovative formulations for potential applications in OA management.

P-28-043
Promiscuous NAD-dependent dehydrogenases enable efficient bacterial growth on the PET monomer ethylene glycol

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Ethylene glycol is widely used as monomer of the ubiquitous plastic polyethylene terephthalate (PET). Its global production amounts to more than 50 million tons per year, and it constitutes an environmental pollutant of increasing concern. Although it is generally accepted that bacteria oxidize ethylene glycol to use it as growth substrate, the enzymes involved in this process are not well understood. Here we show that the soil bacterium *Paracoccus denitrificans* is able to assimilate ethylene glycol efficiently via NAD-dependent alcohol and aldehyde dehydrogenases. Using comparative proteome analysis, we identify a previously unknown gene cluster that is strongly expressed in the presence of ethylene glycol. We report the kinetic parameters and cryo-EM structures of EtgB and EtgA, the key enzymes encoded by this *etg* gene cluster. These novel biocatalysts pave the way for more efficient biotechnological conversion of ethylene glycol. We furthermore show that the transcriptional activator EtgR controls expression of the *etg* gene cluster. Directed evolution of *P. denitrificans* on ethylene glycol

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results in faster growing strains, which is enabled by increased activities of EtgB and EtgA. Bioinformatic analysis reveals that the *etg* gene cluster and variants thereof are widely distributed among Proteobacteria, suggesting a previously underappreciated role of NAD-dependent dehydrogenases in microbial ethylene glycol assimilation.

P-28-044
The red alga *Porphyridium* as a production platform for a chimeric hepatitis B surface antigen with strong cellular and humoral immunogenicity

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Microalgae have emerged as promising production factories for the production of recombinant proteins, including enzymes and proteinaceous biopharmaceuticals. The red microalga *Porphyridium purpureum* displays particularly favorable transgene expression properties, due to the episomal maintenance of transformation vectors at high copy numbers in the nucleus. In this study, we investigated the potential of *Porphyridium purpureum* as a platform for producing a candidate vaccine against Hepatitis B Virus (HBV). Our findings demonstrate that expressing a chimeric HBV surface antigen in the alga enables the efficient formation of virus-like particles (VLPs). Additionally, we developed a purification protocol for these VLPs and conducted vaccination trials in experimental animals. We showed that the HBV antigen produced in *Porphyridium* induces stronger humoral and cellular immune responses than a commercially available yeast-derived HBV vaccine. Moreover, it generates virus-neutralizing antibodies that are effective against various HBV variants, including those containing vaccine-escape mutations. This work highlights *Porphyridium purpureum* as a highly promising platform for the production of vaccines and other protein-based biopharmaceuticals.

P-28-045
Hydrogel-embedded mesenchymal stem cell secretome for enhanced cell proliferation and wound healing

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Mesenchymal stem cell (MSC)-based therapies have emerged as an effective tool for skin regeneration and wound healing due to the inherent ability of MSCs to respond to tissue damage and their high secretory potency. MSC secretome includes a large panel of cytokines, growth factors, extracellular vesicles, lipids, and other bioactive molecules that promote tissue regeneration and modulate the wound microenvironment. It represents a cell-free alternative therapy inducing cell proliferation, migration, and angiogenesis. To achieve targeted distribution and enhanced tissue retention, the MSC secretome can act as cargo incorporated into biocompatible matrices. Here, we demonstrate the key results of the engineering and functional activity of the hydrogel-embedded MSC secretome enriched with cytokines such as GRO, MCP-3, IL-6, IL-8, MCP-1, and G-CSF. The MSC secretome prepared using different tissue sources and varying culturing conditions was incorporated into a hydrogel matrix by a sustained and gentle approach persevering biological activity of the secretome components. The *in vitro* biocompatibility of the resulting secretome-loaded hydrogel using model human MSCs and keratinocyte cell lines revealed induced cellular proliferation and migration. Additionally, *in vivo* studies performed with an ischemic excisional wound model on minipigs showed significantly accelerated wound closure, enhanced re-epithelialization, and reduced scar formation compared to control treatments. The obtained results demonstrate the efficacy of the designed system for wound healing applications and pave the way for its further use as a single therapeutic and a part of complex regenerative technologies for skin restoration. The study was supported by the Russian Science Foundation, grant 24-45-10012.

P-28-046
Protein base-molecular sponges for the elimination of organic and metal pollution of water

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Recently, the development of molecular sponges based on the utilization of proteins for their synthesis has attracted research attention for their potentiality to remove pollutants from water. In our study, we utilize heat-induced human serum albumin (HSA) fibrillation with the utilization of a green cross-linking agent (citric acid) and compare it with the one obtained with formaldehyde to create our molecular sponges. First, we optimize temperature and time to obtain their fibrillation in the range of 65 to 90°C, obtaining the optimum around 80°C for 20 minutes. After freezing and lyophilization, the obtained protein base-molecular sponges showed high porosity, ultralight weight and good deformability. When they are immersed in samples spiked with defined amount (ppm) of metal (Fe^{2+}, Cu^{2+}, Zn^{2+}, Hg^{2+} and Ni^{2+}) and organic (crystal violet, phenol red and n-hexane), both sponges show the potential to remove all the tested metal and crystal violet, creating a completely biocompatible aerogel. Through adsorption by the aerogel, we reached excellent removal efficiencies ranging from 92 to 98% for the different pollutants. The performance is almost superimposable for both distilled and real water samples for the aerogel sponges obtained after cross-linking with citrate, while the performance is of ~30-50% inferior for the one obtained with formaldehyde. The maximum adsorption capacity, for instance, of the aerogel obtained with citrate for Fe^{2+} and crystal violet are 2.3 and 3.6 mg/g, respectively. The characteristics of the protein base-molecular sponges were analysed by Fourier transform infrared spectroscopy, scanning electron microscopy and mechanical assays. Overall, this work shows the potential of HSA-based aerogels as a promising polymeric structure for water purification from different pollutants with completely biocompatibility based on the environmentally friendly procedure of preparation. *The authors marked with an asterisk equally contributed to the work.

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P-28-047
Use of biphasic NADES to evaluate the extraction of biocompounds in hydrophilic phase from okara

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Soybean seeds (*Glycine max L.*) are used in a wide variety of food products. Soybean is recognized as an extremely nutritious food due to its high protein and beneficial compound content. Soybean processing to produce soymilk leaves okara as a by-product. Okara, despite being considered waste, retains a significant amount of biocompounds. Also, green solvents, such as natural deep eutectic solvents (NADES), are relevant for extracting these biocompounds. In a world with growing interest in sustainability, NADES offer environmentally friendly and safer alternatives. They provide numerous advantages, including lower toxicity, recyclability, and biodegradability. This study aims to evaluate the extraction of hydrophilic biocompounds from okara using a combination of hydrophilic and hydrophobic NADES. Isoflavone solubility prediction was performed by using the conductor-like model for real solvents (COSMO-RS) to screen three hydrophobic and three hydrophilic NADES combinations. The extraction was performed using heating-orbital shaker for 45 minutes at 45°C. After extraction, the biocompounds were evaluated as total phenolic content (TPC) and total antioxidant capacity (TAC) in the hydrophilic phase. COSMO-RS screening has simplified the identification of the most interesting combination of NADES formulations, reducing the need for extensive experimental assays. The biphasic NADES composed of camphor:thymol 3:2 and citric acid: glucose 1:1 was the best option for the extraction of biocompounds in the hydrophilic phase (TPC = 24.8139 mg GAE/g; DPPH = 0.039726 mg TE/g; TEAC = 47.80434 µmol TE/g).
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P-28-048
Sodium alginate encapsulation enhances the gastrointestinal stability of miRNA-enriched spinach extract

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Dietary microRNAs (miRNAs) have emerged as potential key regulators of gene expression across kingdoms, with promising implications for human health. However, their stability during digestion, particularly when they are sourced from plant origin, remains relatively unexplored. This study investigated the stability of three spinach-derived plant miRNAs (ath-miR159, ath-miR166, and ath-miR168) and explored the protective effects of sodium alginate encapsulation during *in vitro* digestion. Employing the simulated gastrointestinal digestion model INFOGEST, miRNA levels were quantified after digestion. miRNAs were extracted and then quantified through quantitative real-time PCR. Secondary structure predictions were employed under physiological conditions to correlate miRNA stability with thermodynamic properties. Under our experimental conditions, encapsulation markedly enhanced the stability of ath-miR159 and ath-miR166 compared to the non-encapsulated form. Secondary structure predictions revealed a more stable conformation for ath-miR168 compared to ath-miR159 and ath-miR166. Our findings emphasize the complex interplay between miRNA structure, encapsulation, and digestive system conditions. While sodium alginate encapsulation has been demonstrated to be beneficial for enhancing the stability of certain miRNAs, tailored approaches are essential for accomplishing the most effective miRNA delivery.

P-28-049
Purification of recombinantly produced insect ion transport peptides

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Ion transport peptides (ITPs) are short insect neuropeptides with length of 70–90 amino acids. They influence many different physiological processes, such as homeostastis, molting, diuresis and behavioral processes such as mating. Multiple forms can be expressed from a single gene through the process of alternative splicing, creating ITP and also ITP-like peptides (ITPL). In the present study, we work with five peptides, two originating from *Bombyx mori* – BmITP and BmITPL – and three from *Drosophila melanogaster* – DmITP, DmITPL1 and DmITPL2. All five were cloned in expression vectors containing the gene for thioredoxin, creating fusion genes to promote soluble expression of the peptides in *Escherichia coli*. The ideal growth media and cultivation conditions were optimised for each peptide separately by cultivation in Erlenmeyer flasks and peptides were subsequently produced in 1 litre of growth medium in a benchtop bioreactor. To improve the yield of soluble peptides, we have found that addition of 75 mM L-arginine to sonication buffer increases the amount of peptide in soluble fraction after cell lysis and adding 10% (v/v) glycerol improves it even further. The isolation of all peptides was initially done using ion metal affinity chromatography with subsequent various chromatography steps being currently tested to find the best approach in retrieving peptides with high purity. The finished purified product shall then be used in further studies of the function and structure of the peptides.
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P-28-050
Bump-and-hole engineering of DNA methylation writers in mammalian cells

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The methylation of cytosine in DNA is a crucial epigenetic mechanism in mammalian development and disease, sustained by

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the collaborative function of three distinct AdoMet-dependent proteins (Dnmt1, Dnmt3A, and Dnmt3B). Nonetheless, the catalytic interactions and temporal dynamics of these epigenetic "writers" in establishing and maintaining genomic DNA methylation patterns unique to each cell type are still inadequately understood. To specifically monitor the catalysis of an individual Dnmt enzyme, we used structure-guided engineering of mouse Dnmt1 or Dnmt3A to facilitate the preferential transfer of bio-orthogonal azide groups onto DNA using a synthetic cofactor analog Ado-6-azide. We subsequently established mouse cell lines that express endogenous designed methyltransferases by integrating the appropriate codons into the Dnmt1 or Dnmt3A alleles using CRISPR-Cas9 genome editing. We then used electroporation to facilitate the internalization of the azide cofactor into cells, enabling selective catalysis-dependent azide-tagging of Dnmt-specific DNA targets *in vitro* [1]. Additionally, we established a genetically stable method for the biosynthesis of an endogenous cofactor analog to facilitate metabolic Dnmt-directed DNA labeling in living cells [2]. Most recently, we generated a Dnmt1 N1580A knock-in mESC line at a single allele while retaining functional wild-type protein. A subsequent readout of the Dnmt1-specific epigenetic landscape revealed smooth coverage with WGBS/RRBS profiles, demonstrating the suitability of the established chemical Dnmt1 monitoring for epigenome research. Finally, a thorough investigation of Dnmt1-specific events during the mESC ground state transition indicated that Dnmt1 is essential to maintain methylation of promoter CGIs associated with germ cell genes.
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P-28-051
Development of crosslinked dopamine-alginate nanofibers for enhanced tissue engineering applications

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Natural polysaccharides are known for their biocompatibility, biodegradability, and ability to mimic the extracellular matrix (ECM), making them ideal for tissue engineering, drug delivery, and biomedical applications. Alginate-based scaffolds are particularly notable for forming hydrogels through cross-linking between carboxyl groups and divalent cations, but their long-term stability is limited by cation leakage. To address this, research focuses on modified alginate derivatives, where new functional groups enable covalent crosslinking, enhancing stability and functionality for tissue engineering applications requiring durable hydrogel networks for cell growth and regeneration. In this study, dopamine-modified alginate was synthesized via periodate oxidation and reductive amination. Dopamine derivatives provide an option for efficient cross-linking through highly reactive catechol groups, enabling the formation of stable and functional networks. Dopamine-alginate was used to prepare nanofibers via electrospinning method and this polymer showed improved electrospinnability compared to native alginate. To prevent degradation of the water-soluble polymer nanofibers, obtained nanofibers were crosslinked by dual cross-linking strategy, ionic cross-linking with barium followed by covalent cross-linking with sodium periodate. This approach eliminated toxic cross-linkers, making the fibers suitable for cell culture. SEM microscopy revealed structural changes post-crosslinking, with fibers becoming insoluble in aqueous solutions. The nanofibers were used to cultivate lung fibroblast cells (MRC-5), demonstrating their effectiveness as a cell growth matrix. Cytotoxicity was assessed via the MTT assay, showing increased cell proliferation after 24 hours but a decline after 72 hours. These results highlight the potential of dopamine-alginate nanofibers for tissue engineering applications.

P-28-052
Comparative transcriptomic analysis of decanoate-resistant *Candida tropicalis* ATCC 20962 mutants versus wild-type strains

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Candida tropicalis is renowned for its exceptional ability to ω-oxidize alkanes and fatty acids into dicarboxylic acids (DCAs), industrially valuable compounds used in diverse applications including perfumes, lubricants, adhesives, and antibiotics. These DCAs serve as precursors for polyamide materials with tailored properties through polymerization with diamines. While microbial DCA production has mainly relied on petrochemical-derived hydrocarbons, plant oil-derived fatty acids offer a sustainable alternative. However, fatty acid toxicity poses a significant challenge, limiting the efficiency of bio-based DCA production. Medium-chain fatty acids (MCFAs), essential for producing industrially relevant DCAs, induce cellular stress, including pH fluctuations, oxidative stress, and membrane alterations and fluidity, hindering efficient biosynthesis. Consequently, developing MCFA-tolerant DCA-producing strains is imperative. In this study, we successfully isolated a decanoate-tolerant strain from *Candida tropicalis* ATCC 20962, exhibiting resistance to 0.6 g/L decanoate. The selected tolerant strain showed an approximately 2-fold increase at 0.5 g/L decanoate and a 3.8-fold increase at 1 g/L decanoate compared to the parental strain in substrate toxicity assays. Additionally, sebacic acid conversion efficiency increased by approximately 12-fold at 0.4 g/L decanoate. Furthermore, to investigate the mechanisms of fatty acid toxicity, RNA-seq-based transcriptomic analysis was performed after exposing both strains to decanoate. The analysis identified a total of 1482 differentially expressed genes (DEGs) common to both strains, with increased expression in the mutant strain compared to the parental strain in biological pathways related to amino acid biosynthesis, transcription, translation, DNA replication, cell cycle regulation, and signal transduction. Notably, the GO term that exhibited the greatest change in expression was ubiquitin-dependent protein catabolic process. *The authors marked with an asterisk equally contributed to the work.

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P-28-053
Saccharomyces cerevisiae Mub1 modulates yeast cell wall sensitivity

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Yeasts have evolved an elaborate regulatory network to build and maintain their cell wall, which serves as the primary interface for interactions with the environment. Nevertheless, the link between this regulatory system and important cellular processes remains largely unexplored. This study focuses on Mub1 of *Saccharomyces cerevisiae*, an adaptor protein of the E3 ubiquitin ligase Ubr2, which has previously been implicated in the regulation of proteasome genes via the transcription factor Rpn4. Our results show that Mub1-deficient (mub1Δ) cells exhibit increased tolerance to standard cell wall stressors and perform better than wild-type cells. This protective phenotype results from the activation of several transcription factors that lead to suppression of cell wall remodelling. Although normally protective, this remodelling process becomes detrimental under chronic cell wall stress in laboratory conditions. These results indicate that Mub1 affects not only Rpn4 but also a larger number of transcription factors. Thus, it is a previously unrecognized regulatory hub that directly links cell wall integrity to the ubiquitin-proteasome system.

P-28-054
Investigation of bioactive molecules from novel lactic acid bacteria from Türkiye: antioxidant, antidiabetic, antihypertensive and antibacterial activities

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Lactic acid bacteria (LAB) produce and secrete several molecules during fermentation, including bioactive peptides and small organic molecules. These secreted molecules can be purified from cell free supernatants (CFS). In this study, novel LAB isolated from different dairy and bee products from Türkiye were screened for the presence of antibacterial, antioxidant, antihypertensive and antidiabetic molecules. Five isolates (named as 35, 39, 162, 174, 179) which showed high bioactivity in CFS obtained after fermentation were selected. The CFS were further purified using ultrafiltration columns, resulting in 10 kDa, 3-10 kDa and < 3 k Da fractions. The antioxidant effect was screened by two methods: 2,2-diphenyl-1-picrylhydrazyl (DPPH) and 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid (ABTS) radical scavenging activity. The 3-10 kDa fraction of the isolate 35 showed the highest activity with 496,08 mM Trolox/mg protein. The antidiabetic activity was evaluated through the inhibition of dipeptidyl peptidase IV enzyme activity, and the 3-10 kDa fraction of isolate 174 demonstrated the highest activity, with 85.37% inhibition. The antihypertensive potential was assessed by angiotensin I-converting enzyme inhibition and the 3-10 kDa fraction of isolate 35 showed the highest activity with 42.96% inhibition. Antibacterial activity against *Listeria innocua* was assessed using agar well diffusion method, and all fractions of isolate 174 showed the highest antibacterial activity with an antibacterial zone diameter of 28 mm. These results show that our novel LAB isolates produce bioactive molecules which can be used in pharmaceutical and food industry. Future studies will focus on purification of bioactive peptides, sequence identification and stability tests. Acknowledgements: This work was supported by Ege University Scientific Research Projects Coordination Unit. Project Number: 24139.

P-28-055
Heat shock procedure on *Escherichia coli* enhanced solubility of recombinant autolysin production from *Clostridium tyrobutyricum*

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In cheese production, the growth of non-pathogenic *Clostridium* species and their spores leads to a significant fermentation defect known as "late blowing", which causes the formation of pores, cracks, and fissures in the cheese. Autolysins, which are bacterial peptidoglycan hydrolases, play a role in modifying the peptidoglycan structure during cellular functions such as growth, division, and the elimination of weakened or damaged cells in the population. These autolysins are species-specific enzymes containing peptidoglycan hydrolases with different specificities depending on the bacterium's cell wall structure. In recent years, the use of autolysin enzymes as an alternative method for inactivating pathogens has become a research topic. This study aims to contribute to solving the late-blowing defect by cloning the carboxypeptidase (*cp*) gene, which encodes an autolysin synthesized by *C. tyrobutyricum*, expressing in *E. coli*, purifying the enzyme and investigating its lytic effects on *C. tyrobutyricum* strains. The *cp* gene was cloned into the pQE-30 plasmid and transformed into *E. coli* strains, followed by expression studies. Protein production was performed using different strains, induction temperatures, growth phases and isopropyl-β-d-thiogalactopyranoside (IPTG) concentrations and expression was confirmed by SDS-PAGE and western blot analysis. The highest protein expression was observed in the *E. coli* Shuffle T7 strain, but the produced proteins were found to be insoluble. To overcome this issue, a 2-step heat shock procedure was applied to the cells during induction. This application rendered most of the autolysin soluble, which enabled its handling and continued further analysis. In conclusion, our results present a solution for insoluble proteins expressed in *E. coli* which enhance to recombinant production of unique proteins. This study was supported by TÜBITAK (Project No: 122O031).

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P-28-056
Screening of microorganisms with enzymatic activities leading to the biodegradation of organic matter during the co-composting of dog hair

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The growing worldwide challenges of waste management and environmental sustainability have prompted the adoption of new, environmentally friendly approaches to harnessing the converting of solid waste into valuable product. To overcome this, the enzymatic transformation of waste into high-value products appears to be one of the most worthwhile options for remedying the problem associated with waste mismanagement strategies. For this reason, the current study is dedicated to the use of microorganisms and their enzymes to valorise dog hair and food waste during composting. To achieve this, a mixture of dog hair and food waste was co-composted for 4 months. To investigate the biodegradability of organic matter by compost microorganisms, bacteria and fungi were isolated from this mixture following dilution plating techniques. Isolated and purified microorganisms were screened for their ability to produce proteolytic and cellulolytic enzymes on specific media, including skim milk agar and carboxymethyl cellulose agar. The development of clear zones around the microorganism colonies indicated the production of proteolytic and cellulolytic enzymes. These enzymatic activities were determined spectrophotometrically using the respective substrates, and the identity of the corresponding producents were determined. The results obtained indicated that the mixture studied hosted plenty of distinct microbial species with a high cellulolytic and proteolytic activities. These results were confirmed by shotgun metagenomic sequencing, revealing microorganisms recognised for their ability to degrade cellulolytic and proteolytic materials. Ultimately, these enzymes could be fruitfully used as a promising and sustainable waste management strategy to remedy recalcitrant waste, while simultaneously providing high-value products. Acknowledgment This research was supported by the Centre of Excellence project "Civil Engineering Research Centre" (Grant No. S-A-UEI-23-5).

P-28-057
Optimization of Trx-BmITPL fusion protein production in *Escherichia coli*

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Ion transport peptides (ITPs) are neuropeptides with hormonal functions present in crustaceans and insects. ITPs play important role in water and salt reabsorption as well as in inhibition of acid secretion. In *Drosophila melanogaster* ITPs regulate homeostasis and behaviour. Functions of ITP neuropepepdes are still only partially studied or, as in the case of *Bombyx mori*, completely unknown. The functional studies require purified and soluble peptides in quantities hardly obtainable by simple purification from their natural source. The aim of this work was to design, construct and optimize *Escherichia coli*-based expression system capable of producing sufficient quantities of soluble ITPs and their subsequent purification using protein chromatography. Due to the small size of the peptide (~10 kDa), the approach of fusion to a solubility-increasing partner was chosen. The results show a successful production and purification of soluble *B. mori* ITPL (DmITPL) fused to thioredoxin (Trx-ITPL) providing sufficient a amount of protein for further functional and structural studies. This work is the result of implementation of Slovak Research and Development Agency grants APVV-23- 0393, APVV-21-0227, APVV-21-0215, implementation of the project 101160008 "Fostering Excellence in Advanced Genomics and Proteomics Research at Comenius University in Bratislava – FORGENOM II" funded by the Horizon Europe program and implementation of the project NUKLEUS 313011V387, cofinanced by the European Regional Development Fund.

P-28-058
Enzymatic preparation of phospholipid derivatives of pharmacologically valuable antiviral nucleosides

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Modified analogues of natural nucleosides and nucleotides are traditionally used to treat viral diseases. Classic examples of such compounds include lamivudine (deoxycytidine analogue), telbivudine (L-enantiomer of natural thymidine), and entecavir (modified 2'-deoxyguanosine), which are well-known therapeutic agents for the treatment of hepatitis B, also exhibiting antiviral activity against HIV. One potential approach for developing a new generation of antiviral nucleosides is their conversion into prodrug forms via conjugation with phospholipids. The purpose of this study was to elucidate the possibility and efficiency of the enzymatic synthesis of phospholipid derivatives of the anti-HBV nucleosides mentioned above through trans-phosphatidylation reaction catalyzed by microbial phospholipase D (PLD). Two synthetic phosphatidylcholines containing fatty acid residues of various length in the molecule (C16:0 or C6:0) were used as donors of phosphatidyl groups in trans-phosphatidylation reactions. The enzyme showed higher activity towards phospholipid with long-chain fatty acid residues (C16:0). Lamivudine was found to be the most effective acceptor compared with telbivudine and entecavir. The degree of conversion of nucleosides to liponucleotides varied from 34 mol.% (for 1,2-dihexanoyl-telbivudine) to 97 mol.% (for 1,2-dipalmitoyl-lamivudine). Interestingly, the presence of 4'-oxygen in the nucleoside sugar ring is not required for this enzymatic reaction to proceed. The target conjugates were isolated from the reaction mixture and purified using liquid chromatography on Silica gel columns. As a result, several 5'-phosphatidyl derivatives of antiviral nucleosides lamivudine, telbivudine, and entecavir were obtained for the first time. The enzymatic reaction

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catalyzed by microbial PLD proceeds in one step with high product yields. This work was supported by the BRFFR grant No. 24 -009 dated 02/05/24.

P-28-059
Coupling wastewater treatment with bioelectricity production using microalgae-based microbial fuel cells

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Wastewater treatment and energy production are among the main challenges for sustainable development in developing countries. The work describes the coupling of municipal wastewater treatment to the bioelectricity production using an innovative microalgal-based microbial fuel cells (MFC). Different parameters were studied (nitrate/phosphate consumption and COD abatement) under different conditions (air bubbling, autoclaving wastewater, and addition of laccase). The results showed that non-autoclaved wastewater provided the favorable environment for the installation of benthic MFC. A stable current of 60 mV for up to 140 hours continuous working was coupled to an efficient abatement of DCO (56%). Furthermore, the addition of an immobilized laccase in alginate beads improved the generated electrical potential to90 mV with a significant increase on the nitrate and phosphate assimilation with abatements yields of 51% and 100% respectively. Thus, the use of microalgal MFC can be a promising sustainable alternative in wastewater treatment.

P-28-060
Native nanoparticles from yeast: towards the facilitated drug delivery

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Viruses are inherently organized supramolecular structures designed by nature for viral genome packaging and delivery. Instances of virion application in biomedicine have been substantiated; however, safe and multipurpose delivery systems are notably missing. Yeast is GRAS status organism, highly relevant for human research. *Saccharomyces cerevisiae* L-A and similar virions are genuine transfer systems: the sole capsid protein associates into relatively loosely packed yet firmly structured particles. We cloned and sequenced all known *S. cerevisiae* dsRNA viruses from natural yeast. Gene engineering of capsid has been demonstrated to be compatible with the integrity of virion structure, conferring the loading and targeting options for a particle. The potential application of recombinant ScV-L-BC-1 virus capsid protein-formed VLPs for nano delivery was investigated (1). VLPs were successfully synthesized and encapsulated with mCherry by gene fusion, enabling fluorescence imaging. Gag-mCherry particles successfully enter A549 line cells without a need of transduction facilitators and do not affect cell viability. Introduction of nuclear localization signal into the outer shell of Gag protein resulted in delocalization of mCherry-labeled protein to host nucleus. Screening of other dsRNA viruses from wild yeast led to identifying a novel virus from yeast *Torulaspora delbruecki*, forming VLPs capable for minute-range acquisition times by A549 and HeLa cells. The proof-of-principle of cargo transfer into mammalian cells was demonstrated by assembling of VLPs carrying external nucleic acids and peptides, confirming the perspectives of the different payloads. The bioengineering of capsids is performed to prepare nano scaffolds with precise addressability options. Funded by the Research Council of Lithuania, Project No. S-MIP-23-28. 1. Celitan E. et. al., 2024. DOI: https://doi.org/10.3389/fbioe.2024.1456453.

P-28-061
Novel luminescent method for lipid peroxide detection

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Lipid peroxidation is a spontaneous cyclic process that results in the formation of toxic reactive lipid and fatty acid peroxides under the influence of oxygen. In living organisms, this process leads to damage to cellular structures, including cell death, and underlies many serious diseases. In food products and cosmetics, lipid peroxidation leads to their unsuitability or danger for consumption. Thus, the development of technologies for the detection of lipid and fatty acid peroxides is a relevant and practically important scientific task. Recently, we discovered a new bioluminescent system of the marine polychaete worm *Chaetopterus variopedatus*, in which the substrate is a peroxide of polyunsaturated lipids or fatty acids [1]. Using *Chaetopterus variopedatus* luciferase [2], we developed a novel simple luminescent method for the detection of polyunsaturated lipid and fatty acid peroxides. Briefly, the reaction is performed in PBS buffer. The sample for analysis is mixed with *Chaetopterus* luciferase and bovine cytochrome C, and luminescence is measured immediately. The resulting values are then compared to a calibration curve to calculate the concentration of polyunsaturated lipid and fatty acid peroxides. Non-oxidized lipids and fatty acids do not interfere with the results obtained using this method. References: 1. Zagitova RI et al., Int J Mol Sci. 2023 May 30;24 (11):9466. doi: https://doi.org/10.3390/ijms24119466. 2. Purtov KV et al., Dokl Biochem Biophys. 2019 May;486(1):209-212. doi: https://doi.org/10.1134/S1607672919030104. This project was supported by Russian Ministry of Science and Higher Education, grant number 075-15-2024-681.

P-28-062
Polysulfone membranes enriched with functionalized graphene oxide and carbon nanotubes support human adipose-derived stem cells attachment and neurogenic differentiation

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Carbon-based nanostructures are often used for fine tuning of the biomaterials' structure for tissue engineering (TE)

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applications. They are widely used as coatings of polymer-based scaffolds to enhance cellular activity in terms of adhesion, viability and differentiation due to their structural similarity to the natural extracellular matrix (ECM). In this context, the purpose of this work is to highlight the biological response of newly fabricated polysulfone (PSF) membranes functionalized with reduced graphene oxide (rGO-NH2) and multi-wall carbon nanotubes (MWCNT-COOH) in the context of proliferation, adhesion and neuronal differentiation of human adipose-derived stem cells (hASCs). Cell-scaffold systems were firstly tested for their biocompatibility by MTT assay, LDH assay and Live/Dead staining. Additionally, immunolabeling for α-tubulin and paxillin, together with phalloidin-FITC staining were used to study in-depth cell attachment to the substrates. Next, cells seeded on PSF membranes were treated with a specific cocktail of inducers to obtain the neurogenic differentiation of hASCs. On all composites, hASCs presented a developed cytoskeleton with characteristic elongated shape with emphasis on rGO-NH2, and rGO-MWCNT membranes, where larger groups of adhered cells were captured. Expression of paxillin was present in all cells adhered on all tested composites, and it was distributed in its characteristic manner alongside F-actin filaments, pointing out that the contact with the extracellular environment was fully developed. The tested materials supported hASCs differentiation towards neuron-like cells, by stimulating the expression of neuronal markers nestin, β-III tubulin, NCAM and MAP2. In conclusion, these platforms are biocompatible valid candidates for nervous tissue engineering and regeneration. This work was supported by PN-III-P1-1.1-TE-2019-1191 [197TE/2021]. *The authors marked with an asterisk equally contributed to the work.

P-28-063
Optimization of liposomal formulations for targeted therapy in breast cancer

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Breast cancer is the second most frequently diagnosed malignancy worldwide and ranks first in incidence and mortality rates among women. Currently, conventional treatment approaches such as surgery, chemotherapy, radiotherapy, and hormone therapy are widely employed. However, these methods present significant limitations, including prolonged treatment protocols, high costs, and severe side effects. Therefore, the development of targeted and efficient drug delivery systems is of critical importance in breast cancer therapy. Recent studies have identified liposomes as effective nanovectors that enable the specific and controlled delivery of antineoplastic agents to tumor tissue. In particular, small monolayer liposomes can accumulate in tumor sites through the enhanced permeability and retention (EPR) effect, thereby significantly reducing adverse effects on healthy tissues. Furthermore, key parameters such as liposome size, zeta potential, surface modifications, and synthesis methods play a crucial role in determining treatment efficacy. In this study, the effects of different lipid compositions and synthesis method variations on the structural and functional properties of liposomes were systematically evaluated. The impact of lipid content, synthesis techniques, and formulation differences on liposome characterization was thoroughly analyzed. Fundamental parameters, including zeta potential, particle size distribution, and surface morphology, were comprehensively assessed based on the applied formulation and production conditions. The findings indicate that optimizing liposome design parameters can significantly enhance the effectiveness of breast cancer treatment. Ultimately, the meticulous optimization of experimental design variables – such as lipid composition, cholesterol ratio, and surface modifications – is emphasized as a crucial step in developing safe and highly efficient liposomal treatment strategies for breast cancer.

P-28-064
Diversity of proteolytic enzymes secreted by *Aspergillus ustus*

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Aspergillus ustus is one of the producers of extracellular proteolytic enzymes active against hard-to-degrade fibrillar proteins such as collagen. In this work the effect of various cultivation methods and media compositions on the ability of *A. ustus* to produce proteases was investigated. Micromycete was cultivated in submerged and solid-state conditions on 9 media differing in nitrogen sources. Proteolytic activity of culture liquids was determined spectrophotometrically by reactions with azocasein at pH 8, 6 and 4 and by zymography with casein. The enzymatic activity determined by azocasein hydrolysis was higher after solid-state cultivation: the most significant activities were found on the media containing fishmeal hydrolysate + collagen and $NaNO^{3+}$ casein. The results of casein zymography confirmed the data. The lowest activity during both cultivation modes was observed on the medium without nitrogen sources. Intriguingly, sets of proteases secreted under solid-state and submerged conditions were different. The most active protease discovered under all cultivation conditions had molecular weight of about 50 kDa. After submerged cultivation on some media proteases with weights of 25 and 40 kDa were detected but their activity was low. After solid-state cultivation on all media three novel high-molecular proteases (MW > 80 kDa) were detected. During submerged cultivation azocaseinolytic activity was observed at all substrate pH values but after cultivation on vermiculite it turned out that extracellular proteases were most active at pH 8 and least active at pH 4, which may indicate the secretion of highly active alkaline and neutral proteases by the micromycete. Thus, this work established the most suitable cultivation conditions of *A. ustus* for obtaining highly active extracellular proteases. *The authors marked with an asterisk equally contributed to the work.

P-28-065
Recombinant *Escherichia coli* strain for the production of *Streptomyces netropsis* phospholipase D

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Phospholipase D (PLD) has significant practical applications in biotechnology, medicine, and industrial processes. It is widely used in the enzymatic synthesis of phospholipid derivatives,

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particularly in the pharmaceutical and food industries. Additionally, PLD play a role in drug delivery systems, the development of lipid-based nanocarriers, and therapeutic interventions for diseases linked to lipid signaling dysregulation. The aim of this work was to create a genetically engineered strain of *Escherichia coli* that produces phospholipase from bacteria of the genus *Streptomyces*. The *S. netropsis* BIM B-235 genome was selected as the source of the phospholipase D gene. Plasmid pET28a(+) carrying the *pld* gene was prepared by using the circular polymerase extension cloning method. That plasmid was used to transform *E. coli* BL (DE3)-competent cells. The recombinant strain was grown on LB medium and induces with 0.2 mM IPTG for protein expression. The expression of a 57-kDa protein was determined using protein electrophoresis. The target protein was extracted, refolded, and then purified from inclusion bodies by Ni-NTA-agarose column. The presence of hydrolytic and transphosphatidylation activities was confirmed in the reaction of deoxythymidine with soybean lecithin. The formation of both phosphatidic acid and phospholipid derivative of deoxythymidine was observed. As a result, a recombinant *E. coli* strain expressing *S. netropsis* BIM B-235 PLD was created. The strain could be used for the extraction and purification of PLD in different biotechnological applications.

P-28-066
Challenges and advances in the purification of bacterial Lon protease

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Lon protease is an ATP-dependent enzyme responsible for degrading misfolded and short-lived proteins in bacteria. We tried to produce Lon protease native to uropathogenic *E. coli*, though its heterologous production in *E. coli* expression system presents challenges. Since there is no reversible inhibitor of Lon protease, purification of the active enzyme is also challenging. IMAC purification proved inefficient, likely due to steric inaccessibility of the HisTag in our construct, leading us to explore alternative chromatographic methods. Anion exchange chromatography is often the first step in protein purification because it allows efficient separation of proteins based on their charge. At the same time, it has a high protein binding capacity, which allows processing of a larger sample volume in the initial purification step without the need for protein precipitation. We tested HiTrapQ under various conditions, with the largest fraction of Lon protease eluting at 300–500 mM NaCl. However, separation remained insufficient. When using hydrophobic interaction chromatography (HIC), the initial setting of the conditions is a problem, because there is no universal manual for their setting, and they must be determined case to case. We tested HiTrap FF with butyl residues, eluting at a decreasing ammonium sulphate gradient (1–0 M) into 20 mM sodium phosphate buffer. While resolution remains suboptimal, future work will focus on optimization to obtain stable, active Lon protease. Additionally, we aim to develop a reproducible activity assay, which could serve as a reference for other proteases. This work was founded by grants APVV-21-0215 and APVV-22-0161.

P-28-067
Mushroom-derived polysaccharides as bioactive agents for skin regeneration: evaluation of antimicrobial, wound-healing, and immunomodulatory effects

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Mushroom polysaccharides have emerged as promising bioactive agents for enhancing skin regeneration. This study evaluates three polysaccharide-rich fractions (SB, SC, RD) obtained by hot water and alkaline extractions from *Coriolus versicolor*, *Pleurotus ostreatus*, and *Hericium erinaceus*. Their potential to promote skin repair was investigated by analyzing effects on cell proliferation and migration, immune modulation, and microbial growth inhibition. These fractions were previously characterized by several chemical and structural characterization methods that revealed significant presence of bioactive molecules (e.g., glucans, proteins, phenolics). Cell viability was accessed by PrestoBlue and MTT assays in HaCat and L929 cell lines, revealing the non-toxicity of the compounds at tested concentrations. Furthermore, proliferation assays (BrdU incorporation) and migration assays (scratch assay) in HaCaT cells were conducted at optimized concentrations (0.6 and 0.3 mg/mL). Notably, *C. versicolor* fractions promoted wound closure of the injured monolayer by 95% after 48 h (at 0.6 mg/mL) compared to 66% in the non-treated control. The fractions exhibited broad-spectrum antimicrobial and antibiofilm activity in Gram-positive bacteria (*S. epidermidis*, MRSA), Gram-negative bacteria (*P. aeruginosa, E. coli*) and yeast (*C. albicans, C. tropicalis*). Microbial growth inhibition varied across the three species and extraction method, ranging from 30% to 70% at a concentration of 20 mg/mL. Additionally, immunomodulatory effects are being assessed by measuring key pro-inflammatory cytokines (TNF-α, IL-17A, IL-1β, IL-6) and anti-inflammatory (IL-10) to elucidate how these polysaccharides regulate inflammation during healing and skin repair. These findings highlight the potential of polysaccharides as multifunctional agents in dermatological applications, supporting their role in developing innovative, natural-based therapies for skin regeneration.

P-28-068
Is green chemistry the solution for reducing toxicity of titanium dioxide nanoparticles?

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Titanium dioxide nanoparticles (TiO_2 NPs) have found extensive applications across various industries due to their exceptional mechanical, physical, optical, and electrical properties. From paper and paint to food, cosmetics, and water purification, TiO_2 NPs are ubiquitous in our daily lives. However, their widespread use raises significant concerns regarding their hazardous effects on ecosystems and human health. Studies have shown that these nanoparticles can accumulate in vital organs, causing cytotoxic and genotoxic effects. Therefore, it is imperative to develop TiO_2

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NPs through green chemistry to ensure enhanced stability while minimizing their toxic impact on living organisms. In this context, the present study aimed to compare the *in vitro* cytotoxic effects induced by TiO_2 NPs obtained using the chemical as well as the green synthesis method in the presence of epigallocatechin-3-gallate (EGCG), the major catechin found in green tea (*Camellia sinensis*). Following exposure of MRC-5 lung fibroblasts to 10 and 50 μg/mL TiO_2 NPs for 24 and 72 h, it was observed that both types of NPs induced a decrease in cell viability (up to 30%), with no significant differences between the two doses or time exposure intervals. Changes in reduced glutathione (GSH) levels and catalase (CAT) activity also indicated a short-term cellular defense response against oxidative stress in a dose-dependent manner for both TiO_2 NPs tested. In contrast, chemical TiO_2 NPs caused an increase in MDA levels, a marker for lipid peroxidation, indicating more pronounced oxidative damage. In conclusion, green synthesis of TiO_2 NPs offers a promising approach with enhanced stability and environmental benefits. However, further optimization is needed to improve their biocompatibility and safety for human use.

P-28-069
Enhancing selective itaconic acid synthesis in *Yarrowia lipolytica* through targeted metabolite transport reprogramming

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Itaconic acid (IA) is a valuable chemical building block with numerous industrial applications, especially in polymer synthesis, and can be sustainably produced through microbial fermentation. Advanced insights into the intracellular transport and extracellular export of organic acids in yeasts and mycelial fungi, were used to engineer a highly efficient *Yarrowia lipolytica* strain capable of selectively producing IA. The metabolic engineering strategy aimed to eliminate the accumulation of citric and isocitric acids, the primary by-products of this yeast, by blocking their transport at two levels: mitochondrial (inactivation of the YlYHM2 gene) and plasma (inactivation of the YlCEX1 gene) membranes. Additionally, selective heterologous transporters from *Aspergillus terreus* and *Ustilago maydis* were introduced to facilitate the mitochondrial export of cis-aconitate (MttA and Mtt1) and the extracellular secretion of IA (MfsA and Itp1). For the first time, all components of the IA pathways from *A. terreus* and *U. maydis* were heterologously expressed and functionally compared in strains with similar genetic backgrounds. Remarkably, a synergistic enhancement of IA production was observed when both pathways were co-expressed in a single strain, along with the inactivation of native citric and isocitric acid transport. Unlike previously engineered *Y. lipolytica* strains for IA production, the optimized strain developed in this study does not require complex or nutrient-rich media, achieving the highest reported product yield (0.343 mol IA/mol glucose) and productivity (0.256 g/L/h) in yeast, with minimal by-product formation.

LB-R-28-01
Multi-omics approaches unveiling the survival mode and metabolism of a *Pseudomonas putida* strain on an epoxy matrix

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The crosslinked microstructure of epoxy-based composites represents their main advantage; providing lightness and outstanding mechanical strength, which is ideal for the aeronautic, automotive, and construction industries. However, this structure limits their potential for reprocessing and recycling at the end of their lifecycle. Thus, landfilling or incineration are usually the only alternatives. It is essential to screen and adapt microbes that can degrade specific recalcitrant compounds to facilitate their biodegradation or recycling. Xenobiotics biodegradation is influenced by various factors, such as the chemical nature and structure of the compounds (sometimes making necessary a pretreatment), their surface characteristics, historical presence in the environment (indicating prior microbial interactions that facilitate evolutionary adaptations for degradation), microbial activity, and the containment or release of microorganisms throughout the degradation process. The EU-funded ESTELLA project focuses on strategies for the microbial breakdown of epoxy composites. From a previously cured and milled commercial epoxy resin, a strain identified as *Pseudomonas putida* through Bruker MALDI Biotyper characterization and the sequencing of its 16S rDNA, was isolated. Whole genome sequencing showed a circular chromosome of 6.59 Mb containing 6065 potential coding sequences. Label-free Proteomics and Transcriptomics validation are used to evaluate its ability to survive and utilize the epoxy material as a carbon and energy source. These results offer fascinating insight into the degradation of recalcitrant materials by a novel isolate from a genus known for its metabolic plasticity. Acknowledgements: ESTELLA project (Design of bio-based thermoset polymer with recycling capability by dynamic bonds for bio-composite manufacturing) (project n°: 101058371) funded by the European Union by Horizon Europe program (call: HORIZON-CL4-2021-RESILIENCE-01-11).

LB-R-28-02
Sustainable agriculture based on microbially functionalized bio-plastics for mulching

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European concern about the widespread environmental impact of plastic waste is fuelling one of the top scientific challenges

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nowadays. However, plastic materials are part of countless aspects of our modern life, which makes them difficult to replace, and forecasts predict an increase in the use of these materials in the coming years. Plasticulture is an intensive plastic-consuming practice to increase crop yield, including greenhouses, tunnels, mulching, and auxiliary materials such as irrigation pipelines. Even though the productive advantages (weed suppression, pesticide reduction, humidity preservation, etc), the degradation of these plastic materials in soil leads to the accumulation of nano- and micro-plastics, with harmful effects on the environment. Besides, their post-harvest recycling is a low-efficiency process due to the contaminants (vegetal, soil). Thus, the BioPac project (no. TED2021-131864B-C21), funded by the Ministry of Science and Innovation of Spain, aims to design effective, sustainable and functional materials, promoting a more environmentally friendly agriculture by means of realistically self-degrading plastics. Functionalized plastic materials using microorganisms suitable for agriculture applications represent a promising research field, which presents room for improvement, where traditional microbiology (e.g. selection of microbial candidates), as well as biotechnology (e.g. plastic integration and properties validation), play relevant roles. Acknowledgments: BioPAC project (Development of bioactive and lifespan-controlled bioplastics) (Ref. no. TED2021-131864B-C21) funded by the MCIN (Ministerio de Ciencia e Innovación)/AEI (Agencia Estatal de Investigación)/https://doi.org/10.13039/501100011033 (Digital Object Identifier) and the European Union "NextGenerationEU"/PRTR (Recovery, Transformation and Resilience Plan).

LB-R-28-03
Functionalized biodegradable platforms for sustainable protection of horticultural crops against fungal threats

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Fungal diseases are a persistent threat to horticultural crops traditionally controlled through extensive use of synthetic fungicides. While effective, these treatments lead to environmental contamination. In parallel, the widespread use of plastic mulch films in agriculture has raised concerns over microplastic accumulation in soils. These dual challenges have prompted the exploration of functionalized, bio-based and biodegradable delivery systems. The EU-funded BioBIVE project (No. 101130442) aims to develop eco-friendly platforms for the controlled release of natural bioactive compounds, offering protection against key fungal phytopathogens affecting tomatoes, strawberries, and carrots. The strategy integrates three biodegradable delivery systems (bioplastic mulch films, biochar, and sprayable mulching) functionalized with bioactive agents (including basic substances, antagonistic microorganisms or seaweed extracts). Preliminary results confirmed the antifungal potential of several basic substances, with chitosan, horsetail, and nettle standing out due to their strong inhibitory activity against a wide range of phytopathogenic fungi. To date, their effects have been tested against ten fungal species, including major pathogens such as *Rhizoctonia solani, Sclerotinia sclerotiorum, Verticillium dahliae*, and *Fusarium oxysporum*. Additionally, *in vitro* assays with microbial antagonists revealed noteworthy antifungal properties, particularly some isolates from the genera *Bacillus* and *Streptomyces*, which exhibited activity comparable to that of natamycin in certain cases. Altogether, our findings underline the potential of biodegradable delivery systems to replace conventional fungicides and plastic-based agricultural inputs, advancing both plant health and environmental sustainability. Acknowledgements: BioBIVE project (No. 101130442), funded by the EU under the Horizon Europe Framework Programme (https://cordis.europa.eu/project/id/101130442; www.biobive.eu).

LB-R-28-04
Sonochemical synthesis of bimetallic hybrid nanoflowers with enhanced amylase catalytic activity

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An attractive and novel strategy for enzyme immobilization is biomineralization of enzymes with metal salts in phosphate buffer to form organic-inorganic hybrid nanoflowers (HNFs). This approach has several advantages, like being a simple and mild synthesis method, high surface area, stability and improved enzyme activity. Also, this procedure allows simultaneous immobilization of enzymes with their cofactors and co-enzyme immobilization. A facile and fast approach of sonication was applied to synthesize amylase-$(Co/Mn)_3(PO_4)_2$-HNFs. Firstly, sonochemical synthesis optimization was achieved with a design of experiment approach of Box-Behnken Design with three parameters as sonication time (5, 12.5 and 20 min), total metallic salt concentration (1.0, 2.0 and 3.0 mM) and enzyme concentration (0.05, 0.15 and 0.25 mg mL^{-1}). Then, hybrid structure was obtained under optimized conditions and was applied for further characterization studies. Amylase encapsulation was successfully achieved using this fast method with an approximately 50% enhanced enzyme activity. Both free and immobilized amylase showed the same working temperature and pH of 40°C and 7.0, respectively. The next studies will be focused on molecular dynamic (MD) simulations to probe the assembly process. This study indicates that sonochemically synthesized HNFs are valuable for enzyme immobilization to improve catalytic activity and stability.

LB-R-28-05
Measurement uncertainty in a practical application in a biochemistry laboratory

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Clinical laboratories must produce necessary and analytically reliable results for the accurate use and interpretation of those results. A measurement or test result is not effective enough without an evaluation of its reliability. This research utilized the measurement uncertainty estimation of serum glucose level based on the "top-down" approach as a practical example. The FSM Research and Training Hospital Biochemistry Laboratory

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measurement uncertainty estimation for biochemistry laboratory glucose test with the Roche/Cobas 6000 analyzer was defined. Serum glucose level was calculated by the hexokinase method, and mg/dL unit was used. Performance data based on interlaboratory evaluation, together with quality control data applied in the laboratory, were used to estimate measurement uncertainty. The measurement uncertainty of serum glucose analysis was found to be ± 6% with a 95% confidence interval. According to CLIA88 glucose, the total error is 10%. The Westgard website lists 6.9%. The American Diabetes Association reports it as < 10%. Serum glucose analysis results are reported as ± 5% within the 95% confidence interval, which is within acceptable limits. The effectiveness of measurement performance estimates within the quality improvement components of clinical biochemistry laboratories increases the power of the clinician to participate in patient management across the clinical decision-making spectrum.

Enzymology

P-29-001
Identification and characterisation of novel enzymes potentially involved in hydrocarbon biodegradation

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The environmental contamination of hydrocarbons used as energy sources is an increasing problem as the hydrocarbon compounds can accumulate in soils and waters, which may endanger human health and biodiversity[1]. Thus, the remediation of these polluted areas is of key importance. The identification of novel enzymes contributing to the degradation of these compounds may be a good approach. Metagenomic databases provide a useful platform to identify enzymes from microorganisms adapted to extreme conditions such as hydrocarbon contamination. Therefore, we searched for novel enzymes with potential monooxygenase activity using the previously developed MetaHMM methodology[2]. From the metagenomic hits we selected five gene sequences, which are identified from metagenome present in tar-contaminated soil sample collected in Israel. The enzymes encoded by the selected gene sequences are probably members of Cytochrome P450 enzyme superfamily, which are heme-containing monooxygenases with a wide range of substrate selectivity[3]. To characterise these enzymes, we performed BLAST searches to identify potential substrates and the enzyme subfamilies. To further analyse their function, we carried out protein expression experiments and predicted the three-dimensional structures using AlphaFold. We concluded that the selected enzymes may adapt similar structures as compared to the Cytochrome P450 enzymes sharing high level of sequence similarity with resolved structures. Now we are progressing with the test of these enzymes with reduction assays and molecular docking and preliminary results are promising. In parallel, we also started crystallization experiments for structure determination. References: 1 Abha S, Swaranjit C (2012) in *Introduction to Enhanced Oil Recovery (EOR) Processes and Bioremediation of Oil-Contaminated Sites* (L Romero-Zerón, ed.) pp 185-206, InTech. 2 Szalkai B et al. (2019) Genomics 111 (4):883–5.3 Greule A et al. (2018) Nat Prod Rep. 35(8):757–91.

P-29-002
Structural and functional characterization of CYP109J8 and CYP109G35: novel vitamin D2 hydroxylases from extreme environments

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Vitamin D hydroxylation is essential for converting inactive precursors into active metabolites critical for calcium homeostasis and immune regulation. This transformation is mediated by cytochrome P450 enzymes, particularly those from the CYP109 subfamily, known for their remarkable substrate diversity and regioselectivity. This study investigates the substrate specificity, biochemical properties, and structural characterization of CYP109J8 and CYP109G35, identified via metagenomics from Türkiye's extreme environments. The cyp109J8 and cyp109G35 genes encode proteins of 389 and 408 amino acids, with sequence similarities of up to 45.22% and 41.08% to other CYP109 enzymes. After recombinant production in the *E. coli* C43 host organism via suitable expression vector, CYP109s were purified by affinity chromatography. The oxidized substrate-free forms showed Soret maxima peaks (γ) at 418 nm and 417 nm, along with β and α bands at 536 nm and 569 nm. Type I spectral assays confirmed vitamin D2 binding, with dissociation constants of 12.8 μM for CYP109J8 and 61.2 μM for CYP109G35. CD spectroscopy revealed melting temperatures (Tm) of 61.17°C for CYP109J8 and 69.34°C for CYP109G35 in salt-free conditions. Stability assays showed CYP109J8 stable at 0.5 M NaCl, while CYP109G35 remained stable even at 4 M NaCl. Thermal reassessments with NaCl increased Tm to 70.23°C for CYP109J8 and 77.48°C for CYP109G35, highlighting CYP109J8's thermostable nature and CYP109G35's halo-thermostable characteristics. X-ray crystallography conditions yielded crystals at 25% w/v PEG 4000, 0.1 M MES (pH 6.5), 0.2 M CaCl for CYP109J8, and 26% w/v PEG 3000, 0.1 M MES (pH 6.5), 0.2 M MgCl for CYP109G35. After crystal fishing, diffraction data were collected at P13 beamline at Petra III, DESY Hamburg, using a wavelength of 0.9762 Å. These findings underscore the unique potential of CYP109J8 and CYP109G35, expanding the scope of P450 enzymes for industrial and therapeutic biocatalysis.

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P-29-003
Exploring a potential alkene reductase from *Brucella ovis*: characterization and functional analysis

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Brucella ovis is a Gram-negative bacterium responsible for a reproductive disease affecting all breeds of sheep. Among its flavoproteins, there is a predicted alkene reductase (BoAKR), which holds significant potential as a biocatalyst. We have established solid protocols for BoAKR overexpression in *E. coli* and its purification. Spectroscopic characterization revealed that BoAKR has a redox active FMN as a cofactor, which can get reduced through photoreduction. Furthermore, its redox potential has been determined by both the xanthine oxidase method and cyclic voltammetry. Additionally, fluorescence and circular dichroism spectroscopies have been used to gain further insight into the protein folding, as well as about the protein thermal stability. BoAKR was also successfully crystallized, and its structure was solved. Furthermore, BoAKR has shown the capacity to accept hydride from NAD(P)H, being NADH more efficient as a hydride donor. Catalytic activity was observed with five out of the seventeen compounds tested as potential hydride acceptors. All of them have a C=C double bond conjugated with a carbonyl group. Reactivity is observed both in aromatic and aliphatic compounds. Kinetics and dissociation constant were calculated for four of these five active compounds. Furthermore, their effect on the spectroscopic properties and on the thermal stability of BoAKR were also evaluated.

P-29-004
Rethinking PLA production: harnessing food waste and enzymatic potential for sustainable bioplastics

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Plastic poses a significant threat to both human health and the environment, yet its ubiquitous presence in daily life makes it indispensable. While alternatives like PolyLactic Acid (PLA) offer a biodegradable solution, the current production processes are energy demanding and creating challenging-to-manage waste. Our project addresses these challenges by innovating the PLA production process through the utilization of food waste as a feedstock for engineered yeast for the production of a green plastic precursor. Leveraging machine-learning-assisted directed evolution, we are engineering a yeast enzyme to optimize its catalytic potential for this novel substrate. By redirecting the enzyme's natural catalytic capabilities, we aim to produce the monomer for a more sustainable and efficient manufacturing of PLA. This approach not only mitigates the environmental impact of plastic waste but also adds value to food waste, contributing to a circular economy.

P-29-005
Utilizing ancestral variants of polylactic acid-degrading enzymes to enhance solubility and expression yields

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Over the years, plastic pollution has become an increasing problem for both the environment and human health. The urgent need for sustainable solutions to plastic pollution has led to a surge in research on enzyme-driven biodegradation of synthetic polymers, particularly polylactic acid (PLA). In this study, we identified a novel enzyme, MGY, from a metagenomic database for its PLA-degrading ability. However, limited solubility and low yield of the MGY enzyme hindered further structural and functional analysis. To improve its solubility and yield, we applied ancestral sequence reconstruction, generating three ancestral MGY variants. These ancestral variants retained high PLA-degrading activity, as verified through activity assays on emulsified PLA plates, making them more suitable for practical applications and detailed structural studies. Transformation of electrocompetent bacteria with the vector containing synthetic genes of the specific enzymes, followed by cultivation and protein expression, demonstrated that sequence modification through ancestral reconstruction increases protein solubility and contributes to higher expression yields. The MGY enzyme and its ancestral variants were assayed using differential scanning calorimetry (DSC) and a thermal shift assay with fluorescent dye SYPRO® Orange. The enhanced solubility and expression of these ancestral MGY variants highlight ancestral sequence reconstruction as a powerful tool, providing a pathway for developing effective biocatalysts for PLA recycling and environmental sustainability and facilitating both applied and structural studies.

P-29-006
Characterization of a new phospholipase D from bacteria with unusual enzymatic features

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By identifying new forms of phospholipases D (PLD), we are trying to decipher the structure-function relationships of PLDs, a family of enzymes found in microbes, plants and animals. PLD is an enzyme that hydrolyzes the distal phosphodiester bond of phospholipids (PLs) to generate phosphatidic acid (PA) and a polar head group. This enzyme is involved in various functions, such as cell signaling, tumorigenesis, infection and plant pollination. During a bioinformatic search for novel PLDs, a close homolog (called DaPLD1) of the *Arabidopsis thaliana* PLDα was spotted in the genome of the bacterium *Dechloromonas aromatica* RCB. The gene encoding this putative PLD was amplified from genomic DNA and then cloned for

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recombinant expression in *E. coli*. The His-tagged version of the protein was purified by affinity chromatography followed by gel filtration and characterized biochemically. The optimal temperature and pH of DaPLD1 were found to be 30°C and 6, respectively. However, compared to previously characterized plant or bacterial PLDs, DaPLD1 harbors specific enzymatic features, especially a strong preference for the substrate phosphatidylethanolamine (PE) over all other PL. DaPLD1 also has a typoselectivity for unsaturated acyl chains at both *sn*-1 and *sn*-2 positions. Acyl chain unsaturation was also important for phosphatidylcholine hydrolysis. DaPLD1 had low transphosphatidylation activity in the presence of glycerol using PE as the substrate. Expression of DaPLD1 in *E. coli* resulted in drastic changes in PL composition, with accumulation of PA and reduction of PE and phosphatidylglycerol. In conclusion, we identified and biochemically characterized a novel bacterial Ca^{2+}-independent PLD with a strong preference for PE with unsaturated acyl chains. Crystallization experiments of this unorthodox PLD are underway to understand the molecular features of its substrate specificity.

P-29-007
CyaY and TusA modulate Fe-S cluster assembly by regulating ISC- and SUF-mediated L-cysteine desulfurase activity

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Iron-sulfur (Fe-S) clusters are essential cofactors in numerous cellular processes, and their biosynthesis in *Escherichia coli* is coordinated by the ISC and SUF systems. While the ISC system is the primary Fe-S assembly pathway under normal conditions, the SUF system is primarily activated under oxidative stress and iron-limiting conditions. However, its regulation beyond transcriptional control remains poorly understood. Here, we demonstrate that CyaY, the bacterial frataxin homolog, plays a dual regulatory role in modulating L-cysteine desulfurase activity in both pathways. Under standard conditions, CyaY associates with IscS, where it fine-tunes Fe-S cluster biosynthesis. However, biochemical analyses reveal that under specific conditions, TusA competes with CyaY for IscS binding, redirecting sulfur flux toward tRNA thiolation and molybdenum cofactor biosynthesis, while reducing ISC-mediated Fe-S cluster assembly. This displacement releases CyaY, making it available to interact with SufS, the desulfurase of the SUF system. Using native mass spectrometry and analytical gel filtration, we show that CyaY binds to SufS, both alone and within the SufS-SufE complex, albeit at a lower affinity than SufE. Functional assays reveal that CyaY inhibits the desulfurase activity of SufSE *in vitro*, reducing sulfur mobilization for Fe-S cluster assembly. *In vivo* studies confirm that CyaY overexpression significantly decreases total desulfurase activity, particularly in strains where SufS is the primary desulfurase. Furthermore, the *in vivo* maturation and activity of holo-IscR in presence or absence of an iron chelator suggest that CyaY's inhibitory effect is influenced by intracellular iron levels, providing a potential regulatory switch between ISC- and SUF-mediated Fe-S cluster biogenesis. These findings establish CyaY and TusA as key modulators of Fe-S cluster homeostasis, integrating sulfur distribution and iron availability through dynamic protein–protein interactions.

P-29-008
Regulation of NADPH-dependent glutamate dehydrogenase from *Schizosaccharomyces pombe* by protein phosphorylation

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NADPH-dependent glutamate dehydrogenase from yeast *Schizosaccharomyces pombe* (SpGdh1) is a pivotal enzyme that catalyzes the conversion of 2-oxoglutarate and ammonium to glutamate using NADPH as a coenzyme. Although SpGdh1 is reported to be phosphorylated at several residues, the impact of the phosphorylation on the enzyme activity and the underlying molecular mechanism remains unclear. To elucidate the phosphorylation-mediated regulation of SpGdh1, we determined the high-resolution crystal structure of SpGdh1 binding $NADP^+$ and 2-iminoglutarate at 1.45 Å resolution with low R-factors (R_{work}/R_{free} = 18.1/20.4). The structural analysis revealed that four serine residues to be phosphorylated were located near the active site. In particular, Ser252 directly interacted with the 2′-phosphate group of the adenine ribose moiety of $NADP^+$, suggesting that phosphorylation of Ser252 interfered with $NADP^+$ binding. The results of a kinetic analysis revealed that the replacement of these four residues increased the apparent $K_m^{NADP(H)}$ value and decreased catalytic efficiency, $k_{cat}/K_m^{NADP(H)}$ In contrast, substitutions decreased the apparent $K_m^{NAD(H)}$ value and increased catalytic efficiency, $k_{cat}/K_m^{NAD(H)}$. Therefore, the Ser to Glu replacement caused net shifts in the coenzyme specificities (NADPH to NADH and $NADP^+$ to NAD^+) of 55- and 2900-fold, respectively. We assume that the negative charge of the side-chain carboxy group introduced at position 252 caused a steric hindrance or electrical repulsion against the 2′-phosphate group of the adenine ribose moiety of $NADP^+$, affecting the binding of NADPH. Moreover, Ser252 is highly conserved in NADPH-dependent GDHs from prokaryotes, fungi, and mammal, which may be phosphorylated in other NADPH-dependent GDHs to modulate the GDH activity especially in eukaryotic cells. This study for the first time revealed the phosphorylation-mediated net shift of coenzyme specificity and the structural basis of SpGdh1. *The authors marked with an asterisk equally contributed to the work.

P-29-009
Thermostable metagenomic esterases: discovery and development for polyester depolymerization

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Plastic pollution has become one of the most urgent environmental and health challenges of our time, necessitating the development of innovative solutions to mitigate its impact. Among the promising approaches is enzyme-driven biodegradation, which targets synthetic polymers such as polylactic acid (PLA), a widely used biodegradable plastic. In this study, we identified a novel PLA-degrading enzyme, MGY,

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from a metagenomic database. While this discovery held significant potential, the enzyme's practical application was hindered by several limitations, including low solubility, poor expression, and insufficient thermostability. To overcome these challenges, we employed ancestral sequence reconstruction, a cutting-edge protein engineering technique, to generate three ancestral MGY variants. These variants not only retained high PLA-degrading activity but also exhibited enhanced functional properties. Notably, one variant demonstrated the ability to degrade polycaprolactone (PCL), a structurally distinct polyester. PLA and PCL, though both polyesters, differ significantly in their chemical structures: PLA is derived from lactic acid and possesses a relatively rigid backbone, while PCL, synthesized from caprolactone, is more flexible. This structural divergence makes PLA and PCL an excellent pair of substrates for investigating enzyme adaptability and substrate promiscuity, providing valuable insights into how enzyme structure and flexibility influence substrate recognition and hydrolysis. The ancestral variants exhibited remarkable improvements in key properties, including solubility, expression levels, and thermostability. Differential scanning calorimetry (DSC) analysis revealed that one variant achieved a melting temperature (T_m) of 84°C – the highest reported for any PLA-degrading enzyme to date. These enhancements underscore the potential of ancestral sequence reconstruction as a powerful tool for engineering enzymes with superior functional properties.

P-29-010
New insights over seed-biochemistry: kinetic and thermodynamic characterization of hazelnut lipase–micelle interactions

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Lipases are key enzymes in lipid metabolism, important for food processing as well as the production of biofuels. Their substrate selectivity and interfacial activation, however, remain poorly understood, especially in plants. For food processing, regulation of lipase activity could be at the core of raw material storage, like hazelnuts, allowing producers to prevent lipid degradation. After our recent characterization of a moonlighting hazelnut lipase, we examined its interactions with micelles through stopped-flow absorbance and isothermal titration calorimetry (ITC). Although they are ubiquitous, lipase-micelle interactions remain elusive due to lipid-water interface complexity. Our study sheds light on this much-neglected domain of plant lipid biochemistry. The purified hazelnut lipase was studied via absorbance stopped-flow with 4-nitrophenyl laurate micelles. By varying substrate concentrations, we identified a two-step reaction: (i) reversible enzyme-micelle binding and (ii) rapid hydrolysis yielding 4-nitrophenol. Kinetic analyses support distinct binding and turnover phases. ITC experiments with substrate analogous further revealed substrate-dependent modulation of enzymatic affinity, with dissociation constants and enthalpy changes confirming structural influence on lipase activity. This study presents one of the first comprehensive molecular examinations of lipase-micelle interactions, a challenging but necessary area due to the complexity of lipid interfaces. The scarcity of plant lipase literature highlights the uniqueness of our observations. Due to their role in seed physiology, lipid mobilization, and biotechnological utilization, our interdisciplinary approach advances knowledge of plant lipid metabolism. Later research should investigate if the same principle applies to other organisms and analyze lipid microdomains as regulatory structures in enzymic activity.

P-29-011
Influence of macromolecular crowding on the kinetic of IscS desulfurase

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Although enzymes have traditionally been studied *in vitro* in aqueous solutions, these conditions do not accurately reflect their natural environment. In cells, enzymes operate in a crowded molecular space, where other macromolecules may influence their behavior [1]. To bridge this gap, we studied a well-characterized enzyme under crowded conditions. IscS desulfurase, involved in iron-sulfur cluster biosynthesis and sulfur transfer, was chosen due to its extensive biochemical characterization *in vitro*, and its composite active site, which includes a pyridoxal phosphate (PLP) cofactor and a catalytic cysteine loop [2]. To understand the behaviour of IscS in a more natural environment we used different crowding agents and conditions. Enzymatic kinetics were studied using a colorimetric assay based on methylene blue formation, to quantify spectrophotometrically (650 nm) the sulfide, a product released by the enzyme. Experimental conditions included the use of crowding agents such as polyethylene glycol (PEG) at different molecular weights, dextran, and *Escherichia coli* lysate to simulate the intracellular environment. The results revealed that PEGs and dextran increased the Michaelis Menten constant (K_m) and decreased the maximum reaction velocity (V_{max}), suggesting steric hindrance effects rather than substrate sequestration. The strongest inhibition occurred with cell lysate, which exhibited the highest K_m and lowest V_{max}, implying additional regulatory interactions beyond steric hindrance effects. These findings highlight the importance of macromolecular crowding in modulating enzymatic activity, reinforcing the need to study enzymes under more cell-like conditions. Future research should explore the molecular mechanisms behind these effects to better understand the physiological role of IscS and other enzymes *in vivo*. [1] A. P. Minton (2001) Journal of Biological Chemistry 276, 10577–10580 [2] J. R. Cupp-Vickery et al. (2003) Journal of Molecular Biology 330, 1049-1059.

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P-29-012
Inhibitory effect of vitamin c on lactate dehydrogenase and citrate synthase

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Cancer cells rely on metabolic adaptations to sustain uncontrolled proliferation, particularly through increased glucose consumption and lipid synthesis. Lactate dehydrogenase (LDH) and citrate synthase (CS) are key enzymes in these pathways, supporting anaerobic glycolysis and citrate-dependent lipid production. This study investigates the inhibitory effect of vitamin C on LDH and CS to explore its potential as a metabolic-targeting strategy in cancer therapy. Enzymatic assays were performed to determine the kinetic parameters of LDH and CS *in vitro*, followed by inhibition experiments using increasing concentrations of vitamin C. Lineweaver-Burk plots revealed a competitive inhibition mechanism for LDH, with an IC50 of 15 mM and an inhibition constant (*Ki*) of 11.8 μM. For CS, uncompetitive inhibition was observed, with an IC50 of 12 mM and *Ki* values ranging from 6.8 to 10 mM depending on the substrate. *In silico* molecular docking studies confirmed the binding of vitamin C to the active site of LDH and a secondary regulatory site in CS, supporting its inhibitory effect. By impairing LDH activity, vitamin C reduces lactate production, limiting acidification of the tumor microenvironment, a key factor in chemotherapy resistance. Inhibition of CS disrupts the tricarboxylic acid cycle and fatty acid synthesis, potentially reducing membrane biosynthesis necessary for cancer cell proliferation. These findings suggest that vitamin C-mediated inhibition of LDH and CS may interfere with tumor metabolic flexibility, providing a promising approach for enhancing cancer treatment efficacy. Further studies are required to validate these results *in vivo* and assess their clinical implications.

P-29-013
Enzymatic and functional characterization of a phospholipase D involved in fertilization in plants

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By identifying new types of phospholipases D (PLD), we are attempting to decipher the structure-function relationships of PLDs, a family of enzymes present in microbes, plants, and animals. These PLDs generate phosphatidic acid (PA) by hydrolyzing the distal phosphodiester bond of glycerophospholipids and are involved in cell signaling, tumorigenesis in animals and pollination in plants. In plants, a gene encoding a putative isoform of PLD was recently described in the pollen grain, as playing a fundamental role in the fertilization mechanism in maize. Genomic mutation leads to the formation of haploid embryos which will naturally duplicate their chromosomes to return to the diploid state. This phenotype is highly sought after by seed companies during crossbreeding to quickly create new varieties that are genetically stable and of great agronomic interest. The gene encoding this putative PLD was therefore cloned and expressed in the yeast *Pichia pastoris* for the biochemical characterization of the recombinant enzyme. In addition, PLD activity was measured in wild-type or PLD-mutated pollen grains. Recombinant expression results show that this gene encodes an enzyme with PLD activity. Furthermore, if 14 genes encoding putative PLDs are described in maize, by measuring PLD activity in wild-type or mutated pollen grains in this particular gene, we showed that this single gene is responsible for all of the PLD activity in this tissue. This therefore confirms the fundamental role of this PLD enzymatic activity in the fertilization mechanism in plants. We identified a plant PLD playing a major role in plant fertilization and the PLD activity of this gene was demonstrated. The biochemical characterization of this PLD is underway to understand how this PLD is involved in the haploid induction mechanism.

P-29-014
Modulation of aldose reductase-like 1 enzyme (AKR1B10) activity by thiols

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AKR1B10, also known as aldose reductase-like 1, is a monomeric NADPH-dependent enzyme involved in the redox metabolism of various endogenous and exogenous compounds. Among the endogenous compounds, the enzyme can effectively reduce many reactive carbonyl species (such as 4-hydroxy-2,3-nonenal, HNE), which can influence cellular processes such as proliferation, oxidative stress response, autophagy, ER stress, mitochondrial function, and apoptosis. Moreover, altered expression levels of AKR1B10 in several pathophysiological conditions highlight its key role in chemoresistance and the onset and/or progression of several diseases, thereby promoting extensive research on enzyme inhibitors that may be useful in therapeutic contexts due to their ability to reduce AKR1B10-dependent chemoresistance and/or tumor proliferation. Proteins can undergo post-translational modifications, which can affect their activity. One such modification, glutathionylation, occurs in response to altered cellular conditions, such as increased oxidative stress associated with tumor onset and growth. AKR1B10 has four cysteines (Cys45, 187, 200, and 299), with C299 near the active site, suggesting that its enzymatic activity could be modulated by oxidative stress through glutathionylation. While previous studies have examined the effects of thiols on enzymatic activity with non-physiological substrates, this study focuses on the impact of glutathionylation in the presence of the physiological substrate HNE. Specifically, based on these premises, the *in vitro* effect of glutathionylation induced by oxidized glutathione on AKR1B10 enzymatic activity was investigated, evaluating the potential reversibility of the process and the enzyme's varying susceptibility to known inhibitors, depending on parameters such as thiolating agent concentration, incubation time, and substrate concentration.

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P-29-015
Uncovering diversity and intra-subfamily variations in glycoside hydrolase family 116: fresh perspectives on the function and inhibitor sensitivity of subfamily II

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The glycoside hydrolase family 116 (GH116) encompasses protein sequences from Eukarya, Archaea, and Bacteria, including the human enzyme glucosylceramidase 2 (GBA2), which plays a role in sphingomyelin synthesis [1,2]. Phylogenetic analysis has classified this family into four distinct subfamilies, each exhibiting unique substrate specificities and inhibitor sensitivities [3,4]. Although a subdivision into subfamilies has been proposed, there are very few characterized GH116 members, with most subfamilies having only one characterized representative. Through metagenomic analysis of two geothermal pools at the Pisciarelli Solfatara (Naples, Italy), nine distinct sequences encoding potential novel archaeal GH116 enzymes were identified [5]. In this study, these sequences were examined and categorized into their respective subfamilies, with a focus on comparing the structural motifs across different GH116 subfamilies. Additionally, we performed biochemical characterization and inhibition studies on two enzymes from subfamily 2, which has previously been represented by only a single characterized member. The amino acid residues contributing to the active site structure of subfamily 2 were also mapped, and we explored the characterization and inhibition of two selected mutants. This research offers new perspectives on the structural and functional variability of GH116 enzymes, highlighting the distinct subfamilies and revealing previously unidentified intra-subfamily variations. These findings not only expand our understanding of GH116 subfamilies but may also inform enzyme engineering and inhibitor development strategies. 1. Citterio, A., et al., J Neurol, 2014. 261(2): p. 373-81 2. Cobucci-Ponzano, B., et al., J Biol Chem, 2010. 285(27): p. 20691–703. 3. Ferrara, M.C., et al., Biochim Biophys Acta, 2014. 1840(1): p. 367-77. 4. Shimokawa, M., et al., Nat Commun, 2023. 14(1): p. 6299. 5. Strazzulli, A., et al., FEBS J, 2020. 287 (6): p. 1116-1137.

P-29-016
Exploring the mechanisms and activity of nazolysin, a novel metalloprotease from *Tannerella forsythia*

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Periodontal disease is one of the most prevalent infectious inflammatory conditions affecting teeth-supporting tissues, with proteolytic enzymes playing a critical role in the disease pathogenesis. Here, we identified and characterized a new protease from the M43 family, named Nazolysin, identified in the re-sequenced genome of *Tannerella forsythia* ATCC 43037. The conserved KLIKK motif at the enzyme C-terminus classifies it as another KLIKK protease encoded in the *T. forsythia* genome. Full-length Nazolysin consists of 499 amino acids, including a signal peptide, a catalytic domain of the M43 family of metalloproteases, and a C-terminal domain characteristic of proteins secreted via type 9 secretion system (T9SS). Nazolysin was expressed in *Escherichia coli* as a GST-fusion protein and purified as a 54.4 kDa recombinant enzyme. Spontaneous activation of recombinant Nazolysin involved the cleavage of 24-amino acid N-terminal profragment, maintaining the proenzyme's latency through a cysteine-switch mechanism independent of calcium ions. This mechanism was confirmed using the directed mutagenesis approach. A Cys27Ala variant of Nazolysin transformed into the mature ~36 kDa active enzyme through autoproteolytic cleavage. N-terminal sequencing of autocleavage products revealed that Nazolysin, unlike other M43 proteases, prefers aromatic residues at the P1' position and degrades various physiological proteins related with periodontal disease. The enzymatic activity of Nazolysin was assessed using FITC-casein as a substrate and found to be inhibited by 1,10-phenanthroline, DTT and by divalent cations such as Ca^{2+}, Zn^{2+}, and Mn^{2+}. Interestingly, Nazolysin was inhibited by Potempin N, a protease inhibitor encoded by the ORF directly downstream of the Nazolysin gene. In conclusion, the preliminary characterization of Nazolysin highlights its features, justifying further structural and functional studies to elucidate its role in pathophysiology of *T. forsythia* in periodontitis.

P-29-017
Fungal caffeylpyruvate hydrolase converts oxyluciferin into caffeic acid

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Fungal bioluminescence requires the caffeic acid cycle, a pathway starting from caffeic acid and consisting of four enzymatic reactions catalyzed by hispidin synthase (HispS), hispidin-3-hydroxylase (H3H), luciferase (Luz), and caffeylpyruvate hydrolase (CPH). HispS, H3H, and Luz catalyze formation of hispidin, 3-hydroxyhispidin (luciferin), and caffeylpyruvate (oxyluciferin), respectively. The final enzyme, CPH, has been proposed to catalyze the hydrolysis of oxyluciferin into caffeic acid, but experimental evidence was previously lacking. This reaction is important because it recycles caffeic acid, allowing sustained bioluminescence in engineered organisms without the need to add exogenous caffeic acid. In this work, we studied enzymatic caffeylpyruvate hydrolysis *in vitro* and demonstrated that CPH does indeed catalyze caffeic acid formation from oxyluciferin. *Neonothopanus nambi* caffeylpyruvate hydrolase (nnCPH) was expressed in *Pichia pastoris*, and the recombinant enzyme was purified by affinity chromatography. Without CPH, two simultaneous non-enzymatic reactions occur, neither producing caffeic acid. First, hydroxyl groups (O-H) at the 3rd and 4th positions of the oxyluciferin benzene ring oxidize to carbonyl groups (C = O), resulting in product A (247.0227 Da). Second, oxyluciferin hydrolyzes to product B (3,4-dihydroxybenzylideneacetone, 177.0539 Da). Upon addition of

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purified nnCPH, rapid caffeic acid accumulation alongside a drop in oxyluciferin concentration was observed. Caffeic acid, produced by the CPH-catalyzed reaction, emitted light when combined with yeast expressing HispS, H3H, and Luz, whereas degradation products A and B did not. The rate of CPH-catalyzed oxyluciferin utilization was at least 100 times higher than non-enzymatic degradation. Thus, CPH converts oxyluciferin to caffeic acid, playing a crucial role in fungal bioluminescence pathway. The reported study was funded by RSF project number 24-74-10105, https://rscf.ru/en/project/24-74-10105/.

LB-R-29-01
Novel biomarker for measuring NADPH oxidase activity: a preliminary study

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In immune system cells, NADPH oxidase activity is measured by flow cytometry in the conversion of dihydrorhodamine (DHR 123), which fluoresces as a result of oxidative reaction, to rhodamine (RH123). DHR123 assay plays a particularly important role in the diagnosis of chronic granulomatous disease (CGD). Since CD45 is a transmembrane glycoprotein expressed on the surface of many cells of hematopoietic origin, it is widely used as a pan-leukocyte marker for the differentiation and characterization of cellular populations, especially in flow cytometry analysis. In flow cytometric analysis with CD45 antibodies coupled with APC-Cy7/H7 tandem fluorochromes, new signals in the presence of superoxide and hydrogen peroxide were mentioned and it was shown that tandem fluorochromes degrade [previously published in: Le Roy C et al (2009) Cytometry A 75, 882-890]. The aim of this study was to compare NADPH oxidase activity values by flow cytometry using peripheral venous EDTA blood samples of 20 healthy volunteers with the results obtained by CD45 APC-Cy7 and DHR 123 assay. NADPH oxidase activity in PMA-stimulated neutrophils obtained by flow cytometry was measured with DHR 123 and CD45 APC-Cy7. Flow cytometric images were evaluated with APC channel, the oxidized form of CD45 APC-Cy7. Analyses were performed on a BD LSRFortessa model flow cytometer over 50 000 leukocyte counts with BD FACSDiva Software v8.1 program. There was no significant difference in NADPH oxidase activity measurements obtained from neutrophils using DHR 123 and CD45 APC-Cy7 as a result of flow cytometric gating ($p > 0.05$). In conclusion, it can be stated that NADPH oxidase activity measured with CD45 APC-Cy7 may be an alternative to the DHR 123 assay and may be considered as a novel biomarker that may play an important role in the diagnosis of a primary immunodeficiency such as CGD. This study was supported by grants from Erciyes University Scientific Research Projects BAP Unit as TSA-2024-13659 project. Ethical approval number: 2023/482. *The authors marked with an asterisk equally contributed to the work.

LB-R-29-02
Conserved lysines in the FMN domain of eNOS govern biochemical catalysis through regulation of interdomain electron transfer

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Endothelial nitric oxide synthase (eNOS) is a calmodulin-dependent flavoheme enzyme that catalyzes the conversion of L-arginine to L-citrulline and nitric oxide (NO). NO is a critical regulator of vascular tone, immune response, and cellular signaling. eNOS activity is tightly regulated by post-translational modifications, including acetylation and deacetylation of lysine residues. However, the functional roles of specific lysine sites remain incompletely understood. This study investigates two conserved lysines, Lys^{609} and Lys^{733}, located in the FMN-binding domain. Acetylation was mimicked by lysine-to-glutamine substitutions (K609Q, K733Q) as glutamine structurally resembles acetylated lysine, while deacetylation was mimicked by lysine-to-arginine substitutions (K609R, K733R) which mimic the deacetylated state. Full-length and reductase domain variants were cloned, expressed, and purified to evaluate NO synthesis, NADPH oxidation, cytochrome *c* and ferricyanide reduction, electron flux, flavin fluorescence, and coupling efficiency. K609Q enhanced NO production by ~80% with improved coupling (NADPH/NO ratio ~2.9 vs ~5.0 in WT), increased cytochrome *c* reductase activity (~1.5-fold), and faster flavin autooxidation, suggesting enhanced electron flux and reduced FMN semiquinone stability. K733Q showed wild-type-like behavior. In contrast, K609R and K733R exhibited 38% and 25% reduced NO synthesis, respectively, and severely impaired coupling (ratios ~8.2 and ~8.6), with 3- to 6-fold lower cytochrome *c* reductase activity, reduced flavin fluorescence, and unchanged ferricyanide reduction. These results reveal that acetylation at Lys609 enhances eNOS catalytic efficiency and coupling, while deacetylation disrupts electron transfer, increasing uncoupling and ROS generation. Targeting these sites may offer new strategies for restoring eNOS function in vascular disease.

LB-R-29-03
Evaluation of the thermostability of DNA (cytosine-5)-methyltransferase M.ApeKI with differential scanning calorimetry

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Recently, the aberrant DNA methylation has been associated with several diseases such as cancer, lifestyle diseases, and allergic diseases. Thus, analysis of DNA methylation is significant for understanding the mechanism. Many thermostable proteins have been developed for industrial applications, but little is known regarding the thermostable DNA methyltransferases. In our previous work, we focused on M.ApeKI, which is a DNA methyltransferase from *Aeropyrum pernix* K1, a hyperthermophilic archaeon. From the results, M.ApeKI recognizes the sequence 5′-GC(A/T)GC-3′ and methylates the C-5 position of the second cytosine from the

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5′-end. Moreover, the optimum temperature is over 70°C, and it is strongly tolerant to high temperatures. M.ApeKI is the first highly thermostable DNA (cytosine-5)-methyltransferase. However, the detailed analysis of thermostability is still unknown. In this study, we analyzed the thermostability of M.ApeKI with differential scanning calorimetry (DSC). M.ApeKI was purified using a His-tag purification system. Then, the purified M.ApeKI was dialyzed from the elution buffer to 50 mM phosphate buffer (pH 7.5) containing 500 mM sodium chloride. DSC analysis was performed as follows: raising speed of temperature by 1°C/min from 25°C to 130°C, and the concentration of the dialyzed M.ApeKI was 1 mg/mL. The deletion variant of the disulfide bond in M.ApeKI (C53S/C69S) was also analyzed under the same conditions. As a result, the melting temperatures of M.ApeKI were 106 ± 0.026°C and 108.7 ± 0.083°C, and the Tm of C53S/C69S variant was 97 ± 0.047°C. This result suggests that the disulfide bond contributes approximately 10°C to the thermostability of M.ApeKI. However, the high melting temperature observed in the C53S/C69S variant indicates that other molecular mechanisms are also involved in the thermostability of M.ApeKI. Further studies are needed to identify these mechanisms and understand their contributions to the stability at high temperatures.

LB-R-29-04
Underground bacterial metabolism of Maillard reaction products: degradation of Nε-carboxymethyllysine in *Escherichia coli*

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The Maillard reaction, also glycation, is a prominent non-enzymatic modification of amino acids. Occurring at high temperatures between a reducing sugar and a free amino group, it results in many compounds found in processed foods. One of the best-known glycated amino acids, N_ε-carboxymethyllysine (CML), is consumed widely by humans, reaching a daily intake of 24.6 mg [previously published in: Mark AB *et al.* (2014) Diabetes care 37, 88-95]. It is absorbed in limited amounts by the gastrointestinal tract, which suggests a major role in its degradation by colonic bacteria [as previously published in: Lassak J *et al.* (2023) Curr Opin Microbiol 76, 102393]. *E. coli* produces novel metabolites when exposed to CML, mainly N_ε-carboxymethylcadaverine (CM-Cad), followed by N_ε-carboxymethylaminopentanoic acid (CM-APA) and a piperideinium ion [previously published in: Hellwig M *et al.* (2019) J Agric Food Chem 67, 1963-1972]. We describe the first underground metabolism of CML, proceeding through promiscuous enzymes that accept this compound and its downstream metabolites as substrates. Several decarboxylase-lacking *E. coli* deletion mutants were tested for degradation of CML to CM-Cad via HPLC-MS. The ornithine decarboxylase SpeC was thus discovered as the enzyme that generates CM-Cad, and *in vitro* characterization followed. Furthermore, *E. coli* can utilize CML as a sole nitrogen source. Based on the identified metabolites, a degradation pathway like the one of other biogenic amines was hypothesized. We selected 9 amino acid transaminases to investigate *in vivo* and *in vitro*. The transaminases GabT, PuuE, PatA accept CM-Cad and CML as substrates, with the formation of glutamate, while IlvE accepts CML only. This study reveals a new metabolic route within *E. coli*, generating unique compounds that may influence gut microbiota dynamics and host physiology. The unknown effects of these metabolites on other gut bacteria and the human host warrant further exploration. *The authors marked with an asterisk equally contributed to the work.

Cancer – basic research

P-30-001
Effects of irisin combined with gefitinib on expression levels of miRNAs in the HepG2 cell line

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This study aims to investigate the effects of the combination of irisin and gefitinib on the expression of AKT1, FNCD5, VEGF, Bcl-2, EGFR, PI3K, CDK1, and ERBB2 genes, which play critical roles in the pathogenesis of hepatocellular carcinoma (HCC), as well as the expression of miRNAs targeting these genes in the HepG2 cell line. MTT assay was applied to evaluate the cytotoxicity induced by irisin or gefitinib. Total RNA of the control and treatment groups was isolated using the RNA isolation kit. Subsequently, performed with the cDNA synthesis kit and the synthesized cDNA was used as a template for PCR reactions. miRNAs in the control and treatment groups were isolated using the miRNA isolation kit. Expressions of miR-664b-5p, miR-3922-5p, miR-6843-3p, miR-8085, miR-381-5p, miR-1275, miR-1304, and miR-6801-5p and the expression of target genes of these miRNAs were determined by RT-PCR. The most effective time for the combination of irisin and gefitinib in HepG2 cells was 48 hours and the dose was 3.125 μM for irisin and 10.34 μM for gefitinib. In HepG2 cells, PI3K, Akt, EGF, and ErbB2 expressions were significantly decreased in irisin groups compared to the control group, but this decrease was not significant for VEGF, CycD1, and Bcl-2. Furthermore, the evaluation of miRNA expression profiles showed that irisin, and combination with gefitinib, significantly upregulated the expression of miR-664b-5p, miR-381-5p, miR-3922-5p, miR-1275, and miR-1304. The combined treatment of irisin and gefitinib significantly decreased miR-8085 expression. The expression levels of miR-6843-3p and miR-6108-5p did not demonstrate statistically significant differences. Our study shows that irisin can enhance the anticancer effects of gefitinib in the HepG2 cell line and dysregulated miRNA expressions contribute to HCC pathogenesis by decreasing cell proliferation and inducing apoptosis.

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P-30-002
Dissecting the mechanisms of DNMT3A-R882 mutant clonal haematopoiesis

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DNMT3AR882H/C is commonly mutated in clonal haematopoiesis of indeterminate potential (CHIP) and increases the risk of developing acute myeloid leukaemia (AML). The mechanism of increased clonal fitness for haematopoietic stem and progenitor cells (HSPCs) is incompletely understood. We hypothesize that subtle focal hypomethylation in DNMT3AWT/R882H/C HSPCs is caused by site-specific heterotetramer formation with variable methylation capacity. This would result in altered epigenetic regulation of gene expression and function of mutant cells. We have developed an *in vitro* murine model of Dnmt3aWT/R878C CHIP (the murine equivalent of R882C) in 416B cells using CRISPR/Cas9-editing and prime-editing. This has resulted in single-allele 3xTy1-tagged-Dnmt3a clones with the following genotypes: Dnmt3aWT/WT-3xTy1, Dnmt3aWT/R878C-3xTy1, Dnmt3aR878C/WT-3xTy1, and untagged Dnmt3aWT/WT and WT/R878C. A comprehensive multi-omic analysis is now underway, including whole genome bisulphite sequencing, ATAC-Seq, CUT&Run and RNA-Seq. In particular, anti-Ty1 CUT&RUN will allow precise genomic localisation of Dnmt3a to be correlated with methylation patterns. These clones provide a powerful model from which to interrogate the underlying epigenetic mechanisms of DNMT3AR882H/C CHIP. The correlation between methylation patterns and localisation of WT or mutant Dnmt3a on the genome will offer novel insights into how the dominant negative mechanism of DNMT3AR882H/C functions. Moreover, our comprehensive epigenetic analysis may reveal differences in transcription factor binding and gene expression. Greater understanding of this epigenetic landscape offers the possibility to identify vulnerabilities in mutant cells for diagnostic detection or targeted intervention with the ultimate goal of preventing progression to malignancy.

P-30-003
Investigation of the effect of warfarin on the ferroptosis pathway in MDA-MB-231 and BT-474 cells with different receptor profiles

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Breast cancer is the most commonly diagnosed cancer in women and is divided into cell subtypes based on the expression of estrogen receptor (ER), progesterone receptor (PR), and human epidermal growth factor receptor-2 (HER-2). The presence or absence of these receptors affects important factors such as prognosis, tumor formation, and risk of metastasis. However, no definitive treatment has been found for "triple-negative breast cancer" (TNBC), which does not express all three receptors. Ferroptosis is a form of iron-dependent cell death and is associated with lipid ROS accumulation due to decreased glutathione peroxidase. Warfarin is a widely used anticoagulant in cancer patients and regulates the vitamin K cycle by inhibiting vitamin K epoxide reductase complex 1 (VKORC1). Warfarin is known to induce ferroptosis and suppress tumor growth by inhibiting VKORC1L1. In this study, the comparative effect of warfarin on ferroptosis in BT-474 (triple positive) and MDA-MB-231 (triple negative) breast cancer cell lines was investigated. Optimum doses of the drug were determined and effects on cell proliferation, cell viability, colony formation potential, cell survival and intracellular reactive oxygen species levels were evaluated. In addition, effects on ferroptosis-related protein expressions other than VKORC1 (such as GPX4, NRF2, NCOA4, FTH1, SLC7A11) were analyzed by immunoblotting. Our findings suggest that triple positive breast cancer cell line BT-474 is more sensitive to warfarin-induced ferroptosis, probably due to inherent metabolic differences affecting redox homeostasis. These results may provide important information to fill the gaps in the literature regarding warfarin treatment in cell lines carrying different receptor profiles. In addition, detailed *in vivo* studies were planned with this study and preliminary information was obtained for preclinical modeling. *The authors marked with an asterisk equally contributed to the work.

P-30-004
Investigating the anticancer potential of quercetin and doxorubicin combination in colon cancer cells

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Colorectal cancer is among the most prevalent cancers worldwide, characterized by high incidence rates and poor prognosis, especially in advanced stages. Although chemotherapy remains a cornerstone of treatment, its effectiveness is often hindered by drug resistance. This has sparked growing interest in natural compounds as complementary agents to conventional therapies. In this study, LoVo colon cancer cells were treated with quercetin alone and in combination with doxorubicin to evaluate their anticancer potential. Cytotoxicity was assessed using the MTT assay (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) at 24, 48, and 72 hours, with 48 hours identified as the optimal time point for further experiments. ATP cell viability was measured using a luminometric kit, while ROS (reactive oxygen species) levels were detected using 2′−7′-dichlorodihydrofluorescein diacetate (DCFH-DA) fluorescent probe. Apoptotic cell death was examined through AO/EB staining (acridine orange/ethidium bromide) under a fluorescent microscope. The results indicated that the combination of quercetin and doxorubicin significantly enhanced the cytotoxic effect, increased ROS levels, and promoted apoptosis in the LoVo cells. These findings suggest that quercetin could potentiate the anticancer effects of doxorubicin in colon cancer cells.

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P-30-005
Investigating TREX1 as a biomarker for immune modulation across cancers: insights from a bioinformatics study

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The tumor microenvironment (TME) is a critical regulator of cancer progression and immune dynamics. TREX1 (Three prime Repair Exonuclease 1), a DNA exonuclease, plays a central role in immune homeostasis by preventing aberrant immune activation. While its role in autoimmunity is well-established, its function in cancer immunity remains unclear. This study investigates the association of TREX1 expression with immune infiltration, tumor mutational burden (TMB), microsatellite instability (MSI), and immune marker genes across diverse cancer types to elucidate its impact on the TME. Using TCGA Pan-Cancer and TARGET GTEx data, we analyzed tumor-infiltrating immune cells, TMB, MSI, and immune marker genes. Immune infiltration was assessed via CIBERSORT, and Pearson correlation ($p < 0.05$) was conducted on 150 immune marker genes spanning key pathways. TREX1 expression correlated significantly with immune features across cancers. Immune infiltration analysis revealed associations with T_cells_CD8, Macrophages_M1, and NK_cells_activated in cancers such as GBM, LUAD, BRCA, and STAD. Positive correlations with TMB were observed in COAD, STAD, and KICH, while negative correlations occurred in CESC and LUAD. MSI analysis indicated positive correlations in COAD and STAD and negative correlations in CESC and TGCT. Immune marker analysis identified correlations with chemokines (CCL5, CXCL9) and immunostimulators (CD40) in COADREAD, STAD, and LUAD, and with immunoinhibitory genes (PDCD1LG2, CTLA4) in CESC and TGCT. These findings suggest that TREX1 modulates immune activation and suppression, depending on the TME context. In conclusion, TREX1 demonstrates a complex, context-dependent role in tumor immunity, acting as both an immune activator and suppressor. Its dual regulatory capacity positions it as a potential biomarker for immune-related cancer therapies and a promising target for therapeutic intervention.

P-30-006
Prostate cancer cell lines with different metastatic potential show altered expression of HOX family lncRNAs

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Prostate cancer (PCa) is the most commonly diagnosed cancer in men and the second leading cause of cancer-related deaths after lung cancer, according to 2020 global cancer statistics. Long non-coding RNAs (lncRNAs) are key regulators of gene expression and cellular functions, including proliferation, invasion, and metastasis. This study aims to investigate the role of specific HOX family lncRNAs in modulating the metastatic potential of PCa. To achieve this, a panel of well-characterized prostate cell lines was used, including the non-malignant PNT1A, the androgen-sensitive LNCaP, the moderately metastatic DU145, and the highly metastatic PC3. RNA extraction was performed, followed by qRT-PCR analysis. Expression levels were calculated using the $2^{-\Delta\Delta Ct}$ method, and the relative expression of each gene was normalized to that of GAPDH. RNA expression levels were compared using the Student's t-test in the IBM SPSS Statistics 22 program. In highly metastatic PC-3 and LNCaP cells, HOTAIRM1 expression was found to be statistically unchanged compared to the normal cell line PNT1A, whereas in the DU-145 cell line, it significantly increased. When comparing HOTAIR expression levels, a significant decrease was observed in DU145 and LNCaP cells compared to PNT1A, while in the PC-3 cell line, which has high metastatic potential, HOTAIR expression was significantly elevated. As for HOXA-AS2 expression levels, no significant change was observed in the DU145 cell line compared to PNT1A, whereas a statistically significant decrease was found in LNCaP and PC3 cells. These findings suggest that HOTAIRM1, HOTAIR, and HOXA-AS2 may play a potential role in prostate cancer, and this role may be related to the cell type and behavior. Keywords: prostate cancer; HOTAIRM1; HOTAIR; HOXA-AS2; qRT-PCR; metastasis.

P-30-007
Interplay of m6A methylation and post-transcriptional regulation: the role of RNA-binding protein in hepatocarcinoma cells

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Hepatocellular carcinoma (HCC), the highly lethal form of primary liver cancer, is characterized by complex molecular alterations, including epigenetic and post-transcriptional modifications. One-carbon metabolism, a crucial pathway integrating folate and methionine cycles, generates metabolites such as S-adenosylmethionine (SAM) that serves as methyl donors for RNA methylation, particularly N6-methyladenosine (m6A). This dynamic and reversible RNA modification is a key regulator of gene expression in cancer cells. In HCC, m6A reader proteins (i.e. YTHDF2, ELAVL1 and hnRNPC) play pivotal roles in regulating mRNA splicing, stability and translation, influencing the oncogenic potential of tumor cells. This study explores the interplay between m6A methylation of one-carbon metabolism transcripts, and post-transcriptional regulation in HCC cells. MAT2A and MAT1A which encode the two subunits of methionine adenosyl-transferase, are regulated positively by ELAVL1 and negatively by hnRNPD, respectively in HCC. For the first time, we have identified various RNA-binding proteins (RBPs) in HCC cells, including ELAVL1, hnRNPC, YTHDF2 and hnRNPD, which regulate many transcripts involved in one-carbon metabolism, such as MAT2A, MAT1A, MTR (which encodes methionine synthase), and other mRNAs, through RNA immunoprecipitation analysis. In parallel, we aim to explore the impact of m6A methylation on the regulation of these mRNAs. To this end, we analyzed in HCC cells the m6A methylation

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profiles of specific transcripts related to one-carbon metabolism. Notably, MTR and MAT2A exhibit higher methylation levels compared to MAT1A. Additionally, data from the m6A ATLAS database revealed interactions between these mRNAs and specific RBP (ELAVL1 and hnRNPC) at the same m6A sites. This suggests that a specific m6A methylation site may regulate the coordinated functions of these RBPs, influencing RNA maturation processes such as splicing, stability and transport, a study currently underway.

P-30-008
Modelling neuroendocrine differentiation in bladder cancer and analysis of phenotypic and molecular effects

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Bladder cancer (BC) is one of the most common and deathly malignant diseases worldwide. Nearly 90% of BC originates from the urothelium. Urothelial carcinoma is histopathologically divided into two classes: muscle invasive bladder cancer (MIBC) and non-muscle invasive bladder cancer (NMIBC). Neuroendocrine-like bladder cancer (NE-like BC) is one of the subtypes of MIBC. Although NE-like bladder tumors have a very poor prognosis, information on the molecular characterization of these tumors is very limited. Importantly, although NE-like BC cannot be identified as neuroendocrine-type by initial histopathological examinations, molecular analysis has shown that that these tumors are in the NE-like subgroup. Therefore, an understanding of NE-like BC at the molecular level is of great importance for the diagnosis and treatment of this subtype. In this project, we created bladder cancer cell lines overexpressing mutant β-catenin, KRAS and ARID1A in different combinations to model neuroendocrine differentiation in bladder cancer. We characterized the neuroendocrine differentiation and cell growth in these models using RT-qPCR, immunofluorescence staining and MTT assay. Further, we performed ATAC-seq to investigate the changes in chromatin organization in the different cell lines we established. Our data revealed important changes in some key genes associated with neuroendocrine differentiation both at gene expression and chromatin organization level. We will further pursue the study via application of small molecule drugs which affect the WNT/β-catenin signaling pathway on the cell lines we generated. Ultimately, we envision that the results of these study will put forward the critical pathways involved in neuroendocrine differentiation in bladder cancer. *The authors marked with an asterisk equally contributed to the work.

P-30-009
Amyloid aggregation as promoting factor in cancer: the case of multiple myeloma

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Beta-2 microglobulin (b2m) constitutes the light chain of major histocompatibility complex I and promotes its stabilization on the cell membrane. Abnormal levels of b2m are detected in the bloodstream of multiple myeloma patients. Recent evidence suggests that b2m is engulfed by multiple myeloma tumor-associated macrophages and accumulates within lysosomes. The lysosomal environment favors b2m aggregation into amyloid fibrils, which accumulate and ultimately induce lysosomal damage. The release of lysosomal content triggers the activation of the NLRP3 inflammasome which mediates the maturation of inflammatory interleukin-1β and -18 (IL-1β and -18), thus supporting multiple myeloma progression. Therefore, the aim of the present study is to further characterize this process, thanking advantage of b2m mutants with different stability to investigate their behavior in lysosomal compartment and assess the potential pro-inflammatory effect of their aggregates on cells. The b2m variants were biophysically characterized *in vitro* under both physiological and lysosomal-like conditions. The melting temperature at pH 4.5 was significantly lower than that at pH 7.4 for all the tested proteins which exhibited also an enhanced structural flexibility, and a higher aggregation propensity. The sole exception was W60G – the most stable and low aggregation propensity variant – which was proteolyzed and did not aggregate. For the cellular experiments THP-1 cell line was incubated with wild-type and mutant b2m, collecting samples at different timepoints. All the b2m variants were rapidly engulfed by the cells and activated the NLRP3 inflammasome. Such activation induced proteolytic cleavage of caspase-1 and the subsequent release of IL-1β and -18 at higher levels compared to the untreated cells. Overall, lysosomal pH affects b2m stability, increasing its aggregation propensity. THP-1 treatment with b2m results into activation of NLRP3 inflammasome and a greater release of ILs.

P-30-010
Synergistic effects of oleuropein and paclitaxel on cell cycle, caspase-9 activation, and mitochondrial dysfunction in MCF-7 breast cancer cells

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Breast cancer is a growing global concern due to rising mortality rates, increasing the need for alternative treatments. Recently, polyphenols, including oleuropein (OLE), have gained attention for their anticancer potential. OLE, a natural polyphenol, has antioxidant, anticancer, and heart-protective properties. This study explored the effects of combining OLE with paclitaxel (PTX), a chemotherapy drug commonly used in cancer treatment, on MCF-7 breast cancer cells. MCF-7 cells were

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exposed to OLE (230 μM) and PTX (7.5 μM) at their IC_{50} levels, as well as to combined doses of OLE and PTX at 10%, 20%, 30%, and 40% of their IC_{50} values. The impact on cell cycle progression, caspase-9 activation, and mitochondrial membrane potential (ΔΨm) was assessed. Cell cycle analysis showed 11.56% of cells in the control group in G1 and 16.64% in G2/M. OLE induced G1 phase arrest (29.84%), while PTX caused G2/M phase arrest (53.14%). The 30% PTX combination group increased the proportion of cells in G2/M to 66.84%, resembling the effect of PTX alone. Apoptosis was most pronounced in the 10% and 20% OLE combination groups, with higher doses inducing G2/M arrest. Caspase-9 activity significantly increased in the 10% and 20% OLE combinations. In the ΔΨm analysis, the proportion of JC-1 positive cells was 23.22% in the control group, which increasing to 41.39% in the OLE IC_{50} group and 48.42% in the PTX IC_{50} group. ΔΨm increased to 66.57% at 20% and 72.42% at 40% in the combination groups. Evaluating all results, it was found that the combination of OLE and PTX promoted G2/M phase arrest, activated caspase-9 and disrupted ΔΨm, showing synergistic antitumour effect on MCF-7 cells. The combination reduced the toxic dose of paclitaxel compared to the IC_{50} of PTX alone. 1. Yılmaz, G., & Özdemir, F. (2024). Novel Anti-tumor Strategy for Breast Cancer: Synergistic Role of Oleuropein with Paclitaxel Therapeutic in MCF-7 Cells. Anti-Cancer Agents in Medicinal Chemistry, 24(3), 224-234.

P-30-011
Investigation of cancer stem cell development mechanism in 3D lung tumor models in hypoxic microenvironment

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The aim of this study is to investigate the mechanisms of cancer stem cell (CSC) development in 3D lung tumor models and to identify novel markers for the diagnosis and treatment of non-small cell lung cancer (NSCLC). A549 lung adenocarcinoma cells were cultured in both 2D and 3D cultures. Matrigel and agarose-scaffold methods were used for 3D cultures. Hypoxic conditions were created and optimized. After that presence of hypoxic conditions were verified by qPCR, immunoblotting and immunohistochemistry using known hypoxic markers. Protein isolation (membrane and total proteins) was performed from 2D and 3D cell culture cultured under hypoxic conditions. Proteins were analyzed using LS/MS method and data were identified by UNIPROT database using ProteinLynx Global Server (PLGS) software. The identified proteins were analyzed using Gene Ontology (GO) and DAVID functional annotation tools. Significant differences were observed in protein profiles between 2D and 3D-A549 cell models under both normoxic and hypoxic conditions. A total of 45 proteins were identified in 3D-A549 cells, 24 under hypoxia and 22 under normoxia. 23 proteins were identified in 2D-A549 cells, 7 under hypoxia and 16 under normoxia. The potential of these proteins as biomarkers for NSCLC diagnosis, treatment and their relationship with cancer stem cell mechanism will be investigated in future studies. In particular, plasma proteins and important membrane proteins have been identified, many of which have prognostic importance in cancer development. Additionally, parathymosin, which is a protein thought to play an important role in cancer development, was detected in both matrigel and agarose scaffold 3D cultures under hypoxic conditions, and requires further research.

P-30-012
Investigation of NCOA4-mediated ferritinophagy in cholangiocarcinoma cell line

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Cholangiocarcinoma (CCA) is a rare type of cancer. However, it is the second most common type of liver cancer worldwide, originating from the malignant transformation of cholangiocytes. The majority of CCA cases are diagnosed at advanced stages due to the asymptomatic nature of the disease in its early phases. Consequently, only 30% of tumors are resectable at the time of diagnosis, leading to poor prognosis with five-year survival rates ranging between 7% and 20%. Current treatment approaches include chemotherapy, radiotherapy, and surgical resection; however, curative options are limited to a small subset of patients due to late diagnosis. Ferroptosis, an iron dependent, non-apoptotic cell death mechanism, is regulated through ferritinophagy, a process involving ferritin degradation mediated by NCOA4. Artesunate (ART), a compound primarily used for malaria treatment, has been shown to enhance NCOA4-mediated ferritinophagy and promote cell death in certain cancer types. However, the regulatory mechanisms of ART in cholangiocarcinoma cells remain unclear. In this study, the role of ART in NCOA4-mediated ferritinophagy and its relationship with ferroptosis were investigated in the HuCCT-1 cholangiocarcinoma cell line. After ART treatment at the IC_{50} concentration, malondialdehyde (MDA), glutathione (GSH) and iron levels were investigated in ferroptotic cell death. In addition, immunofluorescence analysis of the localization of FTH1 and NCOA4 proteins revealed notable findings. Moreover, NCOA4 expression regulators are HECW1 and ZNF350 were analyzed for expression alterations under ART effect by qRT-PCR. These results provide significant insights into the regulation of NCOA4-mediated ferritinophagy in CCA cells and establish a foundation for future research in this area. *The authors marked with an asterisk equally contributed to the work.

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P-30-013
Cellular senescence in colon cancer cell chemoresistance: role of hypoxia and autophagy inhibition

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Chemotherapy of colorectal cancer displays limited therapeutic efficacy due to chemoresistance. Autophagy and therapy-induced senescence (TIS) are suspected chemoresistance mechanisms, with TIS believed to induce phenotype changes also present in cancer stem cells. *In vitro* experiments were performed using two cell culture variants: in normoxia (21% O_2) and hypoxia (1% O_2) to determine if autophagy inhibition coupled with oxygen deprivation has a senolytic effect or hinders the ability of senescent cells to divide/produce progeny. The main phenotype-testing experiments were performed on HCT116 cell line treated with irinotecan (IRINO) to induce senescence, and with hydroxychloroquine (HCQ) as well as bafilomycin A1 (BAFA1) to inhibit autophagy. IRINO was capable of pharmacologically inducing senescence, based on increased proportion of polyploid and G2/M phase-arrested cells as well as SA-β-gal positive cells. This coincided with decreased expression of several, surface markers (CD44, CD133, CD166) upon senescence induction. qPCR experiments in HCT116 cell line depict the changes in the level of expression of proliferation- and metabolism-related genes in hypoxic condition and upon treatment with HCQ as well as BAFA1. Additionally, BAFA1 induced a senolytic effect on the senescence-escape HCT116 cell line. Moreover, hypoxia may be a contributing factor for accelerated escape from senescence based on β-gal staining/qPCR results. As such, both regulation of autophagy and oxygen deficiency display an effect in regulating both senescent state and stem features, with hypoxia allowing for senescence escape and autophagy inhibitors having a senolytic effect in specific cases. This indicates the need to consider the role of newly introduced physicochemical parameters in personalized medicine. Funding: NCN SONATA BIS 7 nr 2017/26/E/NZ3/0043 MNISW: Doctoral project "Role of genes involved with fatty acid metabolism in cellular senescence – role of hypoxia" No. 506-1-109-01-24.

P-30-014
Caveolin-1 contributes to sorafenib resistance by enhancing aggressive phenotype in hepatocellular carcinoma

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Hepatocellular carcinoma (HCC) has one of the highest mortality rates among all cancers, primarily due to metastasis, recurrence, and resistance to therapy. Although HCC is one of the most studied cancer types, the diagnosis and treatment options are limited because of its heterogeneous and complex nature. Sorafenib, the first multi-kinase inhibitor, has been used as a first-line treatment for advanced HCC and remains the most preferred and accepted medication. However, prolonged therapy often leads to resistance to sorafenib, and the mechanisms underlying this resistance are not yet fully understood. To investigate the mechanisms of sorafenib-resistant (SOR-Res) HCC, we generated SOR-Res HCC cell lines (HuH-7, SNU-449, and MV). We performed transcriptomic analysis by RNA sequencing and found an increase in caveolin-1 (Cav-1) and caveolae-related genes. Additionally, we confirmed a significant increase in CAV1 expression through RT-PCR, western blotting, and immunofluorescence staining. When we examined caveolae structures using electron microscopy, we observed an increase in the number of caveolar structures in SOR-Res cells. To analyze the biological behaviours of SOR-Res cells, we performed various assays, including proliferation, adhesion, motility, spheroid formation, stress fiber staining, epithelial-mesenchymal transition (EMT) phenotype analysis, as well as *in vivo* zebrafish xenograft and metastasis assays. Our results indicated that SOR-Res cells were less adhesive and less proliferative, whereas they showed increased motility, more stress fibers, a more pronounced EMT phenotype, and larger spheroids. Zebrafish experiments showed that SOR-res cells were more metastatic when injected into zebrafish embryos. Furthermore, we confirmed the role of Cav-1 in sorafenib resistance through overexpression and siRNA silencing studies. This study suggests that Cav-1 plays a significant role in the process of sorafenib resistance in HCC. This project is funded by TUBITAK #118Z156. *The authors marked with an asterisk equally contributed to the work.

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P-30-015
Identifying critical DNA damage response (DDR) regulators for glioblastoma treatment with novel CRISPR/Cas9 library, DDRKOL

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Cells are constantly subjected to DNA-damaging agents and rely on sophisticated DNA damage response (DDR) mechanisms to repair lesions and preserve genomic stability. Impairments in these pathways, along with accumulation of DNA damage, contribute to genomic instability, cancer development, and aging. Chemotherapy and radiotherapy exert their effects primarily by inducing extensive DNA damage in cancer cells, leading to cell death. Glioblastoma (GBM), the most aggressive primary brain tumor with poor survival outcomes, is treated with a combination of radiotherapy and chemotherapy (Temozolomide, TMZ) following surgical resection. This study aimed to uncover novel regulators of cell survival and therapy response in glioblastoma using an *in vitro* CRISPR/Cas9 screen. To achieve this, we designed a targeted sgRNA library, DDRKOL (DNA Damage Response Knock-Out Library), consisting of 9000 sgRNAs targeting 888 DDR-related genes (10 sgRNAs/gene), along with controls. After validating the library's composition and coverage, CRISPR screens were conducted in U87 and A172 glioblastoma cell lines. The screening identified XRCC6, TOP2A, CDK1, and RAD21 as most significantly depleted genes in both cell lines. Functional validation through cell viability assays demonstrated that loss of these genes significantly impaired cell viability and reduced colony-forming ability of both cells, reinforcing their importance in GBM survival. These genes play key roles in DDR pathways, cell cycle regulation, and genomic stability, and their dysregulation has been strongly associated with cancer progression, including GBM. Further studies will investigate mechanisms through which these genes influence cell survival. The essential nature of these genes in GBM, identified with DDRKOL library, is consistent with their established roles in cancer biology. Their overexpression in GBM suggests their potential as therapeutic targets for enhancing treatment effectiveness and overcoming resistance to therapy. *The authors marked with an asterisk equally contributed to the work.

P-30-016
Overexpression of the adaptor protein Ruk/CIN85 in tumor cells leads to increased ROS production dependent on NOX(s) activity

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Reactive oxygen species (ROS), generated by NADPH oxidases in mammalian cells in response to activation of membrane receptors, play an important role in carcinogenesis. It has been shown that the adaptor protein Ruk/CIN85, which contains three SH3 domains, can interact with Pro-rich motifs of the adaptor protein Tks4, which functions as an organizer subunit of the Nox1-mediated NADPH oxidase complex, and overexpression of Ruk/CIN85 in MCF-7 cells correlates with differential system changes in NOX genes expression. To further investigate the role of Ruk/CIN85 in the dynamic control of redox homeostasis in tumor cells of different tissue origins, intracellular levels of ROS production and associated signaling pathways were studied in osteosarcoma cells HOS-2 and SAOS-2, melanoma cells B16/F10 and lung adenocarcinoma cells A549. Flow cytometry with the CM-H2DCFDA probe revealed increased ROS production in tumor cells overexpressing Ruk/CIN85, while apocynin, a potent NADPH oxidase inhibitor, successfully reduce this effect. Overexpression of Ruk/CIN85 in B16/F10 and A549 cells was associated with differential systemic changes in NOX genes expression, increased migration rate and Beclin content, marker of autophagy, all of which were negatively regulated by apocynin. Increased protein levels of the transcription factor c-Myb in B16/F10 cells and p38MAPK in A549 cells overexpressing Ruk/CIN85 were reduced in apocynin-treated cells, which correlates with the data published earlier. The results obtained suggest that the adaptor protein Ruk/CIN85 is one of the key components of redox-dependent signaling in tumor cells, associated with the functioning of membrane NADPH oxidase complexes that produce ROS, and thus regulate cellular responses involved in carcinogenesis. This study was supported by FEBS Ukrainian Shot-term Fellowship. *The authors marked with an asterisk equally contributed to the work.

P-30-017
Role of sodium-dependent vitamin c transporter 2 (SVCT2) in cancer cell migration

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Sodium-dependent vitamin C transporters (SVCTs), encoded by the SLC23 gene family, are key regulators of vitamin C uptake. Beyond their role as transporters, SLC proteins may also function as signaling receptors, underscoring the importance of investigating SVCT2 in cancer signaling pathways and its potential crosstalk with other cellular processes. This study aimed to explore the impact of SVCT2 on cell migration and its interaction with estrogen receptor alpha (ERα) signaling in breast cancer cells. SVCT2-GFP was overexpressed in MCF7 (ERα-positive) and MDA-MB-231 (ERα-negative) cell lines. Single-cell migration was precisely measured using a 2D organoid culture system under controlled conditions (37°C, 5% CO_2) and monitored via time-lapse fluorescence microscopy at 15-minute intervals over 25 time points. Our results revealed that overexpression of SVCT2-GFP significantly increased migration in MCF7 cells compared to Mock-GFP controls. Conversely, no increase in migration was observed in MDA-MB-231 cells overexpressing SVCT2-GFP. These findings suggest that SVCT2-mediated migration enhancement is linked to crosstalk with ERα signaling pathways. Importantly, the data were derived from single-cell migration analyses, offering a high level of precision and reliability compared to macro-scale assays like wound-healing experiments. This study highlights the potential role of SVCT2 in modulating cancer cell migration via interaction with ERα signaling, providing insights into its broader implications in cancer progression and therapeutic targeting.

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P-30-018
Expression of cancer-testis genes in uterine fibroids

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Uterine fibroids (UFs), also known as uterine leiomyomas, are one of the most common diseases in women. However, the etiology and pathogenesis of UFs are not clearly defined. Recently, it has been shown that malignant tumors express cancer-testis (CT) genes which lead to the reprogramming/reactivation of gametogenic program in tumor cells leading to unlimited growth. However, CT gene expression in benign tumors has been poorly studied. The aim of our study was to evaluate the MAGEA1-6, SSX1,2,4, XAGE1, NY-ESO1, MAGEC1 gene expression in UFs patients. The peripheral blood and fibroid tissue samples from 61 UFs patients were obtained from N.A. Semashko Regional Clinical Hospital of Nizhny Novgorod. The peripheral blood samples were collected before surgery. The tumor tissue samples were obtained after surgery. The CT mRNAs were detected by multiplex reverse transcription–polymerase chain reaction. The MAGEA1-6, SSX1,2,4, XAGE1, MAGE 1 mRNAs were detected in 65%, 36%, 16%, 13% of fibroid tissue samples, respectively. NY-ESO1 mRNA was not detected in fibroid tissue. In total, CT mRNAs were detected in 85% of fibroid tissues. In peripheral blood CT mRNAs were detected among 46% of patients. Detection rate of MAGEA1-6, SSX1,2,4, XAGE1, MAGEC1, NY-ESO1 mRNAs in peripheral blood was 29%, 18%, 18%, 3%, 5%, respectively. It should be noted that discrepancy between the set of CT mRNAs detected in the tumor and the set of CT mRNAs detected in the peripheral blood of the same patient was established. In the peripheral blood NY-ESO1 and XAGE1 mRNAs detected more frequently than in the UFs. Thus, it is shown that the CT genes expression is detected both in tumors and peripheral blood of UFs patients. It is possible that activation of CT genes in UFs is associated with hormonal disorders characteristic of this disease.

P-30-019
Targeting glioblastoma: investigating warfarin and C3G as potential therapeutic agents for cell death induction

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Glioblastoma is the most common and aggressive type of malignant brain tumor. Although surgical resection, chemotherapy and radiotherapy are used for treatment, effective treatment models are being developed due to the aggressiveness and invasive nature of the tumor. This study aims to investigate the effects of two agents with different properties on cell death in U87 glioblastoma cells. Warfarin, a commonly used oral anticoagulant, plays an important role in preventing blood clots. Cyanidin-3-glucoside (C3G) is an antioxidant found abundantly in red-purple foods and is a member of the anthocyanin family. In the study, the effects of warfarin or C3G application on cell death pathways in U87 cells were investigated. Initially, the effects of warfarin or C3G on cell viability, survival, and colony formation potential were investigated, and it was shown that these agents decreased cell viability and survival, and impaired colony formation potential. It is known that warfarin triggers the ferroptosis pathway through vitamin K metabolism in melanoma cells. Therefore, the effect of warfarin on the expression of key proteins in the ferroptosis pathway in U87 glioblastoma cells was investigated, and it was found to decrease the expression levels of GPX4 proteins. Furthermore, the effect of warfarin on the apoptosis pathway was also examined, and it was determined that it induced apoptosis in these cells through caspase 3 and caspase 7 activation. In the research with C3G, its effects on the apoptosis-autophagy-endoplasmic reticulum axis were investigated, and it was shown that C3G is effective in triggering cell death mediated by endoplasmic reticulum stress. These studies have shown that new therapeutic agents with potential for aggressive tumors such as glioblastoma may exist. Preliminary information has been obtained for preclinical modeling by planning detailed *in vivo* studies with these agents. *The authors marked with an asterisk equally contributed to the work.

P-30-020
Revealing the role of adipocyte-associated tumor microenvironment in pancreatic cancer and the therapeutic potential of orlistat

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Pancreatic cancer (PC) is an aggressive malignancy with a poor prognosis, primarily due to its tumor microenvironment (TME). Given that the pancreas is surrounded by adipose tissue, adipocytes play a crucial role in tumor progression within the TME. These cells secrete adipokines, hormones and cytokines essential for cellular communication, facilitating interactions between cancer cells and adipocytes which allow tumors proliferation and invasion. Orlistat, an anti-obesity drug (Xenical), functions as a pancreatic and gastric lipase inhibitor and has been found to inhibit the fatty acid synthesis enzyme FASN, suppresses tumor growth and induces apoptosis in cancer cells via lipid metabolism pathways. However, the effects of orlistat on adipocyte-associated pancreatic cancer cells and its influence on TME remain insufficiently explored. This study aims to investigate the role of orlistat on epithelial-mesenchymal transition (EMT) and apoptosis in pancreas cells co-cultured with adipocytes to model tumor microenvironment (TME). The first part of the study, a co-culture system using MIAPaCa-2 pancreatic cancer cells and 3T3-L1 mature adipocytes are established to create a TME-oriented model. The conditioned media (CM) method is utilized, where the medium from 3T3-L1 cells is collected, and applied to MIAPaCa-2 cells to simulate TME interactions. In the second part, orlistat treatment is utilized to this co-culture model, and its molecular effects on pancreatic cancer progression via TME are analyzed. The study explores the role of orlistat on EMT and apoptosis in MIAPaCa/2-3T3-L1 co-culture model by targeting fatty acid metabolism. Unlike conventional molecular studies that exclude TME components, this research provides a comprehensive understanding of pancreatic cancer's molecular mechanisms and therapeutic responses by explaining the role of adipocytes in the TME, offering novel insights for the treatment strategies.

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P-30-021
Investigation of the effects of cyanidin-3-glucoside and metformin on fatty acid metabolism and associated miRNA profiles in pancreatic cancer cells

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Pancreatic cancer (PC) is one of the most malign cancer types, with a poor chance of early diagnosis, a fast prognosis, and an extremely low survival rate. Research points to a direct relationship between type II diabetes mellitus (DM) and PC due to its high risk in diabetic patients. Metformin is a biguanide and a renowned drug prescribed to treat DM due to its effect on inducing the AMPK pathway and glucose uptake. Studies with various cancer types, including PC cell lines such as AsPC-1 and MiaPaCa-2, show that metformin also inhibits cell survival by arresting the cell cycle. Cyanidin-3-glucoside (C3G), an anthocyanin with antioxidant, anti-inflammatory, and anti-cancer properties, improves obesity and triglyceride metabolism in mice partially by inducing AMPK and regulating lipoprotein lipase. Research on different cancer types, such as the liver and stomach, has demonstrated that C3G can impede proliferation, angiogenesis, and invasion. However, its effect on PC has not been elucidated. In this study, we examined the anti-cancer effects of metformin and C3G, as well as their impact on the expression of miRNAs and proteins associated with fatty acid metabolism, in MiaPaCa-2 and Capan-2 PC cell lines grown in high-glucose media to model DM. The combinatorial and individual treatment of metformin and C3G inhibited cell proliferation, cell survival, and colony formation despite the high glucose concentration of the media. Additionally, fatty acid metabolism-associated miRNA and protein expression results align with our previous findings. This study demonstrates how different PC cells with distinct metastatic features respond to therapy. It also provides a foundation for future clinical studies, highlighting the importance of multifaceted treatment strategies while targeting PC as it is associated with other complex disorders, such as DM. *The authors marked with an asterisk equally contributed to the work.

P-30-022
Adipocyte-derived extracellular proteins affect tumor progression and prognosis in obese breast cancer patients

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Studies have shown that excessive obesity in breast cancer patients is associated with increased malignancy and poorer treatment outcomes. Due to the close proximity of breast cancer to adipose (fat) tissue, interactions among breast cancer cells, adipocytes, and immune cells create a complex tumor-adipose microenvironment (TAME) which plays a critical role in breast cancer initiation and progression. Understanding the regulatory mechanisms within the TAME could help address current treatment challenges and guide the development of future immunotherapies. Using the TriNetX database, we found that obesity significantly increases breast cancer incidence, and obese breast cancer patients are more prone to recurrence and distant metastasis. *In vitro* studies demonstrated that conditioned medium (CM) from adipocytes enhances breast cancer cell growth and migration. Co-culturing adipocytes with breast cancer cells successfully induced the transformation of adipocytes into cancer-associated adipocytes (CAAs). Moreover, CM from breast cancer-induced CAAs promoted macrophage infiltration. These findings suggest that CM from CAAs is enriched with factors that drive tumor progression and immune modulation. To further investigate the role of adipocytes in the obese tumor microenvironment, we identified eight extracellular proteins (Ccl6, Thbs1, Serpine1, Sema3f, S100A8, S100A9, Crtac1, and Adamtsl4) that were significantly upregulated in breast cancer-induced CAAs under obese conditions using sequence read archive (SRA) data. Tumor Immune Estimation Resource (TIMER) analysis revealed a strong positive correlation between these extracellular proteins and immune cell infiltration, particularly CD8+ T cells and macrophages. Notably, Serpine1 knockdown significantly reduced adipocyte differentiation and browning, thereby suppressing adipocyte-induced breast cancer growth and migration. Our findings suggest that extracellular proteins from adipose tissue play a pivotal role in breast cancer progression and immune suppression in obese environments. Targeting these adipose-derived extracellular proteins may offer a promising therapeutic strategy to improve breast cancer patient outcomes.

P-30-023
Stable endothelin-converting enzyme-1c enhances metabolic plasticity of colorectal cancer cells

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The isoform c of endothelin-converting enzyme-1 (ECE1c) is upregulated in many types of cancer. We have published that the CK2-dependent phosphorylation of Ser-18 and Ser-20 in cytosolic N-terminal domain of ECE1c improves its stability by blocking ubiquitination at Lys-6 and hence proteasomal degradation. We experimentally increased stability of ECE1c by changing the Lys-6 to the non-ubiquitinable residue Arg, creating a super-stable ECE1c^{K6R} mutant. Thus, we demonstrated that ECE1c^{K6R} promotes the occurrence of a cancer stem cell (CSC) phenotype and thereby aggressiveness traits in colorectal cancer cells, as well as recently in glioblastoma and lung cancer cells. Despite enhanced metabolic plasticity with preference for oxidative phosphorylation (OXPHOS) is a known CSC's trait, whether the stemness phenotype displayed by our colorectal cancer cells is associated to mitochondrial plasticity is unknown. Therefore, we studied whether the expression of ECE1c^{K6R} promotes mitochondrial changes and increases the OXPHOS rate in DLD-1 colorectal cancer cells. Mock (i.e. empty vector), wild-type, and super-stable ECE1c-expressing clones were developed in DLD-1 colorectal cancer cells. Proteins associated with OXPHOS and mitochondrial biogenesis, mtDNA copy number, colony-forming capacity, oxygen consumption rate

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(OCR, Seahorse), and mitochondrial mass were measured. Our results showed that ECE1c^{K6R} mutant promoted increased mitochondrial biogenesis in DLD-1 cells. In addition, ECE1c^{K6R} promoted a colorectal CSC's metabolic dependency for glucose, although cell growth was not affected, as well as maximal OCR was increased in both ECE1c^{WT}- and ECE1c^{K6R}-expressing cells. Altogether, our findings suggest that occurrence of a CSC phenotype is linked to an enhanced metabolic plasticity upon gain-of-stability of ECE1c, which ultimately lead to aggressiveness traits in colorectal cancer cells. This work is granted by FONDECYT 3220604 (ES-P) and 1220353 (JCT). *The authors marked with an asterisk equally contributed to the work.

P-30-024
Association of cancer-testis mRNAs detection with pathomorphological criteria of endometrial cancer

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Cancer-testis (CT) genes exhibit specific expression in immune-privileged tissues and different types of tumor cells, including the most common gynecologic cancer, endometrial cancer (EC). The aim of our study was to explore the expression of the CT mRNA MAGEA1-6, SSX1,2,4, XAGE1, MAGEC1, NY-ESO1 in tumor tissues and peripheral blood and to compare it with EC pathomorphological parameters. CT mRNAs were evaluated in 54 peripheral blood samples and 52 tumor samples from patients with EC using reverse transcription polymerase chain reaction. MAGEA1-6, SSX1,2,4, XAGE1 mRNAs were detected in 53.8%, 38.5%, 23.1% of tumors, respectively. NY-ESO1 and MAGEC1 mRNAs were detected in 7.7 and 5.7% of tumors, respectively. MAGEA1-6 and XAGE1 mRNAs were observed in the blood more often (14.8% in both cases). SSX1,2 and 4, NY-ESO1, MAGEC1 mRNAs were detected in 9.2%, 11.1% and 5.5% of blood samples, respectively. Comparison of the obtained results with the types of tumor growth showed that in tumors with mixed growth, the total detection rate of CT mRNAs reached 90% and was significantly higher than in tumors with endophytic growth ($p = 0.03$). The rate of MAGEA1-6 mRNAs detection was also significantly higher in tumors with mixed growth than in endophytic tumor growth ($p = 0.005$). In the blood of the same patients with mixed tumor growth, the total frequency of CT mRNAs detection was higher than the detection frequency in exophytic ($p = 0.017$) and endophytic tumor growth ($p = 0.035$). The frequency of CT gene mRNAs detection in tumors was not associated with degree of tumor differentiation. However, SSX1,2,4 ($p = 0.019$) and NY-ESO1 ($p = 0.035$) mRNAs were significantly more frequently detected in the blood of patients with poor-differentiated EC comparing with highly differentiated EC. Thus, CT mRNAs are possible biomarkers for EC molecular monitoring, however further studies are warranted to validate our findings.

P-30-025
Killing by iron: harnessing ferroptosis as therapeutic strategy in hepatocellular carcinoma

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Ferroptosis is an iron-dependent cell death which plays a role in the development and progression of hepatocellular carcinoma (HCC). This study aimed to explore the modulation of tyrosine kinase inhibitors (TKIs) sorafenib and lenvatinib in the different key factors of the ferroptosis pathway. Paired human tissue samples ($n = 23$) were collected and RNA expressions of 13 ferroptosis genes were determined by RT-qPCR. Kaplan-Meier curves were drawn to analyze the impact of the genes in patient prognosis. As *in vitro* validation, HCC cells were treated with TKIs at low and high doses. Functional assays were conducted including glutathione peroxidase activity, total iron content, and reactive oxygen species (ROS) assays to confirm mRNA analysis, together with ferroptosis activator erastin as control. Some ferroptosis genes were significantly dysregulated in HCC, including upregulation of *AKR1C1*, *FTL*, *SLC40A1*, *TFRC*, and *TXNRD*1 and downregulation of *MT1G*. The high *SLC40A1* expression endows poor prognosis. In Huh7 and JHH6 cells treated with sorafenib, *GPX4* showed consistent increase in both cell lines. Other ferroptosis genes showed opposing expression patterns, which could be due to inherent differences between the two cell lines. Combining the gene expressions in Huh7 cells treated with TKIs and erastin, *TFRC* and *NCOA4* were evidently downregulated in all treatments, suggesting that the modulation could be dependent to these genes. *HMOX1* (heme oxygenase 1) was upregulated by TKIs and erastin in dose-dependent manner. Moreover, both TKIs also decreased the glutathione peroxidase activity in SLC7A11-meditated blockage of cysteine import while increasing the total iron contents and ROS levels. The activation of ferroptosis could be a therapeutic strategy, especially in HCC tumors that no longer respond to treatment. Here, we show that the TKIs could activate ferroptosis which is mediated by certain ferroptosis gene drivers including *TFRC*, *NCOA4*, or *HMOX1*.

P-30-026
Monitoring of antitumoral properties for some heterocyclic compounds in a noncellular test-system from regenerating rat liver

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Aminoaldehydes are the oxidation productions of polyamines (PAs). They are cytotoxic and have been considered as a cause of apoptotic cell death due to their ability to act as carbonyl agents, conjugating with proteins and nuclear acids. Histochemical studies of animal and human malignant tissues showed the decrease of amine oxidase activity in NDEA-induced hepatomas,

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Guerin's carcinoma, human nephroblastomas, other cancer types. The oxidative deamination (OD) of PAs is decreased or almost arrested in tumor cells. Thus, the activators of PA catabolism are potential antitumor agents. The effects of some heterocyclic compounds on activity of the main enzymes of PA OD – spermine oxidase, polyamine oxidase (PAO) and diamine oxidase – were calculated in the acellular testing system of the rat regenerating liver. Only azafluorene and aniline derivatives were activators of PA catabolism, especially: 1-amino-9-phenylamino-4-azofluorene, 1-amino-2-bromo-4-azofluorene-9, 3-(4-iodinanilino)-1-phenylpropanone-1, 3-(1-phenyl-2-fluorinanilino)-propanone-1. The antiproliferative activity was demonstrated at prostate cancer cell line PC-3 by these compounds as well. QSAR analysis with Chemic-Descript software demonstrated the significant correlation of cytotoxicity with the Balaban topological index (R2 = 0.7). Docking with yeast PAO enzyme Fms1 using Molegro Virtual Docker (MVD) software was performed with flexibility in torsion angles of ligands. The lowest energy pose of each compound was described by energies of ligand interaction with different residues of enzyme. Decomposition of total docking score into partial ligand-residue terms was performed using the 'energy inspector' tool embedded in MVD. There were identified few critical atoms in the sphere radius 12 Å of the PAO active center: His67, Tyr450, His191, Trp174, Gly487. Results revealed the structural basis for the design and synthesis of novel activators of PA catabolism as potential antitumor substances.

P-30-027
The diagnostic significance of the correlation between inflammatory indices and body mass index in obese women with breast cancer

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Breast cancer is a complex disease influenced by various biological and lifestyle factors, including body weight and systemic inflammation. Among these, body mass index (BMI) is a common measure of obesity that is often correlated with breast cancer risk and outcomes. Inflammatory markers such as the systemic immune-inflammation index (SII), neutrophil-to-lymphocyte ratio (NLR), monocyte-to-neutrophile ratio (MNR), and neutrophil-to-lymphocyte and platelets ratio (NLPR) have garnered attention for their potential prognostic value in cancer patients. Elevated levels of these inflammatory indices are frequently associated with poorer survival outcomes, suggesting a link between obesity-related inflammation and breast cancer progression. In our study, we found statistically significant correlations between various inflammatory indices and BMI in obese women diagnosed with breast cancer. Specifically, higher BMI was associated with increased values of SII, NLR, MNR, and NLPR ($p < 0.05$). These findings underscore the role of inflammation in breast cancer among obese women, indicating that elevated inflammatory markers are linked to worse prognostic implications. Identifying these correlations allows us to leverage basic laboratory results to enhance prevention strategies. Routine assessments of these inflammatory markers, alongside BMI measurements, may provide valuable insights into an individual's risk profile, which can be useful in the context of malignant diseases. This proactive approach could facilitate early interventions aimed at reducing obesity and managing inflammation, ultimately contributing to better outcomes in breast cancer prevention.

P-30-028
Exploring aquaporin-5 in pancreatic ductal adenocarcinoma: tumor microenvironment interactions and therapeutic implications

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Pancreatic ductal adenocarcinoma (PDAC) is one of the most aggressive and lethal cancers with a highly immunosuppressive tumor microenvironment [1]. Despite advances in immunotherapy, these treatments have shown limited success in PDAC [2]. Aquaporins (AQPs), a family of membrane water channels, are overexpressed in PDAC, having a role in aggressiveness and reduced overall survival [3]. Therefore, this study aims to investigate the role of AQPs in cancer immunotherapy, evaluating their impact in modulating TME components, critical for immunotherapy efficacy, to further disclose its application as potential target or prognosis factor to improve PDAC patients outcomes. For this purpose, we established and characterized two murine PDAC cell lines: Panc02 and KPC. In qPCR and WB assays, AQP5 emerged as the most relevant isoform, expressed in Panc02, at values commonly observed in tumorigenic cell lines. However, in immunofluorescence and stopped-flow assays, KPC cells seemed to have superior AQP5 expression than Panc02, with higher permeability rate, suggesting differences in protein turnover between both cell lines and different AQP5-mediated transport dynamics. Since AQP5 was the most relevant isoform observed in cell lines tested, Lentivirus and CRISPR-Cas9 are being used to generate AQP5-overexpressing and AQP5-silenced variants, which will be implanted in murine models to evaluate tumor growth, immune infiltrations and responses. Elucidating the role of AQP5 on immune infiltration and therapeutic response, may help identifying new biomarkers and therapeutic strategies to enhance immunotherapy efficacy, laying the foundation for a more effective and personalized treatment to PDAC patients.
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P-30-029
The antidepressant sertraline impairs proliferation and invasiveness of human glioblastoma cells

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Glioblastoma is the most aggressive and highly invasive primary brain tumor, with current multimodal treatments resulting in a mean survival of only up to 15 months. Repurposing existing drugs that are capable of crossing the blood–brain barrier can offer a solution to improve the poor survival rate in glioblastoma. In this context, this study investigated the anti-carcinogenic and anti-invasive potential of sertraline, a selective serotonin reuptake inhibitor (SSRI), in glioblastoma treatment. We examined the effects of sertraline on U87 and U251 glioblastoma cell lines using BrdU cell proliferation, colony formation, and 3D spheroid cell proliferation assays, as well as flow cytometry for apoptosis detection. Metastatic cell behaviors were assessed through wound healing, Matrigel invasion, and adhesion assays. Results demonstrated that sertraline significantly suppressed cell proliferation and induced apoptosis in U87 cells. Both cell lines exhibited dose-dependent decreases in colony formation. In 3D culture models, sertraline showed anti-proliferative effects, significantly reducing spheroid volume and viability. Regarding metastatic cell behaviors, sertraline impaired migration, invasion, and adhesion abilities of U87 and U251 cells. These findings suggest that sertraline has the potential to be used as not only an anti-carcinogenic but also anti-invasive agent for glioblastoma treatment.

P-30-030
Loss of the MCPIP1 protein in hepatocytes leads to β-catenin phosphorylation and liver tumor formation

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The MCPIP1 protein (monocyte chemotactic protein-1 induced protein) is involved in the negative regulation of inflammation due to its RNase activity. A growing number of publications suggest that the MCPIP1 protein may influence the development of cancer by regulation of factors involved in angiogenesis, proliferation and cell death. However, the role of MCPIP1 protein in the progression of hepatocellular carcinoma (HCC) has not yet been investigated. The main aim of our research is to study the importance of MCPIP1 for liver tumor formation and phosphorylation of β-catenin in hepatocytes. Our research model is based on the chemical induction of HCC in mice with MCPIP1 knock out in hepatocytes. We have shown that mice lacking MCPIP1 in the liver develop significantly higher number of liver tumors compared to control mice with increased expression of epithelial to mesenchymal transition markers. Moreover, the results obtained so far indicate that progression of HCC depends on different signaling pathways, for example β-catenin, STAT3 and NFκB. In addition, we found a decrease in the level of MCPIP1 in tumor tissues compared to healthy adjacent liver tissue in samples from patients with HCC. Our data indicates that the MCPIP1 protein may have a protective role during HCC progression. We believe that the MCPIP1 protein may play a key role in neoplastic transformation by regulating the changes in cell phenotype and phosphorylation of β-catenin. This study was supported by National Science Center grants no. 2017/26/E/NZ5/00691, 2022/47/B/NZ5/02724.

P-30-031
Tripterygium wilfordii-derived compounds induce pro-apoptotic and anti-migratory effects against medulloblastoma subtypes

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Medulloblastoma is a common cause of childhood cancer, accounting for 20-25% of paediatric brain tumours worldwide. Whilst current treatments are effective, they are associated with off-target effects, impacting patients' quality of life for decades after remission. This necessitates the development of new therapies, with phytochemicals offering potential novel sources. Recent evidence suggests compounds from *Tripterygium wilfordii* exert anti-cancer effects. However, there is limited evidence of this in medulloblastoma. To address this, we sought to elucidate the fundamental anti-medulloblastoma effect of T*ripterygium wilfordii*-derived compounds. The effect of two *Tripterygium wilfordii*-derived compounds (celastrol and triptolide) on medulloblastoma (HD-MB-03 & DAOY) viability was determined using MTT and washout assays. Time-lapse live-cell microscopy was used to examine cell morphology, cell fate profile and cellular migration. Flow cytometry examined the cell death mechanism (Annexin V/Propidium Iodide). This study was ethically approved by the University of Salford Ethical Review Board (ID:10513). Both celastrol and triptolide induced irreversible concentration and time-dependent decreases in both HD-MB-03 and DAOY viability with IC_{50} values of 151-232 nM and 18-169 nM respectively. Cell fate profiling highlighted significant increases in morphological hallmarks of apoptosis (cell shrinkage & blebbing). Flow cytometry revealed significant increases in apoptotic cells following exposure to both compounds ($P < 0.001$). No effect on cell migration was observed following exposure to triptolide. Celastrol significantly decreased bulk cell migration by $50.4 \pm 3.8\%$ ($P = 0.0002$). Single-cell migration analysis revealed significant decreases in cell migratory distance and speed ($30.4 \pm 4.1\%$, $P < 0.0001$). These data highlight the potential of celastrol and triptolide as treatments for medulloblastoma. Further work is now required to elucidate the molecular mechanisms of action.

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P-30-032
Anticancer effects of indoleamine 2,3-dioxygenase 1 inhibitor 1,3-dimethyl-6-amino-1H-indazole on FaDu hypopharyngeal carcinoma cells: biological activity evaluation and molecular docking

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Hypopharyngeal carcinoma (HPC) is a rare type of head and neck cancer with a high risk of metastasis. In this report, we investigated the anticancer effects of C15, a novel indoleamine 2,3-dioxygenase 1 (IDO1) inhibitor, on FaDu hypopharyngeal carcinoma cells by evaluating biological activities and conducting molecular docking simulations. C15 effectively suppressed IDO1 expression, induced caspase-dependent apoptosis, and inhibited cell proliferation, migration, and invasion. Additionally, it downregulated key cell cycle mediators, such as cyclins and CDKs, in a dose-dependent manner, disrupting mitotic progression. Mechanistically, C15-induced apoptosis was mediated by severe DNA damage and inhibition of the PI3K/AKT/mTOR signaling pathway, coupled with overactivation of ERK1/2, which played a critical role in promoting programmed cell death. Molecular docking simulations further revealed that C15 binds more tightly to the IDO1 active site compared to the previously reported compound C7, forming additional hydrogen bonds with key residues due to its 2,3-dimethoxy substituents. Collectively, these findings highlight the potential of C15 as a promising therapeutic agent for cancer treatment, targeting both IDO1-mediated immune suppression and essential oncogenic pathways. *The authors marked with an asterisk equally contributed to the work.

P-30-033
Modulation of DNA damage response and chromosomal changes by synthetic ssRNA in etoposide-treated osteosarcoma cells: the effect of TRDMT1 methyltransferase gene knockout

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The applications of RNA-based interventions in biomedicine are increasing, but still little is known about the effects of the presence of synthetic RNA in the cell, especially if and how extracellular/exogenous RNA may affect cancer cell short and long-term responses during chemotherapeutic treatment involving DNA damaging agents. 5-methylcytosine RNA methyltransferase TRDMT1, implicated in the DNA damage response (DDR) and other stress responses, is considered as a novel target of anticancer therapy. In the present study, we used several cellular models of osteosarcoma *in vitro* and *TRDMT1* gene knockout (KO) approach to reveal the role of TRDMT1 during RNA-based response upon stimulation with DNA damaging anticancer drug etoposide. The effect of synthetic ssRNA (complementary to *BCR-ABL* fusion gene) and *TRDMT1* KO on selected markers of nucleic acid sensing, DDR, and inflammatory response was investigated, namely the levels of RIG-I, pATM, pH2AX, RAD51, RAD52, PARP1, 53BP1, XRCC1, HuR, and IFN-β. TRDMT1 binding RNA partners such as p21, IL-6, NSUN2, and MYC were identified. Array comparative genomic hybridization (aCGH) was also applied to document long-term effects (30 days upon stimulation) of synthetic ssRNA on karyotype features (gain and loss analysis) and fluorescence *in situ* hybrydization (FISH) to detect Bcr-Abl signals. We postulate that synthetic ssRNA may modulate osteosarcoma response to drug treatment that may be regulated by TRDMT1 methyltransferase and have potential therapeutic implications. This study was supported by the National Science Centre (NCN, Poland) grant OPUS 22 no. 2021/43/B/NZ2/02210.

P-30-034
ATP-binding cassette transporter A1 (ABCA1), as a prognostic marker of melanoma progression

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Plasma membrane lipid rafts are dynamic structures rich in cholesterol and sphingolipids, acting as signaling platforms controlling many cellular processes such as proliferation, growth or survival. Dysregulation of cholesterol homeostasis in the plasma membrane is associated with changes in its properties (fluidity, integrity), which may affect the reprogramming of signaling pathways, such as MAPK, JAK-STAT or NF-kB, initiating the tumorigenesis processes. Moreover, recent literature data indicates significant disturbances of lipid content (especially cholesterol) in cancer cells. The composition and distribution of lipids in the plasma membrane are under the control of several proteins. Among them, ATP-binding cassette transporter A1 (ABCA1) plays an important role in the removal of cholesterol from cells and therefore regulates cellular cholesterol homeostasis and plasma membrane organization directly affecting lipid rafts nanodomains. A recent study conducted on a group of 110 melanoma patients indicates significant differences in the ABCA1 protein expression level depending on the cancer clinical stage, suggesting the role of ABCA1, as a predictive molecule of melanoma development [Wu et al. (2023) Biol Res. 56: 32]. Our goal is to further investigate the function of the ABCA1 transporter in the carcinogenesis process using patient-derived melanoma cell lines. For this purpose, we inhibited pharmacologically the activity of the ABCA1 protein and examined some characteristic features of melanoma cells (proliferation, growth or migration). We also generated an ABCA1 knock-out cell lines. Our preliminary experiments indicate that inhibition of ABCA1 protein activity significantly reduces cell migration capacities *in vitro* and affects tumor xenografts growth *in vivo*. Currently, we are examining how ABCA1 activity influences selected kinases involved in the oncogenic processes of melanoma, to have a deeper insight in the role of this protein in the process of carcinogenesis.

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P-30-035
Effect of synoviolin suppression on the conditions of cell lines obtained from brain tumors and astrocytes

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Cancer represents one of the most significant challenges in human medicine. A key characteristic of cancer cells is their ability to spread to other tissues. ER associated degradation (ERAD) and unfolded protein response (UPR) are two key mechanisms of protein quality control in cell. ERAD system is an important determinant of normal cellular physiology and pathogenesis. Disruptions in ER functions lead to ER stress, characterized by the accumulation of unfolded proteins that must be eliminated by ERAD to ensure cell survival. Signaling pathways are then initiated to improve protein folding. There are three UPR sensors: ATF6, IRE1α and PERK. The IRE1α pathway is commonly activated in tumor cells. One consequence of its increased activity is the elevated expression of synoviolin (SYVN1) which is an essential component of ERAD. The objective of this project was to examine the effect of SYVN1 inhibition on various cell lines including SH-SY5Y, T98G, U87, as well as astrocytes NHA and K1884. Our findings show that inhibition SYVN1 leads to reduced cell survival. Astrocytes K1884 exhibit the highest sensitivity to LS-102, while T98G glioblastoma cells were the most resistant to LS-102. We also observed a significant increase in SYVN1, SEL1L and GRP78 expression. Treatment of T98G cells with LS-102 results in activation of the IRE1α-XBP1 pathway and subsequent activation of IRE1α ribonuclease activity leading to splicing of XBP1 into XBP1s. In U87 cells treated with LS-102, SYVN1 was overexpressed but IRE1α-XBP1 pathway was not activated. We also observed changes into networks of endoplasmic reticulum and mitochondria. After treatment of a stress specific inductor, we observed presence of stress granules. Our results suggest that T98G and U87 cells respond to SYVN1 inhibition by increased expression of SYVN1. We hypothesize that upregulation of SYVN1 represents a compensatory mechanism by which the cells try to cope with inhibition of SYVN1.

P-30-036
VHL isoforms unveiled: dual roles in androgen receptor regulation and clear cell renal cell carcinoma (ccRCC) aggressivity

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Von Hippel Lindau syndrome is an inherited disorder characterized by the abnormal growth of both benign and cancerous highly vascularized tumors in many parts of the body. The harbouring of this condition is due to pathogenic mutations in the von Hippel Lindau tumor suppressor protein (VHL) resulting in the accumulation of Hypoxia inducible factor proteins (HIF1a, HIF2a) and thus in the transcription of genes promoting tumor development, vascularization and growth. *VHL* gene encodes for two isoforms, VHL19 and VHL30, the short and the canonical form respectively. VHL's most studied function is when it acts as the recruitment subunit of the E3 ubiquitin ligase complex, favouring targets' ubiquitination and proteasomal degradation. Our investigation focused on the clear cell renal cell carcinoma (ccRCC), which is a leading cause of mortality in VHL patients. Retrospective and epidemiological analysis showed a doubled incidence in RCC for men compared to women, suggesting androgenic characteristics of these tumors. We investigated the androgen receptor (AR)–VHL interaction. From *in vitro* preliminary experiments, it was found that the two isoforms have opposite effects on AR faith. VHL30 increases AR cellular turnover, suggesting that AR is a target of VHL for protein degradation process. On the other hand, VHL19 promotes AR stability and activity. To further investigate the mechanisms of these interactions, we generated, evaluated and analyzed interaction models through AlphaFold3 and state-of-the-art structural tools. Specifically, CRL2-VHL30-AR ubiquitin ligase complex to explain the increased turnover and VHL19 interacting with AR during the last stage of its folding process for the increased stability and activity. The structural parameters applied in our model's analysis successfully described the dual role of VHL isoforms having a significant effect on AR faith, suggesting that the VHL loss could drive ccRCC aggressivity also in non-VHL syndrome patients.

P-30-037
The role of MCPIP1 protein in hepatocellular carcinoma initiation and progression by transcriptomic time-course analysis in a mouse tumor model

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The incidence of hepatocellular carcinoma (HCC) is rising, with projections exceeding one million cases by 2025. Chronic inflammation leading to fibrosis or cirrhosis is a hallmark of HCC progression. Men are three to five times more likely to develop HCC than women, a disparity that is also observed in mouse models. Monocyte chemotactic protein-1 induced protein (MCPIP1), encoded by *ZC3H12A*, is a key negative regulator of inflammation, and its absence in hepatocytes promotes fibrosis, immune activation, tissue remodeling, and tumor formation, particularly in female mice. We utilized a hepatocyte-specific *Zc3h12a* knockout (KO) mouse model with wild-type (WT) controls. Diethylnitrosamine (DEN) was administered to two-week-old female mice to induce tumorigenesis. Mice were sacrificed at 3, 6 and 9 months post-injection, with a 0-month control group. To assess MCPIP1 knockout effects, liver RNA was subjected to next-generation sequencing and analyzed with DESeq2, revealing the most significant transcriptomic changes at 6 months post-DEN. Gene set enrichment analysis identified key pathways affected by MCPIP1 loss, including EMT, WNT beta-catenin signaling, and inflammatory response, crucial for tumor development and metastasis. The time-course analysis provided insights into the induction of tumor transformation and its dynamic progression at different stages. Despite the binary difference between WT and KO mice at 9 months—where nearly all KO mice developed tumors—gene expression changes at this time point were minimal. The difference in tumor occurrence enabled the identification of potential HCC biomarkers, highlighting MCPIP1's role in liver tumorigenesis. The 6-month

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time point emerged as critical for transformation, providing a basis for further mechanistic studies on MCPIP1 as a therapeutic target. Supported by National Science Center grants no. 2017/26/E/NZ5/00691, 2022/47/B/NZ5/02724.

P-30-038
The combined administration of a TLR9 inhibitor and ruxolitinib may improve RNA exosome complex activity in Philadelphia chromosome-negative myeloproliferative neoplasms

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Philadelphia chromosome-negative myeloproliferative neoplasms (Ph-MPNs) occur due to mutations, including an activation mutation, JAK2V617F, occurring in the *JAK2* gene in hematopoietic stem cells in the bone marrow. This leads to the production of many different mRNAs in the cytosol because of the stimulation of the associated pathways. Exosome complex (ExosC) is involved in the processing of stable RNAs, transcriptional stabilization, and genomic stability by degrading unstable RNAs. We hypothesized that co-treatment with a TLR9 inhibitor and ruxolitinib in Ph-MPNs improves RNA homeostasis by providing ExosC modulation and may be a new treatment approach to alleviate the symptoms of the disease. Single-stranded DNA (ssDNA) was isolated from the supernatants of HEL and SET2 cell lines, and from the plasma of cord blood (CB). Healthy CB-mononuclear cells (MNCs) were co-cultured with 2 ng HEL, SET2, or CB-ssDNA for 48 h following application of TLR9 inhibitor (300 nM) for 30 min and ruxolitinib (300 nM) in the last 4 h of culture. qRT-PCR was performed to examine the expression of the ExosC genes. EXOSC8, which plays a role in erythroid maturation, was suppressed under inflammatory conditions and in MNCs treated with both TLR9 inhibitor and ruxolitinib. DIS3 encoding both exo- and endoribonucleases, which was overexpressed after culture with CB-ssDNA, was downregulated in MNCs treated with the TLR9 inhibitor, whereas further downregulation was observed in cells treated with the TLR9 inhibitor and ruxolitinib. The increased expression of EXOSC10 in cells treated with the TLR9 inhibitor and CB-ssDNA decreased after ruxolitinib administration. EXOSC8, EXOSC10, and DIS3 were affected by inflammatory conditions, and their expression decreased in cells treated with the TLR9 inhibitor and ruxolitinib. In conclusion, the use of a TLR9 inhibitor, in addition to ruxolitinib treatment, in MPNs may be promising; however, further studies are required. *The authors marked with an asterisk equally contributed to the work.

P-30-039
Nature's answer to cancer: cyanopeptolines from *Nostoc edaphicum* CCNP1411 inducing apoptosis in neuroblastoma cells

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Neuroblastoma, one of the most common solid tumors in children, presents significant therapeutic challenges due to its aggressive nature and resistance to conventional treatments. Natural compounds, particularly those produced by *Cyanobacteria*, have gained attention as promising candidates in cancer therapy. Among them, there are cyanopeptolines, produced by the *Nostoc edaphicum* strain CCNP1411. These compounds belong to one of the most prevalent classes of cyanobacterial nonribosomal peptides and have been shown to affect cancer cell viability. Therefore, the aim of this study was to evaluate the effects of cyanopeptolines on viability of neuroblastoma cells and to identify a potential mechanism of cell death. For this purpose, neuroblastoma SH-SY5Y cells were treated with cyanopeptolines for 24 h, 48 h, and 72 h, and cell viability was assessed using the MTT assay. To evaluate the safety of these compounds, analogous experiments were conducted with healthy human fibroblasts (HDFc). To determine the mechanism of cell death induced by cyanopeptolines, the Guava Muse Analyzer was used to assess the fractions of apoptotic, early and late apoptotic, dead, and viable cells. Additionally, fluorescence microscopy was employed to further analyze cell death mechanism. Cyanopeptolines exhibited strong anticancer effects on neuroblastoma cells, causing a 50% reduction in cell viability after 24 h, with the effect intensifying at 48 h and 72 h after the treatment onset. Importantly, these compounds were found to be safe for healthy child fibroblasts. Furthermore, cyanopeptolines increased the proportion of apoptotic cells, suggesting the induction of the programmed cell death. These findings highlight the potential of cyanopeptolines as therapeutic options for neuroblastoma treatment and warrant further investigation into their mechanisms of action and potential clinical applications.

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P-30-040
Targeting USP47 enhances the efficacy of KRAS inhibitor in KRASG12C mutated non-small cell lung cancer by controlling deubiquitination of c-Myc

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FDA-approved KRASG12C inhibitors, like Sotorasib, target G12C-mutated KRAS (Kirsten rat sarcoma virus) in NSCLC (non-small cell lung cancer). However, issues with insensitivity and drug resistance have emerged, requiring the development of new combination therapies to overcome these limitations. USP47 (ubiquitin specific peptidase 47) has been identified as a regulator of cancer-related signaling pathways such as Wnt, Hippo, and p53. However, its role in the KRAS signaling pathway remains largely unexplored and USP47 inhibitors are less developed than those targeting its homolog, USP7. Here, we identify USP47 as a novel therapeutic target in KRASG12C-mutated NSCLC and report K-552, a selective USP47 inhibitor, as a potential treatment strategy. We demonstrate that USP47 stabilizes c-Myc by preventing its proteasomal degradation through deubiquitination, thereby promoting NSCLC cell proliferation. Additionally, the compound K-552, a USP47 inhibitor identified through virtual screening, effectively destabilizes c-Myc and inhibits KRASG12C-mutated NSCLC cell proliferation. Furthermore, USP47 inhibition—either by siRNA knockdown or K-552 treatment—enhances the efficacy of Sotorasib *in vitro* and *in vivo*. Together, our findings establish USP47 as a promising therapeutic target in KRASG12C-mutated NSCLC and introduce K-552 as a USP47 inhibitor with potential for combination therapy with KRASG12C inhibitors. *The authors marked with an asterisk equally contributed to the work.

P-30-041
Leptin modulates protein expression in endometrial adenocarcinoma cells with distinct localization patterns

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Endometrial cancer, the second most frequently diagnosed malign neoplasm in the female genital system after cervical cancer, presents poor survival and association with high body mass index. Leptin, an adipokine elevated in obesity and a key risk factor for endometrial cancer, may modulate tumor progression through angiotensin-converting enzyme 2 (ACE2), a member of renin angiotensin aldosterone system with emerging roles as a potential biomarker in cancer biology [1]. Here, we investigated leptin's effects on ACE2 expression and observed its localization in HEC-1-A endometrial adenocarcinoma cell line (HTB-112). The cells were treated with 100 ng/ml leptin and 24 hours after treatment, ACE2 levels were assessed via immunostaining with ACE2 polyclonal antibody (bs-1004R). Analysis of ACE2 expression in endometrial adenocarcinoma cells revealed that leptin treatment significantly upregulated ACE2 levels compared to untreated controls, as quantified by corrected total cell fluorescence (CTCF) across all cells ($p = 0.0162$) and specifically in non-mitotic cells ($p = 0.0037$). Notably, ACE2 localized predominantly to the nucleus in non-mitotic cells, while exhibiting a cytoplasmic granular pattern in mitotic cells, suggesting cell cycle-dependent distribution [2]. This study provides the first evidence that leptin upregulates ACE2 in endometrial adenocarcinoma, a novel finding that may link obesity-driven mechanisms [3] to tumor behavior. This leptin-ACE2 interaction unveils a previously unrecognized pathway, meriting further exploration of its role in cancer progression. References: [1] Yang J, Li H, Hu S, Zhou Y. Aging (Albany NY). 2020;12(8):6518-6535. doi:10.18632/aging.103100 [2] Ray I, et al. Cancer Metastasis Rev. 2022;41(1):211-242. DOI: 10.1007/s10555-021-10002-6 [3] Dalamaga M, et al. Curr Obes Rep. 2021;10(3):214-243. DOI: 10.1007/s13679-021-00436-y.

P-30-042
Do salivary cullin7 gene expression and protein levels provide advantages over plasma levels in diagnosing breast cancer?

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In addition to the tumor suppressor role of Cullin 7 (Cul7), one of the proteins belonging to the Cullin (Cul) family, studies have also suggested that Cul7 may act as an oncogene under certain conditions. The role of the Cul7 molecule in breast cancer is still unclear, and understanding its function could have significant implications for identifying novel therapeutic targets or improving diagnostic strategies in breast cancer management. In this study, the levels of the Cul7 molecule in plasma and noninvasive material saliva were investigated, and its possibility as a marker for breast cancer was discussed. Protein levels of blood and saliva samples taken from breast cancer patients and a healthy control group were measured by the ELISA (Enzyme-Linked Immunosorbent Assay) method. Gene expression levels between the two groups were analyzed by the qPCR (quantitative polymerase chain reaction) method. In our study, Cul7 mRNA and protein expression levels were examined in 60 breast cancer patients and 20 healthy female controls, and a statistically insignificant difference was found between the patient and control groups in both plasma and saliva samples ($p > 0.05$). No correlation was found between the clinical characteristics of the patients and plasma and saliva Cul7 gene expression and protein levels ($p > 0.05$). Considering the possibility of Cul7 being a biomarker at the protein and mRNA levels, plasma is thought to be a better study material for Cul7. Our findings suggest that in the context of a study on salivary material, the expression of Cul7 at the mRNA level may have better potential utility as a biomarker.

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P-30-043
Cytotoxicity of cyclodextrin-complexed cannabidiol/cannabinol on colon and breast cancer cell lines and their intestinal bioavailability

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Cancer remains one of the leading causes of death worldwide and there is a need for further research into new treatments. Non-psychoactive cannabinoids are gaining significant attention due to their potential therapeutic benefits without the psychoactive effects associated with tetrahydrocannabinol (THC). Cannabidiol (CBD) has shown to increase cell death by various mechanisms in some cell lines, but the anticancer activity of cannabinol (CBN) remains still unexplored. Also due to its poor solubility it is difficult to obtain a bioavailable dosage form of some cannabinoids. The ability of cyclodextrins to improve the physicochemical properties of bioactive compounds opens up new possibilities in the design and development of innovative and effective products in multiple sectors, including the design of anticancer drugs. The use of cyclodextrins to complex bioactive compounds would improve their solubility and allow a controlled release by increasing the concentration of the compound at the site of action. In this work, we have tested the cytotoxic activity of CBD and CBN complexed with HP-β-cyclodextrin in triple-negative breast cancer cells and colon cancer cells. Oral bioavailability of both complexes was also tested by simulated digestion and intestinal absorption in Caco2 cells. Complexation of the cannabinoid compounds CBD and CBN in HP-β-cyclodextrins increased the bioaccessibility of both compounds after simulated gastrointestinal digestion. Complexation therefore protected the compounds from the digestion process and increased the portion of the complex that crossed the intestinal barrier. CBD and CBN showed cytotoxic activity in MDA-MB 231 breast cancer cells and Caco2 colon cancer cells. Complexation with HP-β-Cyclodextrins increased cytotoxicity in MDA cells, but not in Caco2 cells. In conclusion, complexation of CBD and CBN significantly increased their bioavailability and cytotoxic capacity, especially against MDA-MB 231 breast cancer cells.

P-30-044
Evaluating Tofranil® as a potential therapeutic approach in colorectal cancer mice models

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Preclinical models are essential for studying metastasis and testing therapies. Previously, our group identified imipramine (FDA-approved) as an inhibitor of fascin-1 (FSCN1) in colorectal cancer cells, reducing invasion *in vitro* and in a zebrafish model. We hypothesized that imipramine could prevent tumor escape by interfering with invasion mechanisms. Additionally, we explored its synergy with a chemotherapeutic agent, 5-fluorouracil (5-FU), confirming its therapeutic potential. HCT-116 cells (2×106 cells/100 μL) were subcutaneously inoculated with matrigel to establish a xenograft model in mice. Mice ($n = 29$) were divided into control and treatment groups, monitoring tumor progression through *in vivo* imaging system (IVIS), caliper measurements, tumor dissection, immunohistochemistry, and droplet digital PCR (ddPCR). Tofranil® (imipramine) was administered via oral gavage (95 mg/kg) in mice to evaluate its effects on the primary tumor, while 5-FU (25 mg/kg) was delivered intraperitoneally. Tofranil® treatment significantly reduced tumor weight (54–55%) in both chemotherapy-treated ($p = 0.011$) and untreated groups ($p < 0.001$), suggesting an antitumor effect independent of 5-FU. Despite these effects, ddPCR analysis showed no changes in the KRAS-mutated to wild-type copy ratio in plasma cfDNA and Immunohistochemical analysis revealed no differences in FSCN1 or cytokeratin expression, but Ki67 staining indicated that tumors in treated mice were encapsulated, lacking an infiltrative pattern ($p = 0.008$), while lung micro-metastases were only observed in untreated mice ($p = 0.008$). Our findings confirm that combination therapy can be implemented, as the effect of Tofranil® remains unaltered after the combination of both treatments. This could be due to distinct mechanisms of action, where Tofranil® may exert its antitumor effects through pathways that do not directly interact with those modulated by 5-FU. *The authors marked with an asterisk equally contributed to the work.

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P-30-045
The role of CHIP/STUB1 U-BOX domain in p53 and autophagy-lysosomal regulation in colorectal cancer cells

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The STUB1/CHIP, an E3 ubiquitin ligase essential for protein homeostasis, comprise an N-terminal tetratricopeptide repeat (TPR) domain mediating interaction with HSPs as a cochaperone, a central coiled-coil domain, and a C-terminal U-BOX domain responsible for substrate ubiquitination. To elucidate the role of the U-BOX domain, we generated HT-29 (p53 R273H) and HCT116 (p53 WT) colorectal cancer cell lines with targeted U-BOX domain deletions or complete CHIP/STUB1 knockout. We found that increase of p53 aggresome in the U-BOX domain deleted cells compared to parental cells examined by immunofluorescence microscopy, which was corroborated by western blot analysis showing an increased insoluble fraction of p53, irrespective of p53 mutation status. This finding underscores the potential tumor-suppressive function of STUB1/CHIP, primarily mediated by the E3 ubiquitin ligase activity of its U-BOX domain, which facilitates p53 ubiquitination and subsequent degradation. Furthermore, we observed inhibition of autophagy flux, evidenced by activation of the AKT-mTOR pathway and decreased expression of LC3-II, Rab7, LAMP2, and CTSB, as determined by western blotting and immunofluorescence microscopy. This suggests that U-BOX domain deletion impacts multiple cellular processes beyond protein degradation. Our results indicate the intricate interplay between the ubiquitin-proteasome system and autophagy. The impairment of CHIP's E3 ligase activity not only affects proteasomal degradation but also influences autophagy, potentially as a dysregulated compensatory mechanism. Further investigations are ongoing to elucidate the precise molecular pathways governing these regulatory interactions.

P-30-046
Syntenin-1 promotes triple-negative breast cancer metastasis via stabilization of BACH1 by blocking E3 ubiquitin ligase FBXO22 activity

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Syntenin-1 plays role as a positive regulator of cancer cell progression in several human cancers. Here, we report that syntenin-1 positively regulates protein expression of BACH1, which is well known as a biomarker of triple-negative breast cancer (TNBC). We also investigated novel mechanisms of action that E3 ubiquitin ligase FBXO22 mediates the K48-degradative ubiquitination of BACH1 and syntenin-1 could inhibit FBXO22 activity to stabilize BACH1 protein. In detail, syntenin-1 could directly bind on FBXO22 and block its enzymatic activity via disruption of linkage between E3 ligase and its substrate. Our patient samples showed a positive correlation between syntenin-1 and BACH1 at the translational level, but not mRNA expression at the transcriptional level. Moreover, knockout of syntenin-1 increased expressions of several tumor suppressor in list of BACH1 target genes. knockout of syntenin-1 also boosted the anticancer effect of metformin in treatment of triple-negative breast cancer via BACH1-dependent-mitochondrial metabolism. This study demonstrated that syntenin-1 promotes TNBC metastasis via stabilization of BACH1 by blocking FBXO22 and its potential as a new target for cancer therapy in TNBC. *The authors marked with an asterisk equally contributed to the work.

P-30-047
Transmissible endoplasmic reticulum stress in temozolomide-resistant glioblastoma cells: the potential role of 24-epibrassinolide

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Glioblastoma (GBM) is the most aggressive primary brain tumor of the central nervous system, with an average survival time of less than two years. The standard treatment includes temozolomide (TMZ) administration following surgical resection. The complex GBM microenvironment promotes TMZ resistance, leading to tumor recurrence in most patients within the first year. 24-Epibrassinolide (EBR), a member of the brassinosteroid family, has demonstrated anti-tumor activity in cancer cells. Our previous studies showed that EBR induces ER stress-mediated apoptosis in GBM cells, particularly in TMZ-resistant cells. Recent research suggests that transmissible endoplasmic reticulum stress (TERS) occurs between tumor and microenvironmental cells, potentially sensitizing or rendering cells resistant to therapy. This study aims to investigate whether EBR, in combination with TMZ, can trigger TERS. TMZ-resistant LN18 cells were treated with EBR (30 μM) and/or TMZ (200 μM) for 24 hours. The conditioned media from these cells was then transferred to TMZ-sensitive U87 cells. Cellular effects of TERS were evaluated using cell viability assays, cell survival analysis, 2D and 3D colony formation assays, and wound healing analysis. Additionally, expression levels of ER stress-related genes and apoptotic markers were analyzed by immunoblotting and flow cytometry with annexin V/PI staining. The conditioned medium obtained from LN18 cells significantly increased ER stress markers in recipient U87 cells. Furthermore, apoptosis rates were significantly higher in the treated groups compared to control groups. This study is one of the first to show that EBR has the potential to trigger TERS. The data obtained provide valuable insights into the potential effects of TERS in the tumor microenvironment and its role in therapeutic responses. Future studies could contribute to the identification of molecules involved in the TERS process and the discovery of new therapeutic targets for GBM treatment. *The authors marked with an asterisk equally contributed to the work.

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P-30-048
Bone morphogenetic protein 2 promotes osteogenesis in lentivirus mediated reprogrammed osteosarcoma stem cells

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Cancer stem cells (CSCs) are emerging model towards understanding cancer progression, such as highly metastatic osteosarcoma (OS), attributable to their role in promoting malignancies. Thus, osteosarcoma stem cell (OSSCs) reprogramming presented new approach for malignancy reversal. Besides promoting osteogenesis, bone morphogenetic protein (BMP) involves in modulation of tumour microenvironment (TME) crucial in cancer progression. However, metastatic cascade by CSCs plasticity and TME dysregulation in OS remains elusive. Hence, this study aims to investigate the role of BMP in OS metastasis using lentiviral mediated reprogrammed OSSCs, a novel approach with regards to OS research. Briefly, OS cell (MG63) reprogramming was performed via lentivirus transduction comprising pluripotent genes (Oct4, Klf4 and Nanog) at multiplicity of infection (MOI) 6. The established OSSCs was treated with 100 ng/mL of BMP-2 for 7-, 14-, 21-, and 28-days, followed by characterisation and validation of the dedifferentiated cells at pre- and post-treatment. Morphological assessment showed prominent shift from adherent, fibroblast-like feature to spheroid-form appearance, indicating transition of stem-like phenotype. Immunocytochemistry with TRA-1-60 and TRA-1-81 pluripotency markers showed high level of expression in the reprogrammed cells, substantial of induced stem cells pluripotent properties. The alkaline phosphatase activity was observed at post 21- and 28-day BMP treatment, suggestive of reverse plasticity potential towards osteogenesis. Similar BMP effect was apparent within the cytoskeletal network of actin and tubulin, seen via immunofluorescence analysis. In conclusion, our reprogrammed OSSCs render potential approach for malignancy reversal via BMP-2 modulation on the TME. To the best of our knowledge, these findings present the first fundamental insights on the dynamic interplay between OSSCs plasticity, and the TME in OS metastasis pathway. *The authors marked with an asterisk equally contributed to the work.

P-30-049
NEK2 phosphorylates RhoGDI1 to promote cell proliferation, migration and invasion through the activation of RhoA and Rac1 in colon cancer cells

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Rho guanine nucleotide dissociation inhibitor 1 (RhoGDI1) plays a critical role in regulating the activity of Rho guanosine triphosphatases (GTPases). Phosphorylation of RhoGDI1 dynamically modulates the activation of Rho GTPases, influencing cell proliferation and migration. This study explored the involvement of Never In Mitosis A (NIMA)-related serine/threonine protein kinase 2 (NEK2) in phosphorylating RhoGDI1 and its implications in cancer cell behavior associated with tumor progression [1]. We employed GST pull-down assays and immunoprecipitation to investigate the interaction between NEK2 and RhoGDI1. Truncation fragments identified the region of RhoGDI1 responsible for binding with NEK2. Phosphorylation assays determined the site of NEK2 mediated phosphorylation on RhoGDI1. Functional assays were conducted using overexpression of the RhoGDI1 substitution mutant to assess their impact on cancer cell behavior. NEK2 directly bound to RhoGDI1 and phosphorylated it at Ser174. This phosphorylation event facilitated cancer cell proliferation and motility by activating RhoA and Rac1. The RhoGDI1 aa 112–134 region was critical for the binding to NEK2. Disruption of the NEK2–RhoGDI1 interaction through overexpression of a RhoGDI1 truncated fragment (aa 112–134) led to diminished RhoGDI1 phosphorylation and RhoA/Rac1 activation induced by NEK2, resulting in reduced cancer cell proliferation and migration. Moreover, *in vivo* studies showed reduced tumor growth and lung metastasis when the NEK2–RhoGDI1 interaction was disrupted. This study indicates that NEK2 promotes the metastatic behaviors of cancer cells by activating RhoA and Rac1 by phosphorylating RhoGDI1.
Reference: [1] Lim, J.; Hwang, Y.-S.; Kim, J.-T.; Yoon, H.-R.; Park, H.-M.; Han, J.; Kwon, T.; Lee, K.-H.; Cho, H.-J.; Lee, H.-G. NEK2 Phosphorylates RhoGDI1 to Promote Cell Proliferation, Migration and Invasion Through the Activation of RhoA and Rac1 in Colon Cancer Cells. Cells 2024, 13, 2072. https://doi.org/10.3390/cells13242072.

P-30-050
Differential expression of receptor kinases in epithelial and mesenchymal hepatocellular carcinoma cell lines: potentials for targeted therapy

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Hepatocellular carcinoma (HCC) treatment remains challenging due to tumor heterogeneity and drug resistance. Tyrosine kinase inhibitors (TKIs), such as lenvatinib, cabozantinib, sorafenib, and regorafenib, provide systemic treatment options; however, their effectiveness varies among patients. This emphasizes the necessity of molecular profiling-guided, individualized treatment approaches. In this study, we characterized a mesenchymal HCC cell line and compared it to an epithelial HCC cell line counterpart by evaluating clonality, proliferation, tumorigenicity, and drug resistance. To better understand tumor heterogeneity, we examined drug responses in a co-culture system, where mesenchymal cells displayed a growth advantage, suggesting a more aggressive phenotype. Transcriptomic profiling and gene set enrichment analysis (GSEA) were performed to identify molecular differences. Using the Caruso dataset, we analyzed 35 HCC cell lines to determine potential therapeutic targets. A systematic evaluation of receptor tyrosine kinases (RTKs), serine/threonine kinases, and their ligands revealed two main subgroups (S1 and S2), with S2 further divided into S2a and S2b. Notably, some receptors functioned through paracrine

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mechanisms, while others relied on autocrine signaling, a common feature in cancer progression. Our findings emphasize the unique molecular and functional characteristics of mesenchymal and epithelial HCC cells. They also highlight the impact of cellular competition on tumor progression and the crucial role of molecular profiling in designing personalized and effective treatment strategies. These insights could contribute to the development of more targeted and efficient therapeutic approaches for HCC.

P-30-051
GLI2 transcription factor regulates PSA expression in androgen-sensitive prostate cancer cell line

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Prostate cancer (PC) is the most frequently diagnosed cancer in men, with increasing mortality. Prostate-specific antigen (PSA) serves as a diagnostic biomarker for PC. PSA expression is regulated by the androgen receptor (AR), which is overexpressed in PC. Recent studies have shown that AR interacts with GLI2, a transcription factor in the Hedgehog-GLI pathway (HH-GLI), which is activated in various cancers, including PC. This interaction may regulate the expression of certain genes in PC, although its effect on PSA expression remains unclear. This study aims to determine whether PSA expression is regulated by AR, GLI2, or their interaction in PC. Protein array analysis of the supernatant from GLI2-overexpressing LNCAP cells demonstrated an upregulation of KLK3 (PSA) in GLI2-transfected cells compared to mock-transfected controls. An analysis of the genomic region of KLK3 gene in the UCSC database revealed several GLI2 consensus binding sites. Further investigation involved treating LNCAP cells with various agents: enzalutamide (AR inhibitor), sonidegib (Hedgehog-GLI pathway inhibitor), dihydrotestosterone (DHT, AR activator), and their combinations. qPCR analysis revealed that PSA expression was downregulated in cells treated with enzalutamide, as expected, but also in cells treated with sonidegib, suggesting that GLI2 contributes to the regulation of PSA expression. DHT treatment upregulates the expression of both AR and PSA, which are reduced to control levels upon addition of sonidegib, indicating a potential interaction between Androgen receptor signaling pathway and HH-GLI signaling pathway in PSA regulation. These findings give novel insight into PSA regulation. A better understanding of PSA regulation in PC could lead to the detection of possible new targets for prostate cancer therapies.

P-30-052
Investigation of nutrient limitation on mTORC1 pathway, mRNA translation control and integrated stress response in colorectal cancer cells

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The mTORC1 pathway regulates essential cellular processes such as protein translation and cell growth. It is activated by signals from MAPK and PI3K pathways, and phosphorylates downstream targets like p70S6K, 4EBP1 and rpS6. rpS6 is a component of the small subunit of the ribosome. Phosphorylated 4EBP1 regulates translation initiation, facilitating eIF4E to form the eIF4F complex. Furthermore, mTORC1 acts as an amino acid (AA) sensor upon translocation to lysosomes when TSC2 inhibition is relieved by growth factors. The integrated stress response (ISR) is a key translational program that is activated under AA deprivation. ISR enhances eIF2α phosphorylation, which upregulates ATF4 and drives the transcription of genes involved in nonessential amino acid (NEAA) synthesis and transport. mTORC1 can also upregulate ATF4 via mechanisms distinct from ISR and results in transcription of AA uptake, synthesis, and tRNA charging genes (Torrence ME et al. (2021) eLife 10:e63326, 1-33). Our lab identified that colorectal cancer cells that survive under nutrient depletion (10% of complete medium nutrients, ND) show increased motility and drug resistance. These cells also showed decreased 40S- and polysome-to-total volume ratios suggesting slower translation, although 60S and 80S (monosomes) levels were not affected. Of note, although typical markers of starvation were observed, rpS6 phosphorylation via the MAPK axis remained active. Additionally, eIF4E phosphorylation persisted through 48 h of ND. Supplementation of the depletion medium with NEAA could not rescue ISR, suggesting alternative mechanisms for the persistence of stress. These results indicate a rewiring of stress related signaling for continuing translation under nutrient depletion.

P-30-053
Mechanisms of cell-specific regulation of the proteotoxic stress response

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Proteotoxic stress leads to the activation of the heat shock transcription factor 1 (HSF1), which induces the expression of molecular chaperones (e.g. heat shock proteins) enabling cell survival. HSF1 can also trigger mechanisms leading to cell death by increasing the expression of the proapoptotic *PMAIP1* (*NOXA*) gene. Based on cell survival tests under heat shock (HS), we selected two human myeloid leukemia cell lines: K562 (as heat-resistant) and HL-60 (as heat-sensitive) to study the mechanism of HS-induced apoptosis. Only in HL-60 cells, was the intrinsic/mitochondrial pathway (related to cytochrome c release) activated, leading to caspase 3-dependent apoptosis. The expression level of HSF1 in HL-60 cells is several times lower than in K562 cells, which translates into the lack of cytoprotective HSPA1 (inducible HSP70) under basal conditions. Moreover, different HSF1 phosphorylation patterns (as determined by Phos-Tag method) in the two lines suggest its different activity. In the regulatory sequences of the *PMAIP1* gene, we identified two HSF1 binding sites: in the intron and about 34 kb upstream of the transcription start site (TSS). HSF1 binding after HS occurs at these sites in both cell lines, while an increase in transcription is observed only in the HL-60 line. Chromatin accessibility assays (by ATAC-seq) showed that under control conditions chromatin is open around the intron binding

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site in both lines, while differences are observed 34 kb upstream of the TSS and downstream of the 3'UTR of *PMAIP1* (open chromatin only in HL-60). The direction of chromatin opening in response to heat stress in this chromatin locus suggests that enhancer RNA transcription may occur here in the opposite direction to the gene transcription. We postulate that this RNA enhancer may be responsible for the differential *PMAIP1* activation under stress in heat-sensitive HL-60 and heat-resistant K562 cells. Funded by the National Science Center, grant number 2021/43/B/NZ3/02161.

P-30-054
Determination of the relationship between cytidine deaminase enzyme activity and cell toxicity of gemcitabine and intracellular metabolites of gemcitabine in cancer cells

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Gemcitabine, a deoxycytidine analog, is an antimetabolite widely used in cancer treatment. The two main mechanisms responsible for the toxicity of gemcitabine are the inhibition of replication or transcription by the diphosphate/triphosphate forms and the inhibition of the ribonucleotide reductase enzyme. It has been suggested that 2′,2′-difluoro-2′-deoxyuridine (dFdU), which is formed due to the deamination of gemcitabine by the cytidine deaminase (CD) enzyme, may contribute to the toxicity and/or antitumor activity of gemcitabine (1). Our study investigated the effect of CD enzyme activity on gemcitabine and dFdU conversion and cell toxicity in MDA-MB-436, A549, and C33A cancer cells. Gemcitabine and dFdU were analyzed using the Singh method (2), CD activities using Amici's modified method (3), and protein values using the BCA method. The levels of lactate dehydrogenase (LDH) released into the medium in MDA-MB-436, A549, and C33A cells treated with gemcitabine (10 μM) for 48 hours were calculated as 26%, 5%, and 8%, respectively, and these values were statistically significant compared with control cells ($p < 0.001$). CD activity, gemcitabine, and dFdU concentrations were measured in MDA-MB-436 cells, which were determined to have the highest LDH level. The specific activity (SA) of CD was calculated as 3.20 ± 1.53 in MDA-MB-36 cells. The concentration of gemcitabine and dFdU in the cells was determined as 1.18 ± 0.05 μM and 6.13 ± 2.73 μM, respectively. A student-t test was used to analyze cell viability and LDH levels statistically. These results suggest that the significantly higher level of dFdU, the deaminated form of gemcitabine, compared to gemcitabine in MDA-MB-436 cells may be responsible for toxicity. References: (1) Veltkamp S et al. (2008) Mol Cancer Ther 7, 2415-2425. (2) Amici A et al. (1997) FEBS letters 419, 263-267. (3) Singh R et al. (2015) Int J Anal Chem 2015:862592.

P-30-055
Flt3ITD specific inhibitors based on azaindole proved antiproliferative not cytotoxic effect in MV4-11 cells

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Between 20 and 30% of patients with acute myeloid leukemia bear the Flt3ITD mutation, which is the negative prognostic marker of the disease. The following work aims to evaluate the antiproliferative activity of newly synthetised azaindole-derived Flt3 inhibitors with selectivity towards Flt3ITD. Based on the specificity ratio established via MTT assay on MV4-11 (Flt3ITD) and THP-1 cells (Flt3wt), three hit compounds from a series of 60 were selected for evaluation of Flt3ITD-linked antiproliferative activity. All three compounds caused a significant decrease in the proliferation of MV4-11, which was linked with cell cycle arrest in G1/S phase and diminished DNA synthesis. Contrarywise, the drop of viability in treated cells was only modest, which was confirmed by weak increase in caspases 3/7 activity. The inhibitory effect of synthetized compounds on Flt3 and subsequent signaling pathways was proved via electrophoresis of Flt3, ERK, Akt and STAT5 proteins. To conclude, we present a pattern for synthesis of Flt3ITD-specific inhibitors with proved antiproliferative activity.
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P-30-056
New insights into ZNF224 functions in melanoma cell plasticity and tumor progression

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ZNF224 is a Krüppel-like zinc finger transcription factor with an oncogenic role in different types of tumours. In melanoma, ZNF224 mediates the pro-oncogenic function of transforming growth factor-β (TGF-β) by establishing a positive feedback loop with the cytokine and its receptors, leading to sustained activation of the signalling pathway, and the upregulation of epithelial-mesenchymal transition (EMT)-related genes. Given the crucial role of EMT in tumour progression, both in regulating cell plasticity and gene expression, here we investigate the involvement of ZNF224 in these events by analysing its functional relationship with vimentin, a key EMT marker. Functional experiments and immunofluorescence assays revealed that ZNF224 overexpression induces morphological changes in melanoma cells due to vimentin filament reorganisation, and it also promotes the acquisition of stem-associated features. Notably, these alterations also affected neighbouring cells, suggesting that ZNF224 promotes the release of soluble factors that drive phenotypic and molecular changes in adjacent cells.

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To explore this, we analysed the effects of conditioned medium from ZNF224-overexpressing cells on A375 melanoma cultures. These cells show vimentin structural reorganisation and exhibit increased expression of key stemness-related genes. Beyond its role in cytoskeletal remodelling, we investigated the impact of ZNF224 on vimentin subcellular localisation. Consistent with recent studies on unusual vimentin functions, our preliminary experiments show that vimentin is present in the nucleus of A375 melanoma cells and that ZNF224 influences its distribution in the nuclear compartments. Moreover, co-immunoprecipitation assays demonstrate an interaction between ZNF224 and vimentin. Taken together, this evidence suggests that ZNF224 may regulate cellular structural dynamics and gene expression through vimentin, potentially contributing to melanoma cells plasticity and tumour progression.

P-30-057
DNA methylation regulates the expression of the cysteine cathepsins in normal and pathological conditions

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Human cysteine cathepsins are represented by 11 proteases of the C1A family. They are involved in a wide variety of biological processes. Their expression has been shown to be dysregulated during the development of several pathological conditions, in particular tumor diseases. The mechanism of this dysregulation is not known yet. This highlights the importance of further investigation into the mechanisms that control their expression, in particular epigenetic regulatory processes. In this study, we investigated the role of CpG island methylation in the promoter regions of cysteine cathepsin genes and its effect on the expression. Our study was performed in cell lines of both embryonic and tumor origin. First, bioinformatic analysis of the promoter regions revealed the presence of CpG islands in eight out of eleven cathepsin genes. Quantitative analysis of mRNA expression and bisulfite sequencing of the promoters of the cysteine cathepsin genes were then performed. The results demonstrated that methylation of promoter regions was correlated with mRNA accumulation for certain cysteine cathepsins. A significant effect of methylation on gene expression was found for cathepsins Z, H and F in the 769P kidney cancer, MCF7 breast cancer and HEP-G2 liver cancer tumor lines. These results suggest the importance of DNA methylation as a key epigenetic mechanism regulating the expression of cysteine cathepsins. Moreover, this regulation differs between embryonic and tumor cell lines. Differences in promoter methylation may be caused by specific molecular mechanisms that require further investigation. Understanding these mechanisms may help to identify new molecular markers and therapeutic targets, providing new opportunities for cancer diagnosis and treatment.

P-30-058
Comparative targeted metabolomics and proteomics analysis in human lymphoma model cell lines for the study of metabolic rewiring and differential diagnosis

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Metabolic rewiring is essential for sustaining cancer cell nutrition, growth, and proliferation. Studying metabolic reprogramming is critical for understanding and diagnosing cancers with high clinical and histological diversity, such as lymphomas. In the present work, intracellular metabolomic and proteomic profiles of human lymphoma cell lines were integrated, to explore lymphoma metabolism and support differential diagnosis. Three model cell lines were used, corresponding to specific Hodgkin and non-Hodgkin lymphoma subtypes. Gas chromatography-tandem mass spectrometry and a targeted method was employed to target 54 organic acids (OAs) involved in key metabolic pathways within cell extract samples. Metabolomics data were combined with proteomics data, generated in our laboratory. Databases and bioinformatics tools (MetaboAnalyst, Perseus, Rstudio) were used for data integration, analysis and visualization. 20 OAs were identified and quantified, the majority of which exhibited significant differentiation between lymphoma subtypes. Key oncometabolites, such as lactic acid, succinic acid and GABA were successfully quantified. Metabolomics and proteomics data integration highlighted that these 20 OAs, along with 20 differentially quantified proteins, participate in deregulated metabolic and signaling pathways in cancer cells (e.g., Warburg-effect, glutamate metabolism, citric-acid cycle, HIF-1 signaling pathway). These findings indicate specific pathway deregulations in the lymphoma subtypes studied. Moreover, the applied methodology demonstrated the ability to differentiate the lymphoma subtypes both in metabolite and protein level. Our results underscore the potential of integrating trans-omics data as a promising approach to support differential diagnosis and advance understanding of metabolic and signaling pathways in lymphoma pathophysiology. Mouskeftara T. et al. (2021) J Chromatogr A 1658, 462590 Psatha, K. et al. (2023) Cancers, 15, 3903.

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P-30-059
Accurate measurement of fifteen estrogen metabolites in urine samples by GC-MS method

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Estrogens and their bioactive metabolites play key roles in regulating various processes in health and disease that affect breast cancer risk [Denver N et al. (2019) J Steroid Biochem Mol Biol 192, 105373]. Estrogen metabolism occurs when the parent estrogens, estrone and estradiol, are irreversibly hydroxylated at the 2-, 4-, or 16-position of the steroid ring. Laboratory studies suggest that 16-hydroxylated metabolites can bind covalently to the estrogen receptor with potent estrogenic effects, and conversely, 2-hydroxylated metabolites bind weakly to the estrogen receptor with only modest proliferative effects and are rapidly cleared from circulation [Moore S. et al. (2016) JNCI J Natl Cancer Inst 108(10): djw103]. Identification of estrogens and their metabolites is one of the most critical steps in human physiological and pathological diagnosis, especially in risk assessment of certain cancers [Cheng C. et al. (2018) J Chromatogr Sep Tech, 9:3]. In this study, GC-MS method was developed for the precise/accurate determination of estrogen metabolites in urine samples. According to the in-house analysis method, urine samples were prepared using a "derivatized" approach. Quantification of estrogen metabolites was conducted using calibration curves established on the concentrations of the calibrators, compensating for matrix effect according to yields of internal standards. The total run time was 15.0 min. The methodological verification results showed that the method met the expected requirements and objectives in terms of sensitivity, accuracy, and precision. The percent recoveries calculated were found between 101.0–104.4% with very low percent relative standard deviation values. This method successfully separates the fifteen main estrogen metabolites.

P-30-060
Evaluation of the effects of sulforaphane on hepatocellular carcinoma cell lines

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Hepatocellular carcinoma (HCC) accounts for 85–90% of liver cancer cases and is the leading cause of liver cancer-related deaths. Liver resection, ablation, or liver transplantation are potentially lifesaving, but only if the disease is diagnosed at an early stage. Despite the availability of more pharmacological drugs for HCC treatment, a significant proportion of patients experience side effects, and drug resistance arises frequently. Hence, there is a need to evaluate the effects of natural phytochemicals as safer alternatives against HCC. Sulforaphane (SFN), an antioxidant found in vegetables such as broccoli, is considered a promising preventive and therapeutic agent for cancer. Epithelial-mesenchymal transition (EMT) occurs when epithelial cells undergo changes, acquiring a mesenchymal phenotype characterized by increased invasion, metastasis, and resistance to apoptosis. In this study, we aimed to delineate effects of SFN on HCC cell proliferation and EMT mechanisms. Given the complex molecular background of HCC, we used a panel of HCC cell lines with distinct characteristics to evaluate SFN's antiproliferative effects on cells and regulatory effects on EMT mechanisms. We used HepG2, Hep3B, and Huh7 cells as representatives of epithelial-like cell lines and SNU182, SNU449, SNU475, FOCUS, and Mahlavu as mesenchymal-like cell lines. MTT assays were performed by incubating these cells with different doses of SFN for 24, 48, and 72 hours, and IC_{50} values were determined for each time point. We then analyzed several EMT markers, including E-cadherin, vimentin, SNAIL, and β-catenin, via immunoblotting after SFN treatment of HCC cell lines. Based on cell proliferation and EMT marker analysis assays, Huh7 and SNU449 cell lines were the ones affected most by SFN treatment. In conclusion, SFN showed a promising potential as a natural phytochemical against HCC cell lines with different characteristics.

P-30-061
Collagen-rich tumor microenvironment promotes bladder cancer progression via COL1/DDR1-ANXA6 signaling axis

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The tumor microenvironment (TME) plays a critical role in cancer progression, with collagen being a major component of the extracellular matrix (ECM). Discoidin domain receptor 1 (DDR1), a receptor tyrosine kinase, binds to collagen and is subsequently cleaved by ADAM10 upon activation. Our previous study demonstrated that the COL1/DDR1 signaling axis promotes infiltration and distant lung metastasis in bladder cancer. In this study, we identified that Annexin A6 (ANXA6), a Ca^2-dependent membrane-binding protein, is downregulated upon Type I collagen (COL1) treatment. ANXA6 functions as a multifunctional scaffold protein, interacting with phospholipid membranes and various signaling proteins to regulate tumor progression. Therefore, this study aims to explore the pathological role of ANXA6 and the impact of COL1-mediated ANXA6 downregulation in COL1/DDR1-driven bladder cancer progression. Bioinformatics analysis revealed that ANXA6 expression was lower in bladder tumors compared to normal tissues. Upon COL1 treatment, ANXA6 protein levels decreased in a dose- and time-dependent manner, and DDR1 silencing further reduced ANXA6 expression. However, treatment with a proteasome inhibitor restored ANXA6 levels, indicating that COL1-mediated ANXA6 reduction occurs via the ubiquitin-proteasome pathway. DDR1 activity was not directly involved in ANXA6 downregulation, as DDR1 inhibitors failed to restore expression. However, ADAM10 inhibition reversed collagen-induced DDR1 cleavage and restored ANXA6 levels, suggesting DDR1 shedding regulates ANXA6. Functionally, ANXA6 silencing enhanced cell proliferation and migration. ANXA6 regulates Ca^2-dependent processes, including autophagy. Since Ca^2 signaling and autophagy crosstalk is essential for cellular homeostasis and survival. We found COL1 treatment increased intracellular calcium levels and autophagy, as evidenced by the upregulation of autophagy markers and LC3-positive puncta aggregation by immunofluorescence staining.

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P-30-062
Proteomic insights into tocotrienol supplementation: modulation of the complement system and glycolysis in a syngeneic mouse model of breast cancer

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Breast cancer remains the second most prevalent cancer worldwide, with ongoing research focused on mitigating tumour progression while minimising the adverse effects of conventional therapies. Natural compounds are increasingly explored for their potential anticancer properties with fewer side effects. Gamma-tocotrienol (γ-T3), a vitamin E isoform abundantly found in palm oil, has gained significant attention due to its anticancer, anti-angiogenic and immunomodulatory effects in breast cancer models. Additionally, γ-T3 has been reported to modulate key proliferation-regulating proteins in breast cancer cell lines. However, the precise molecular mechanisms through which γ-T3 exerts these effects within the tumour microenvironment remain unclear. Identifying γ-T3-induced molecular targets in a syngeneic mouse model of breast cancer could provide valuable insights into its anticancer mechanisms and therapeutic potential. This study employed label-free quantitative proteomics using LC-MS/MS to analyse serum proteomes of BALB/c mice. Five-week-old female BALB/c mice were supplemented daily with γ-T3 or vehicle (soy oil) for two weeks before inoculation with 4T1 cells, followed by continued supplementation for 35 days post-inoculation. Blood samples were collected for serum isolation and analysed using label-free proteomics at study completion. A total of 250 differentially expressed proteins were identified following γ-T3 supplementation. Functional and pathway analyses revealed that γ-T3 modulated proteins involved in extracellular matrix interactions, the complement system, anti-tumour immune responses and glycolysis—key pathways implicated in tumour progression and metastasis. These findings offer a comprehensive molecular perspective on the anticancer properties of γ-T3, underscoring its potential as a therapeutic agent for breast cancer.

P-30-063
High-resolution respirometry reveals distinctive mitochondria utilization among mesenchymal and proneuronal glioblastoma multiforme cell lines

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Glioblastoma multiforme (GBM) is the most aggressive forms of brain cancer, characterized by a poor prognosis and high resistance to the currently available chemotherapies. Genetic alterations in cancer cells alter amino acid metabolism, affecting both cell proliferation and survival. Among the deregulated genes, those encoding membrane proteins, that act as nutrients transporters, are often the most affected. Voltage-dependent anion-selective channel (VDAC) is a family of pore-forming proteins located in the outer mitochondrial membrane. The most abundant isoform, VDAC1, serves as the primary mitochondrial channel for ions and metabolites, including glutamine, NAD^+/NADH, and ATP/ADP, contributing to energy homeostasis. Additionally, VDAC1 interacts with the cytosolic proteins Hexokinases (HKs) and Bcl-2 family members (Bak, Bax), participating in the regulation of glycolysis and apoptosis. Notably, the VDAC1-HKs interaction inhibits apoptosis while simultaneously enhances metabolism via the Warburg effect, giving a preferential access of HKs to the mitochondrial ATP. To assay the contribution of glycolysis and/or oxidative phosphorylation to cell proliferation, high-resolution respirometry (HRR) was employed in a panel of commercially available GBM cell lines that mimic different tumor grade and phenotypes (mesenchymal, proneuronal). To this end, specific protocols using substrates, inhibitors and uncouplers, were pointed out to measure oxygen consumption in both intact and permeabilized cells. HRR revealed high heterogeneity between cell lines and suggested a possible correlation between VDAC1 expression, assessed here by real-time PCR, and mitochondria utilization. Given that VDAC1 is a key pharmacological target in cancer therapy, our preliminary results may pave the way for the use of small synthetic VDAC1-antagonist molecules for the treatment of GBM. Acknowledgments: PRIN 2022 PNRR E53D23021700001 (Funded by European Union NextGeneration UE) to A. Magrì.

P-30-064
MicroRNA-133b has tumor-suppressive effects in colorectal cancer cells *in vitro*

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Colorectal cancer (CRC) is the third most common cancer and the second leading cause of cancer mortality worldwide. CRC cells are characterized by dysregulation of a spectrum of genes and microRNAs that promote their survival, uncontrolled proliferation and invasiveness. MiR-133b was shown to regulate cell

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proliferation, differentiation, and apoptosis. In CRC, miR-133b basal expression is low and its effects remains unclear. Here, we investigated the influence of miR-133b on CRC cell proliferation and gene expression. HCT116 colorectal cancer cells were transfected with either a miR-133b mimic (30 nM), inhibitor (50 nM), or their respective negative controls. Cell proliferation was evaluated by BrdU and MTT assays at 0 h, 24 h, 48 h, and 72 h post-transfection. Expression of genes related to cancer cell proliferation/apoptosis (*HDAC4*, *IL-6*, *IL-8*, *LIF*, *SIRT1*, *SRF*, *TIMP1*, *TP53*), metabolism (*CS*, *PRKAA1*) and angiogenesis (*VEGF*) was analysed by qPCR 48 h after transfection. MiR-133b mimic reduced cancer cell proliferation at 72 h, as shown by both MTT and BrdU assays ($n = 3$; $p < 0.01$). This was accompanied by a decrease in lactate release into the media ($n = 3$; $p < 0.01$), indicating a reduced cell number. Gene expression analysis ($n = 3$) demonstrated downregulation of *SIRT1* and *TP53* ($p < 0.01$), while *HDAC4*, *LIF*, and *SRF* were upregulated ($p < 0.001$). No changes were observed in *IL-6*, *IL-8*, *TIMP1*, *CS*, *PRKAA1* or *VEGF*. In contrast, transfection with the miR-133b inhibitor had no effect on HCT116 cell proliferation, likely due to inherently low levels of miR-133b in cancer cells. However, it resulted in increased expression of *TP53*, *SRF*, and *VEGF* ($p < 0.05$). These findings suggest that myomiR-133b inhibits colorectal cancer cell proliferation, potentially through the regulation of transcription factors (SRF), deacetylases (SIRT1, HDAC4), and genes associated with cancer cell proliferation and apoptosis (*TP53*, *LIF*). Grant support: VEGA-2/0144/23, APVV 19-0411, APVV 23-0604.

P-30-065
Effects of liquid anaesthetics on spheroid models of glioblastoma and neuroblastoma

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In vitro models have been evolving in search of a suitable replacement for *in vivo* research. 3D cell culture models have been widely accepted due to their ease of use. We grew spheroids from two available glioblastoma cell lines (D54 and U251 respectively) and one neuroblastoma cell line (SH-SY5Y) in low adherence plates to achieve relative uniformity in size. Spheroids were exposed to 3 liquid anaesthetics indicated for local and short-term sedation (propofol, hypnomidate and levobupivacaine) for periods of 15 to 30 minutes in concentrations of 10 μg/mL for propofol and hypnomidate and 100 μg/mL for levobupivacaine. All three applied anaesthetics induce a significant change of membrane potential in the cells immediately after application (up to 40% reduction in the first 5 minutes after application). The potential remains low with minor changes during the entire treatment period (measured in 5-minute intervals up to 30 minutes). The recovery, although observed after replacing the treatment with fresh media, is slower (average 20% increase after 10 minutes in fresh media). Despite large changes in membrane permeability there is minimal cytotoxicity observed for the short treatment periods. Cytotoxic effect can be registered after prolonged exposure. In our initial research all tested anaesthetics show similar effects on glioblastoma and neuroblastoma spheroids regardless of differences in structure. Further mechanism investigation will show if these compounds have significant differences in affecting the tested cells. We intend to validate that spheroids can be an informative starting *in vitro* model by presenting new spheroid specific effects while at the same time confirming the previous findings made in 2-dimensional cell culture systems.

P-30-066
Tumor-derived CTF1 is a potent activator of cancer-associated fibroblast phenotype

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Autophagy is a cellular stress response and recycling mechanism that is active in all eukaryotic cells. Furthermore, autophagy has been studied in the context of different diseases, including cancer. Recent studies indicate that autophagy plays a role in tumor-stroma interactions. A major component of tumor stroma is cancer-associated fibroblast (CAF). We identified a tumor-derived cytokine, namely cardiotrophin 1 (CTF1) as a major activator of CAF, in our hands as potent as TGF-β. Recent studies shown that there are several subtypes of CAF. In our study, we compared the characteristics of CTF1- versus TGF-β-induced fibroblast activation and CAF formation. We dissected signaling pathways that were activated by individual cytokines. We observed that fibroblast activation-related pathways were stimulated by both cytokines. Yet, a number of cytokine-specific changes were observed as well. For instance, there were differences in signal transduction pathways, transcription targets and growth factors produced in CTF1- versus TGF-β-activated fibroblast. Additionally comparative proteomic analyses were performed on CTF1 versus TGF-β treated fibroblasts providing us with a broader picture of these divergent CAF phenotypes. Outcome of these specific phenotypic characteristics on cancer-CAF interactions have been studied in detail. References: 1 Akkoc Y, Dalci K, Karakas HE, Erbil-Bilir S, Yalav O, Sakman G, Celik F, Arikan S, Zeybek U, Ergin M, Akkiz H, Dilege E, Dengjel J, Dogan-Ekici AI, Gozuacik D. Tumor-derived CTF1 (cardiotrophin 1) is a critical mediator of stroma-assisted and autophagy-dependent breast cancer cell migration, invasion and metastasis. Autophagy. 2023 Jan;19(1) 306-323. 2. Sari D, Gozuacik D, Akkoc Y. Role of autophagy in cancer-associated fibroblast activation, signaling and metabolic reprograming. Front Cell Dev Biol. 2024 Jan 3;11:1274682.

P-30-067
Targeting tumor microenvironment *in vitro* with hydroxytyrosol

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Tumor microenvironment (TME) is a complex ecosystem that plays a crucial role in solid tumor progression. Tumor associated fibroblasts (CAFs) contribute to tumor growth through paracrine signaling and extracellular matrix remodeling. Hydroxytyrosol (HT) is a phenolic compound, with a variety of biological activities and displays great antioxidant potential. How novel

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nutraceuticals interact with the TME is currently under intense and widespread investigation. Breast tumoral cells (MDAMB231, MCF7, SKBR3), preteated with 100 ds: μM HT, were later stimulated with 1 ng/mL IL-18 and 1 mM DL-Buthionine-sulfoximine. Conditioned media was subsequently applied to HT pretreated fibroblasts (MRC5CCL171). A panel of immunomodulatory and oxidative state genes has been applied for cDNA and designed for this study. Western blot was assessed for NFkB and ERK pathway phosphorylation in cancer and fibroblast cells. Principal component analysis (PCA) was used to visualize the results. HT showed upregulation of antioxidant genes such as HMOX1, TXNRD1, MT2A, GLRX, specifically in tumoral cells. The combination of HT and IL18 did not revert the results compared to IL18 alone. The PCA results for treated fibroblasts were less well-defined, possibly indicating more complex interactions between the cell lines and treatment conditions. HT reduced the inflammatory state in breast cancer cells by decreasing TNF□ levels, while it also enhanced ERK phosphorylation in fibroblasts treated with conditioned media. These findings highlight a clear distinction in HT′s mechanism of action: in tumoral cells, its effects are observed at transcriptional level, whereas in fibroblasts, the impact occurs through post-transcriptional or post-translational modifications rather than direct gene regulation. TME has been recognized as a therapeutic target, however, the current findings highlight the complexity of tumor-stroma interactions and that HT may exert its effects on the TME predominantly at protein level.

P-30-068
C-jun oncogene expression during experimental liposarcoma progression followed by a novel jun-responsive fluorescent reporter construct

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Liposarcoma is a rare tumour of adipocytic lineage. The dedifferentiated liposarcoma shows a complete loss of adipocytic differentiation and tends to be both locally aggressive and metastatic. Developing mostly as a progression of well-differentiated tumours, it presents some unique genetic aberrations like the amplification of the 1p32 locus harbouring the *c-jun* oncogene. This is reflected in a unique liposarcoma cell line progression series composed of normal murine preadipocytes 3T3L1, their transformed derivative cells, and thereof derived murine dedifferentiated liposarcoma cells LM3D [Previously published in: Mariani O. et al. (2007), Cancer Cell 11, 361-374]. C-Jun protein expression shows a gradual increase in expression as analysed by indirect immunofluorescence, being absent in nuclei of normal 3T3L1 cells, with a heterogenous medium-level nuclear expression in transformed 3T3L1 cells and high-level uniform nuclear expression in LM3D cells. To explore this functionally, we constructed a jun-responsive fluorescent reporter plasmid, by systematically replacing the ubiquitous EF1A promoter of the commercially available reporter pRP-Hygro-EF1A-EGFP (Vectorbuilder) by several jun-responsive promoters. The JAC gene promoter, carrying one consensus (5′TGACTCA-3′) and one non-consensus (5'-TGAGTAA-3′) AP-1-recognition motif, gave the most faithful jun-responsiveness. Upon transient transfection, it mirrored endogenous c-jun immunofluorescence patterns in our cell line series. We believe that functional characterization of liposarcoma cells featuring graded levels of c-jun activity reveals new aspects of liposarcoma progression. Supported by the Bilateral Cooperation Project Czech Republic – Austria jointly financed by the Ministry of Education, Youth and Sports, Czech Republic, and Federal Ministry of Education, Science and Research, Republic of Austria, by the project 4EU + and by the Cooperatio Program, research area SURG, Charles University in Czech Republic.

P-30-069
Coordinated role of non-coding RNAs ADAMTS9-AS2 and HAND2-AS1 in regulating common targets and pathways in breast cancer

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Breast cancer (BC) is the most common cancer in women, but many molecular pathways of its development remain unclear. This study aimed to identify new lncRNA-miRNA-mRNA interactomes and their contribution to signaling pathways in BC. The expression and methylation levels of lncRNAs and miRNAs were assessed by RT-qPCR and MS-qPCR. Since epithelial-mesenchymal transition (EMT) is a key player in BC progression and metastasis, we focused on lncRNAs associated with EMT. Based on NCBI GEO datasets (GSE22820 and GSE57297) and the GeneCards database, we selected 4 lncRNAs: ADAMTS9-AS2, HAND2-AS1, MAGI2-AS3, and ZNF667-AS1. All 4 lncRNAs showed significant hypermethylation in 129 BC tissues. Comethylation was observed in pairs: ADAMTS9-AS2 – HAND2-AS1, HAND2-AS1 – ZNF667-AS1, HAND2-AS1 – MAGI2-AS3, and ZNF667-AS1 – MAGI2-AS3 ($r_s > 0.6$; $p < 0.001$). ADAMTS9-AS2 and HAND2-AS1 showed the biggest comethylation correlations. ADAMTS9-AS2 and HAND2-AS1 expression significantly decreased in 50 BC tissues. We analysed the expression of miR-17-5p predicted to interact with ADAMTS9-AS2 and HAND2-AS1. A significant negative correlation was found between ADAMTS9-AS2 and miR-17-5p expression (r_s = -0.41, $p = 0.04$) in 50 BC samples. Using bioinformatic analysis, 14 common mRNA targets were predicted for ADAMTS9-AS2, HAND2-AS1, and miR-17-5p. The studied lncRNAs can act as competing endogenous RNAs for miR-17-5p, thereby indirectly regulating such mRNA targets as STAT3, EGLN1, ANKRD52. Functional enrichment analysis revealed shared pathways for ADAMTS9-AS2/HAND2-AS1 – miR-17-5p – STAT3/EGLN1/ANKRD52 axis, including Focal Adhesion, PI3K-Akt, p53, ErbB, Hippo, and MAPK. This confirms their coordinated role in regulating common targets and suggests competition between HAND2-AS1 and ADAMTS9-AS2 for miR-17-5p binding. These findings provide insights into the molecular mechanisms of BC and suggest potential therapy targets. This work was supported by the Russian Science Foundation grant no. 24-75-00159.

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P-30-070
Deciphering novel players and pathways through investigating cancer-associated fibroblasts in the tumor microenvironment

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Cells within the same tumor can exhibit surprising heterogeneity through genetic and/or epigenetic changes. This heterogeneous structure could unexpectedly reduce the efficacy of therapeutics that initially provided promising results. Tumors do not consist only of cancer cells. Fibroblasts trained and activated by cancer, called cancer-associated fibroblasts (CAFs), constitute the largest subgroup of cellular components of the tumor microenvironment (TME). Cancer programmed TME and especially CAFs are shown to help cancer cell adaptation, survival, progression, and metastasis through secretions and cell-to-cell interactions. In this context, rather than only focusing on cancer cells, it is very important to reveal the novel regulators and pathways in the CAF-to-TME crosstalk to investigate their possible use in cancer diagnosis and treatment. Previously, in our laboratory we discovered a factor CTF1, cardiotropin-1, derived from cancer, affects invasion, migration, and metastasis capacity of breast cancer by increasing autophagy and CAF phenotype in fibroblasts [1]. Within this scope, we aim to understand fibroblast activation and CAF formation and identify key pathways in the nexus of TME interactions. We performed comparative proteomic and secretomic analyses using naïve fibroblasts and CTF1-activated fibroblasts and investigated novel factors and pathways on the role of CAFs in TME. Among others, autophagy, extracellular matrix remodeling pathways, and cytokines related with immune cell infiltration were identified as one of the key players in this context. Therefore, our data might help us better understand the roles of CAFs and the TME by discussing the implications of targeting tumor stroma, which may facilitate the development of more efficient cancer treatment.
This work was supported by TUBITAK (Grant #122Z227)
Reference: [1] Akkoc et al., (2023) Autophagy 19(1):306–323.

P-30-071
The impact of PD-1/PD-L1 inhibitors and chemotherapy on drug resistance in an immune-integrated breast cancer microenvironment

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Breast cancer remains the most prevalent malignancy among women, with immunotherapy emerging as a key targeted treatment strategy. The tumor microenvironment, particularly immune cell interactions, plays a crucial role in modulating therapeutic responses. The PD-1/PD-L1 axis is of growing significance in cancer immunotherapy, influencing drug resistance mechanisms. This study investigates the impact of PD-1/PD-L1 inhibition on drug resistance protein expression in an *in vitro* breast cancer model incorporating immune cells. In our study, an *in vitro* model was established by co-culturing breast cancer cell lines (MDA-MB-231 and MCF-7) with activated immune cell lines (THP-1 monocytes and Jurkat lymphocytes). The IC_{50} values of paclitaxel and doxorubicin, commonly used chemotherapeutics, were determined via MTT assays. Cell viability was assessed following treatment with PD-L1 inhibitor IV and BMS-1166, which target PD-1 and PD-L1, respectively. Flow cytometry was employed to analyze PD-1 receptor, PD-L1 ligand, and the expression of drug resistance proteins ABCG2, MDR-1, and MRP-1. According to the obtained results, Doxorubicin decreased PD-L1 expression in all co-culture groups except MDA-MB-231:THP-1 and led to a reduction in drug resistance, except in the MDA-MB-231:Jurkat co-culture. BMS-1166 significantly reduced cell viability across all co-cultures, further enhancing the cytotoxic effect of chemotherapy. Notably, in the MDA-MB-231:Jurkat co-culture, both inhibitors downregulated PD-L1 expression; however, drug resistance was increased. The impact of Paclitaxel on PD-L1 expression varied depending on the co-culture conditions. These findings highlight the differential effects of chemotherapy and PD-1/PD-L1 inhibitors on PD-L1 and drug resistance-related proteins in breast cancer, emphasizing the influence of tumor-immune cell interactions on therapeutic responses.

P-30-072
Thymol in combination with docetaxel enhances therapeutic response in prostate cancer cells

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Thymol, a bioactive natural compound, is a monoterpenoid phenol that is the active ingredient of *Thymus* species. It has a high industrial value and exhibits a wide range of biological activities such as anti-microbial, anti-nociceptive, anti-inflammatory, and anti-cancer effects. Prostate cancer is one of the most significant malignancies affecting men. The complex biology of the disease requires treatments such as surgery, androgen deprivation therapy, chemotherapy, and, more importantly, combinational approaches. This study aimed to reveal the synergy profile and efficacy of thymol and docetaxel against DU145 prostate cancer cells. Cell viability analysis was performed by the WST-1 assay for 24, 48, and 72 h for both thymol and docetaxel. Cellular analysis was further assessed through real-time, label-free iCELLigence technology and cell morphology alterations. Drug combination analysis was performed in a 5×5 dose matrix and dose-response data was analyzed with the SynergyFinder tool. The activities of the drugs on the migration ability of cells were carried out by *in vitro* scratch assay both as single treatments and in combination. Thymol inhibited the growth of DU145 cells in a time- and dose-dependent manner. Combination therapy revealed that two compounds exhibit additive to synergistic effects according to different synergy models. Real-time cell analysis confirmed these findings and revealed a phenotype suggesting that thymol enhances the efficacy of docetaxel. The migration ability of cells decreased significantly with these treatments. In conclusion, this study demonstrated both the independent activity of thymol and

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its supportive role when combined with docetaxel in prostate cancer. Thus, considering the integrative impact of its diverse biological activities, thymol stands out as a remarkable combination for the prevention and treatment of cancer and other cancer-related pathophysiologies.

P-30-073
Effects of two copper(II) complexes with chromone-2-carboxylic acid on the 3D cell model of colon adenocarcinoma: visualization and differences between Caco-2 and HT-29 sferoids

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Colon cancer is the second most frequently diagnosed malignant disease. Organometallic compounds containing copper have attracted the interest of researchers due to their diverse biological activities. In the conducted research, the antitumor activity of two new copper complexes with chromone-2-carboxylic acid (L_1) as a ligand was investigated ($[Cu_2(\mu\text{-}OH)_2(L_1)_2(2H_2O)]$ – complex I; $[Cu_2(\mu\text{-}OH)_2(L_1)_2(4H_2O]$ – complex II). The antiproliferative effect was investigated by the MTT assay (1×10^{-5}; 1^{5}; 1×10^{-6} mol/dm^3) on tumour (Caco-2; HT-29) and normal (MRC-5) spheroids. Spheroids were cultured for 72 h before complexes I and II were applied at two time points: single exposure (72 h; 1×) and multiple exposures (72 h + 72 h; 2×). The same spheroids were also grown on an agarose matrix (1.5%) for MALDI-TOF-MSI analysis (Schimadzu IMScope TRIO MALDI-IT-TOF, Japan). When comparing the inhibitory concentrations (IC_{50}: μmol/dm^3) and selectivity index (SI), the Man-Whitney test and Student's t-test ($P < 0.05$) were selected. The study showed that Caco-2 spheroids are more sensitive to the presence of complex I (inhibition: 60.4%; $P < 0.05$) compared to complex II (1.7%). HT-29 spheroids are more sensitive than Caco-2. With a single application of complex I, the growth inhibition is over 50% ($P < 0.05$), while with repeated application a growth inhibition of 54% ($P < 0.05$) was only measured at a higher concentration (1×10^{-5} mol/dm^3). MRC-5 spheroids were less sensitive to the presence of complexes I and II than tumour spheroids. The IC_{50} values were: HT-29 < Caco-2 < MRC-5 (1×) and HT-29 < MRC-5 < Caco-2 (2×). SI showed that HT-29 spheroids were more sensitive than Caco-2 to the presence of both complexes, regardless of whether the application was 1× (SI = 3.323 (I); 1.274 (II)) or 2× (1.415 (I); 1.580 (II)). The visualization showed different signal distribution densities in the range of 136-562 (m/z) with 53 signals (MRC-5), 114-159 (Caco-2) and 90-147 signals in HT-29 spheroids.

P-30-074
Erastin overcomes paclitaxel resistance in SKOV3-TR ovarian cancer cells by inducing apoptotic cell death

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Paclitaxel resistance and recurrence remain major challenges in the treatment of ovarian cancer, which remains the leading cause of death among gynecologic cancers. However, the mechanisms underlying paclitaxel resistance are not fully elucidated, and effective therapeutic strategies to overcome this resistance are limited. In this study, we investigated that erastin, a ferroptosis inducer, could overcome paclitaxel resistance in SKOV3-TR ovarian cancer cells. Unexpectedly, the combined treatment of erastin and paclitaxel primarily induced apoptosis rather than ferroptosis. The combined treatment significantly reduced cell viability and increased PARP cleavage, indicating enhanced apoptotic cell death. Furthermore, ferroptosis inhibitors, including ferrostatin-1 and liproxstatin-1, failed to restore cell viability, confirming that the observed cell death is not driven by ferroptosis. Mechanistically, the combined treatment induced phosphorylation of Bcl-2 and Bcl-xL in a time-dependent manner, which correlated with CDK1 activation. CDK1 knockdown or inhibition attenuated Bcl-2/Bcl-xL phosphorylation and rescued cells from erastin- and paclitaxel-induced cell death. These findings suggest that erastin may serve as a potential strategy to overcome paclitaxel resistance in ovarian cancer.

P-30-075
Regulation of nuclear protein 1 (NUPR1) expression in response to amino acid deprivation in lung cancer cells

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Nuclear protein 1 (NUPR1) is a stress-responsive transcription factor that plays a critical role in cellular adaptation to metabolic stress. Given its involvement in stress resistance, several studies have highlighted NUPR1 as a promising therapeutic target in cancer. However, its regulation under amino acid-restricted conditions remains poorly understood. In this study, we investigated the regulation of NUPR1 expressions in response to amino acid deprivation. NUPR1 mRNA levels were evaluated under the depletion of each amino acid, and significant upregulation was particularly observed upon the deprivation of arginine, glutamine, lysine, and methionine. In particular, the NUPR1 inhibitor ZZW115 further increased cell sensitivity induced by glutamine deprivation. Additionally, ZZW115 increased cell sensitivity induced by V-9302, a glutamine transporter inhibitor, indicating a strong link between glutamine metabolism and NUPR1-mediated survival mechanisms. These findings suggest that NUPR1 plays an important role in cellular responses to amino acid stress and may serve as a potential therapeutic target, particularly under glutamine-deprived conditions.

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P-30-076
Regulatory mechanisms of PD-L1 expression under prolonged glucose deprivation: lack of involvement of the ER stress response

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The tumor microenvironment poses significant challenges for cancer cell survival and growth but can also drive more aggressive tumor behavior, including resistance to anticancer treatments and immune evasion. The impact of glucose deprivation within the tumor microenvironment on anticancer immune responses, particularly its role in regulating PD-L1 expression, remains poorly understood. Studies have reported conflicting effects of short-term (24-hour) glucose deprivation on PD-L1 expression in renal and hepatocellular carcinoma cells (Cell Death Discov 7:15, 2021; Sci Rep 14:1751, 2024). Moreover, the mechanisms regulating PD-L1 expression under prolonged glucose deprivation conditions are not well characterized. In A549 lung adenocarcinoma cells, short-term glucose deprivation (< 7 days) did not significantly alter PD-L1 protein levels, despite an increase in BiP protein level. However, prolonged glucose deprivation (≥ 4 weeks) progressively increased PD-L1 mRNA and protein levels, including cell surface PD-L1, with a corresponding resistance to cytotoxic T lymphocyte (CTL)-mediated apoptosis including granzyme B-induced cell death. Our findings demonstrated that Janus kinase 2 (JAK2), histone deacetylase 2/6 (HDAC2/6), and EGFR/MEK/ERK signaling critically contribute to enhanced PD-L1 expression under prolong glucose deprivation. Mechanistically, JAK2 inhibition reduced PD-L1 protein level by promoting its proteasomal degradation, whereas suppression of HDAC6 and EGFR signaling primarily modulated PD-L1 transcription, independent of proteasome activity. Notably, no correlation was observed between PD-L1 regulation and the activity of BiP or IRE1α-XBP1 signaling, indicating that ER stress response is not involved in this process. In summary, prolonged glucose deprivation enhances PD-L1 expression through dual mechanisms: JAK2-mediated stabilization of PD-L1 protein and transcriptional activation regulated by HDAC6 and EGFR signaling (supported by 2022R1F1A1071397).

P-30-077
HERPUD1 links palmitic acid-induced ER stress and UPR activation to doxorubicin survival and aggressiveness in TNBC via CK2 phosphorylation

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Endoplasmic reticulum (ER) stress is a hallmark of many tumors, including triple-negative breast cancer (TNBC). Palmitic acid (PA), a dietary saturated fatty acid and tumor risk factor, induces ER stress and activates the unfolded protein response (UPR). HERPUD1, an ER stress-responsive protein, has been linked to stress tolerance in cancer cells. While its role in UPR activation by chemical agents like thapsigargin (TG) is well-documented, its function in lipid-induced ER stress and TNBC progression remains unclear. We analyzed HERPUD1 expression in breast cancer (BC) biopsies via immunohistochemistry and immunofluorescence, revealing significantly higher levels in BC, including luminal A and TNBC, compared to non-malignant tissue. In TNBC, HERPUD1 correlated with inflammatory infiltrates, highlighting its potential role in tumor progression. Given the impact of PA and high-fat diet-induced obesity in cancer, we examined PA's effect on TNBC cells, finding that it specifically upregulates HERPUD1 in MDA-MB-231 cells, along with increased XBP1s, a key UPR factor. In contrast, TG treatment elevated HERPUD1 across all cell lines. Functionally, HERPUD1 silencing reduced TNBC cell proliferation, migration, and invasion, while its loss enhanced doxorubicin (DOX) cytotoxicity in both 2D and 3D models. Interestingly, while HERPUD1 ablation elevated UPR activation under TG, PA-induced stress led to reduced UPR activation and lower IL-6 and IL-8 levels. Mechanistically, we identified CK2 as a kinase regulating HERPUD1 stability via Ser-59 phosphorylation. CX-4945, a CK2 inhibitor, reduced HERPUD1 levels, but its cytotoxic effect was diminished in HERPUD1-S59D phosphomimetic mutants, linking HERPUD1 stability to CX-4945 sensitivity. Fondecyt 1211261.

P-30-078
PLK1 phosphorylates RhoGDI1 and promotes cancer cell migration and invasion

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Rho guanine nucleotide dissociation inhibitor 1 (RhoGDI1) plays an important role in diverse cellular processes by regulating Rho guanosine triphosphate (GTP)ases activity. RhoGDI1 phosphorylation regulates the spatiotemporal activation of Rho GTPases during cell migration. In this study, we identified polo-like kinase 1 (PLK1) as a novel kinase of RhoGDI1 and investigated the molecular mechanism by which the interaction between RhoGDI1 and PLK1 regulates cancer cell migration. Immunoprecipitation, GST pull-down assay, and proximity ligation assay (PLA) were performed to analyze the interaction between RhoGDI1 and PLK1. *In vitro* kinase assay and immunoprecipitation were performed with Phospho-(Ser/Thr) antibody. We evaluated RhoA activation using RhoGTPases activity assay. Cell migration and invasion were analyzed by transwell assays GST pull-down assays and PLA showed that PLK1 directly interacted with RhoGDI1 *in vitro* and *in vivo*. Truncation mutagenesis revealed that aa 90–111 of RhoGDI1 are critical for interacting with PLK1. We also showed that PLK1 phosphorylated RhoGDI1 at Thr7 and Thr91, which induces cell motility. Overexpression of the GFP-tagged RhoGDI1 truncated mutant (aa 90–111) inhibited the interaction of PLK1 with RhoGDI1 and attenuated RhoA activation by PLK1. Furthermore, the overexpression of the RhoGDI1 truncated mutant reduced cancer cell migration and invasion *in vitro* and suppressed lung metastasis *in vivo*. Collectively, we demonstrate that the phosphorylation of RhoGDI1 by PLK1 promotes cancer cell migration and invasion through RhoA activation. This study connects the interaction between PLK1 and RhoGDI1 to the promotion of cancer cell behavior associated with malignant progression, thereby providing opportunities for cancer therapeutic interventions.

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P-30-079
Role of peroxisome proliferator-activated receptor α-dependent mitochondrial metabolism in ovarian cancer stem cells

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Peroxisome proliferator-activated receptors (PPARs), including PPAR-α, PPAR-β/δ, and PPAR-γ, are involved in various cellular responses, including metabolism and cell proliferation. Increasing evidence suggests that PPARs are closely associated with tumorigenesis and metastasis. However, the exact role of PPARs in energy metabolism and cancer stem cell (CSC) proliferation remains unclear. This study investigated the role of PPARs in energy metabolism and tumorigenesis in ovarian CSCs. The expression of PPARs and fatty acid consumption as an energy source increased in spheroids derived from A2780 ovarian cancer cells (A2780-SP) compared with their parental cells. GW6471, a PPARα inhibitor, induced apoptosis in A2780-SP. PPARα silencing mediated by small hairpin RNA reduced A2780-SP cell proliferation. Treatment with GW6471 significantly inhibited the respiratory oxygen consumption of A2780-SP cells, with reduced dependency on fatty acids, glucose, and glutamine. In a xenograft tumor transplantation mouse model, intraperitoneal injection of GW6471 inhibited *in vivo* tumor growth of A2780-SP cells. These results suggest that PPARα plays a vital role in regulating the proliferation and energy metabolism of CSCs by altering mitochondrial activity and that it offers a promising therapeutic target to eradicate CSCs.

P-30-080
Ozone exposure modulates Bax and Bcl-2 expression in colorectal adenocarcinoma cell lines: implications for apoptosis-based cancer therapy

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Colorectal adenocarcinoma remains a major therapeutic challenge due to resistance to conventional treatments. The study examines how ozone therapy might affect apoptotic pathways through modulation of Bax (pro-apoptotic) and Bcl-2 (anti-apoptotic) protein expression in Colo-320 and Colo-741 colorectal adenocarcinoma cell lines. Cells were cultured in RPMI-1640 medium with 10% fetal bovine serum and treated with 20 μg/mL ozone for 72 hours. Morphological changes were analyzed by inverted microscopy; immunocytochemistry and semi-quantitative H-SCORE evaluation were done for Bax and Bcl-2. The analysis of morphology showed no considerable changes in cell morphology after the ozone treatment. By contrast, the expression of Bax was strongly upregulated in Colo-320 and Colo-741 (Colo-320: 225 ± 10 to 358.3 ± 10.41; Colo-741: 254.5 ± 13.87 to 311.7 ± 18.89; $p < 0.05$) was activated. On the contrary, both Colo-320 and Colo-741 decreased the expression of Bcl-2 significantly (Colo-320: 306.7 ± 5.77 to 238.3 ± 7.64; Colo-741: 288.3 ± 17.56 to 251.7 ± 10.41; $p < 0.05$), indicating the suppression of anti-apoptotic mechanisms. These findings suggest ozone shifts the Bax/Bcl-2 ratio towards apoptosis without changes to the cellular morphology, thereby illustrating its specificity for particular molecular pathways. The data are in line with other studies proving that ozone may induce mitochondrial-mediated apoptosis (1). These data suggest that ozone therapy may complement existing cancer treatments, inducing selective apoptosis in malignant cells. Further mechanistic studies should be undertaken to design optimized therapeutic protocols and evaluate clinical efficacy. References: (1) Xu L et al. (2021) Exp Ther Med 22, 803.

P-30-081
Liquidambar orientalis suppressed ROS formation by inhibiting HDAC1 and ATG4 expression in MDA-MB-231 breast cancer cells

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Breast cancer is a leading cause of female cancer mortality, with triple-negative breast cancer (TNBC) posing a significant challenge due to its aggressive nature and receptor absence. *Liquidambar orientalis* is an oil with demonstrated anticancer properties that has been utilized in herbal therapy. The generation of reactive oxygen species (ROS) can induce the hyperacetylation of histones, leading to various cellular outcomes, including the activation of apoptotic pathways and the initiation of autophagy. Consequently, the utilization of epigenetic targets holds significant potential as a promising approach in breast cancer therapy. This study investigated the epigenetic effects of *Liquidambar orientalis* on the MDA-MB-231 TNBC cell line, focusing ROS production and histone deacetylase (HDAC) activity. MTT assays determined drug doses. ROS production was quantified using H_2DCFDA staining. The effects on HDAC and ROS were quantified using western blot analysis. ImageJ was used for band intensity analysis. The IC_{50} of *Liquidambar orientalis* was determined to be 250 μg/ml. As a result of *Liquidambar orientalis* treatment, the suppression of ROS production was achieved by inhibiting HDAC1 and Atg4 protein expression levels. These findings support the anticancer potential of *Liquidambar orientalis* and provide valuable insights into the utilization of herbal therapies in breast cancer treatment.

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P-30-082
Determination of the proteins that play a role in aggressive features of isocitrate dehydrogenase 1 silenced cells

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The isocitrate dehydrogenase (IDH), which important key enzyme in the TCA cycle, has an active role in cancer development. Our previous study showed that IDH1 knockout results in G0/G1 arrest, and reduces proliferation, invasion, migration, and colony formation abilities of the colon adenocarcinoma cell line (SW620). However, how the aggressive features of colon cancer cells were decreased is unknown. In this study, the protein's role in pathways related to these features was investigated in IDH1 KO cells by using LC-MS/MS analysis. It was found that the levels of integrin alpha-V (ITGAV), cell adhesion molecule 1 (CADM1) and intercellular adhesion molecule 1 (ICAM1) in cell adhesion and vimentin (VIM) in cell shape and cell organization and biogenesis were increased in IDH1 KO cells compared to control cells. Also, the levels of cadherin-1 (CDH1), catenin alpha-2 (CTNNA2) and claudin-11 (CLDN11) in the cell adhesion, cell organization and biogenesis were decreased IDH1 KO cells compared to control cells. Our findings reveal that ITGAV, CADM1, ICAM1, VIM, CDH1, CTNNA2, and CLDN11 proteins can be directly related to the IDH1 enzyme role in aggressive features of cancer. In the future, these proteins can be targeted to combat cancer cells after IDH1 silence.

P-30-083
Exploring the antitumor potential and mechanisms of action of novel DNA-intercalating heterocyclic fused naphthalimides

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1,8-Naphthalimides are a class of heterocycles known for their strong antiproliferative effects, which are primarily attributed to their ability to intercalate into DNA. Here, we introduce conceptually novel structures with no known analogues in the scientific literature. These molecules were designed to enhance the therapeutic efficacy of the cytotoxic agents mitonafide and amonafide by combining their scaffold with the DNA intercalating tricyclic planar chromophore dibenzodioxin into a single entity. This study aims to assess the biological activity of these newly synthesized compounds. A549 and NCI-H1299 human non-small cell lung carcinoma cell lines, along with MRC-5 normal lung fibroblasts, were utilized for analysis. A series of experiments were conducted to investigate the cytotoxic and antiproliferative characteristics of the novel derivatives. Selected candidates underwent further analysis to determine their effects on the cell cycle and the mechanisms underlying cell death. Results demonstrated that benzodioxin-1,8-naphthalimides exhibited greater cytotoxic potency compared to mitonafide and other intercalating agents. Notably, mitonafide-based derivatives displayed higher toxicity than their amonafide-based counterparts. Cell cycle analyses revealed distinct treatment responses between the two cancerous cell lines. These novel compounds induced apoptosis, inhibited DNA replication, and triggered the accumulation of genetic damage. The present study demonstrates that benzodioxin-1,8-naphthalimides exhibit potent cytotoxic activity by inducing apoptosis and disrupting DNA replication. These findings support their potential as promising anticancer agents. Acknowledgments: This work was supported by grant KP-06-H61/1 from 13.12.22 of the Bulgarian National Science Fund.

P-30-084
Metabolic adaptation and multidrug resistance in FLT3 inhibitor resistant acute myeloid leukemia

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Acute myeloid leukemia (AML) is a blood cancer marked by the expansion of immature myeloid blasts. FLT3 mutations (ITD/TKD) occur in 30% of AML cases and are linked to poor prognosis, prompting the development of FLT3 inhibitors (FLT3i). Although FLT3i have improved patient outcomes, additional FLT3 mutations (e.g., D835) contribute to resistance and relapse. The molecular and metabolic mechanisms of FLT3i resistance remain unclear. This study aims to analyze cellular adaptations in FLT3-ITD+ AML cells after prolonged quizartinib (AC220) exposure. MV4-11 (FLT3-ITD+) cells were treated with increasing AC220 concentrations for six months until resistant clones emerged. Resistant cells (MV4-11QR) were authenticated by STR and next-generation sequencing (NGS). Cell viability, apoptosis, and cell cycle progression were assessed. Proteome analysis and metabolic profiling were performed using Seahorse XF96 Pro. NGS revealed FLT3 D835H (VAF: 41%) and TP53 R248W (VAF: 100%) mutations in MV4-11QR. These cells exhibited decreased AC220 sensitivity (IC_{50}: wt: 0.95 nM vs. QR: 34.3 nM) and minimal clonogenicity and cell cycle disruption. MV4-11QR cells were also resistant to other FLT3i (midostaurin, gilteritinib) and AML therapies like venetoclax and cytarabine, suggesting limited treatment options. At the molecular level, MV4-11QR cells showed reduced sensitivity to AC220 with diminished FLT3/ERK inhibition, reduced γH2AX expression, and PARP cleavage. Proteomic analysis identified 86 upregulated proteins (e.g., BCL2L13 and PTPN1) and 111

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downregulated proteins (e.g., GLUL, SOD2, and ACOX1). Gene enrichment analysis (GSEA) revealed changes in glucose metabolism and oxidative stress pathways. Mitochondrial analysis showed increased oxygen consumption and reduced spare respiratory capacity, indicating metabolic reprogramming. Our findings suggest prolonged AC220 exposure induces cellular reprogramming, providing insights for combination therapies to overcome FLT3i resistance.

P-30-085
The role of miR-548ah-3p in ovarian cancer stemness and chemoresistance

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Ovarian cancer (OC) is the most lethal gynecological malignancy, with high-grade serous ovarian cancer (HGSOC) being the predominant histological subtype. Ovarian cancer stem cells (OCSCs) play a crucial role in poor prognosis and aggressive disease progression due to their ability to self-renew, remain quiescent, re-enter the cell cycle, and transition between epithelial and mesenchymal states. A deeper understanding of the regulatory mechanisms governing OCSCs could provide valuable insights into these processes and offer potential therapeutic targets. One key regulatory element of interest is microRNAs (miRNAs), a class of small non-coding RNAs that modulate gene expression at the post-transcriptional level. To investigate their role, spheroids were generated from the HGSOC cell line OVCAR-3 using ultra-low attachment plates. CSC trait enrichment in this population was confirmed through flow cytometry, RT-qPCR, and western blot analysis. RNA-Seq analysis identified differentially expressed miRNAs, with hsa-miR-548ah-3p emerging as a significantly downregulated candidate with potential regulatory functions in CSC traits. Mimic treatment of CSC-enriched OVCAR-3 cells led to a reduction in key stemness markers, including SOX-2, ALDH1A1, and c-MYC, as well as CSC-associated surface markers CD44 and CD133. Additionally, miR-548ah-3p overexpression impaired migration and invasion abilities of CSC-enriched cells. Notably, it also enhanced the sensitivity of these cells to chemotherapeutic agents commonly used in HGSOC treatment, including carboplatin, olaparib, paclitaxel, and niraparib. In conclusion, miR-548ah-3p expression was found to be inversely correlated with cancer stemness, suggesting that it may target key regulators of CSC enrichment during post-transcriptional regulation. These findings highlight its potential as a therapeutic target for combating CSC-driven drug resistance and disease progression in HGSOC.

P-30-086
Thymoquinone-loaded exosomes reduce drug resistance and induce apoptosis in colon cancer cells and spheroids

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Colorectal cancer (CC) is the third most common cancer type worldwide and the second leading cause of cancer-related deaths. Despite significant advances in classical cancer treatments such as endoscopic surgery, robotic surgery, and chemotherapy, the 5-year overall survival rate in patients with locally advanced or metastatic CC is extremely low. The development of drug resistance, which significantly contributes to poor clinical prognosis, is a major obstacle to CC treatment. Developing natural product-based compounds may be an attractive strategy for treating and preventing CC. In this study, thymoquinone (TQ), the main component of *Nigella sativa's* essential oil and a low-bioavailable but effective molecule, was packaged into exosomes, and its potential therapeutic effects were investigated. These effects were examined in 2D-monolayer and 3D-spheroid (better physiologically reflect cell-cell and cell-matrix interactions in tumors) Caco-2 cell cultures. In 2D-culture, cells treated with thymoquinone in exosomes (EXO-TQ) showed more sensitivity to 5-FU. Immunofluorescent labeling showed decreased nuclear localization of NF-κB in treated cells. The treatments resulted in alterations in cell signaling pathways (ERK, PI3K, Akt), induction of apoptosis (Bax, Bcl-2, caspase-3, PARP1), reduction of cellular stress (Hsp70, Hsp90), and attenuation of drug resistance mechanisms (MRP1, MGMT). In 3D-culture, EXO-TQ reduced the growth and viability of spheroids. Overall, these findings provide proof that natural products with low bioavailability, but anticancer properties have enhanced therapeutic effects via exosome-mediated delivery and that EXO-TQ may be a promising strategy for treating CC against drug resistance.

P-30-087
TanIIA-induced necroptosis requires activation of the JNK pathway in malignant mesothelioma cells

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Necroptosis, also known as programmed necrosis, has gained attention from cancer researchers due to its potential as a novel treatment strategy. Limited treatment options for malignant pleural mesothelioma (MPM), an aggressive cancer linked to asbestos exposure, highlight the need for alternative therapeutic targets and treatments. TanshinoneIIA (TanIIA) is a natural compound derived from the *Salvia miltiorrhiza* plant and shows promising potential for treating various types of cancer. This study aims to investigate whether TanIIA induces necroptosis in MPM cells and to clarify the molecular mechanisms involved. Initially, we assessed the changes in indicator proteins related to necroptosis and the activation of stress pathways in cells exposed to TanIIA. Afterwards, the interaction between stress and necroptotic signals was evaluated in SPC212 cells. The results indicated that TanIIA induced necroptosis in a dose-dependent manner in MPM cells. Additionally, an increase in JNK protein phosphorylation was observed in the cells treated with this flavonoid. Moreover, the phosphorylation of RIPK1, RIPK3, and MLKL induced by TanIIA was inhibited using a pharmacological JNK inhibitor, SP600125. This finding strongly indicates that necroptotic activation requires stimulation by JNKs. Our next goal is to elucidate the role of the p38 MAPK molecule in this cell death mechanism. *The authors marked with an asterisk equally contributed to the work.

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P-30-088
Metal nanoparticles can influence the paracrine communication between macrophages and tumor cells

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Tumor-associated macrophages (TAMs) contribute to an immunosuppressive environment and promote tumor progression through reprogramming driven by tumor and stromal cells. Macrophages generally polarize into either classically activated (M1) or alternatively activated (M2) phenotypes, which differ in their secreted cytokines, surface markers, and biological functions. This study aimed to determine whether gold (AuNP) or silver nanoparticles (AgNP) could disrupt cellular interactions and modulate the TAM phenotype. Murine breast cancer cells (4T1) were treated with AuNP or AgNP and co-cultured with murine macrophages (J774 or bone-marrow derived macrophage (BMDM)). The co-culture led to a significant increase in the RNA and protein expression of several M1 and M2 markers. However, when 4T1 cells were treated with nanoparticles (NPs), certain M2 markers in J774 and BMDM samples showed a notable reduction compared to untreated co-culture samples. Additionally, co-culturing enhanced the total matrix metalloproteinase (MMP) activity, the migration, and the levels of cell migration-related factors of macrophages, however they were significantly diminished upon NP treatments. The co-culture system strongly influenced M1 and M2 marker expression, while the NPs primarily suppressed TAM-associated traits, such as enhanced migration and MMP activity. These findings suggest that this *in vitro* co-culture model is suitable for studying paracrine interactions between macrophages and tumor cells.
This project was supported by the NTP-NFTÖ-22-B-0100, NTP-NFTÖ-21-B-0157 grants of the Ministry of Human Resources, by the János Bolyai Research Fellowship of the Hungarian Academy of Sciences (BO/00351/22/8), by the University Research Scholarship Program of the Ministry for Culture and Innovation (EKÖP-24-4-SZTE-626), by the Incubation Competence Centre of the Centre of Excellence for Interdisciplinary Research, University of Szeged, and by OTKA-NKFIH (K142371) of the Hungarian Government.

P-30-089
From apical to basal extrusion: E-cadherin and filamin A in the trajectory of gastric cancer cells

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E-cadherin germline variants are causative of hereditary diffuse gastric cancer (HDGC), a highly invasive cancer syndrome with a lethal outcome. In HDGC, E-cadherin dysfunctional cells initiate invasion by spreading beneath wild-type cells, as a result of a switch from apical to basal extrusion. Nonetheless, the molecular mechanisms that allow E-cadherin dysfunctional cells to invade instead of being eliminated are still not fully understood. To explore the fate of E-cadherin mutant cells, AGS gastric cells were transfected with both wild-type and E-cadherin variants, affecting different protein domains. A monolayer system, encompassing labelled mutant cells surrounded by non-labelled wild-type counterparts, was then established and monitored to determine extrusion phenotypes. Structural organization, protrusion formation, and cell spreading were also evaluated in a 3D culture system. A comprehensive analysis was implemented to examine the distribution patterns of integrins and cytoskeletal components. Variants disrupting the juxtamembrane and intracellular regions showed higher basal extrusion rates, when compared to those affecting the extracellular domain. Interestingly, the R749W juxtamembrane and V832M intracellular variants were associated with decreased Filamin A activity and actin remodeling. The role of Filamin A in cell extrusion was further validated by its inhibition in wild-type cells, inducing significant changes in cytoskeletal networks, basal positioning of nuclei, and upregulation of integrins, ultimately leading to increased capacity to migrate deep into the extracellular matrix. Our findings suggest that E-cadherin and filamin A operate within a common pathway that determines extrusion phenotypes, revealing potential therapeutic avenues for early intervention in E-cadherin mediated gastric cancer.

P-30-090
Impact of tanshinoneiia treatment on NSCLC cell proliferation with MEK5 knockouts

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Non-small cell lung cancer (NSCLC) is one of the most prevalent subtypes of lung cancer. Studies indicate that NSCLCs exhibit elevated mitogen-activated protein kinase (MAPK) signalling, which contributes to the uncontrolled proliferation of cells. MEK5/ERK5 pathway is the latest route discovered in the MAPK family that influences lung cancer proliferation and represents a potential "escape route" for cancer cells. TanshinoneIIA (TanIIA) is a flavonoid isolated from the roots and rhizomes of the Chinese medicinal herb *Salvia miltiorrhiza* Bunge (Danshen). The anticancer, anti-inflammatory, and antioxidant properties of TanIIA were reported in several

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studies. In this study, the effect of TanIIA was investigated in NSCLC cells by targeting the MEK5/ERK5 pathway. MEK5 gene knockout cells were constructed using the CRISPR/Cas9 system. The proliferation profile of MEK5-silenced cells was compared to their non-silenced counterparts following TanIIA treatments. The anti-proliferative effects of TanIIA in different NSCLC cells were indicated formerly. Consistent with these results TanIIA inhibited cell proliferation in a dose-dependent manner in our cells. Significantly, the proliferation assay showed that genetic silencing of the MEK5 pathway has altered the growth kinetics of the H1975 cell line. Relevantly, the IC_{50} value of TanIIA was reduced almost twice in MEK5-silenced cells, indicating increased sensitivity of these cells to this flavonoid and the importance of MEK5 signals for the survival and proliferation of NSCLC. Furthermore, our assessment of the effects of TanIIA on key proteins involved in cell proliferation and survival revealed that TanIIA significantly suppressed the phosphorylation of ERK1/2 and AKT proteins in both cell types. Moving forward, we plan to analyze the phosphorylation and expression of ERK5 in cells exposed to TanIIA to confirm our findings on proliferation, and to evaluate any potential interactions with ERK1/2 and AKT. *The authors marked with an asterisk equally contributed to the work.

P-30-091
Exploring aryl hydrocarbon receptor signaling regulation by microRNAs in acute myeloid leukemia microenvironment

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Aryl hydrocarbon receptor (AHR) is a conserved cytosolic transcription factor that regulates the body's response to environmental stimuli, acting as a xenobiotic sensor and controlling cytochrome P450 (Cyp) enzyme activity. MicroRNAs (miRNAs) released by mesenchymal stromal cells (MSCs) circulate in the bone marrow and are taken up by niche cells, influencing their behavior. AML (acute myeloid leukemia) progression is not only driven by genetic changes but also shaped by microenvironmental alterations. AHR inactivation is linked to AML development, suggesting that circulating miRNAs may regulate AHR signaling and impact disease progression. However, the role of miRNAs in AHR regulation within the bone marrow remains largely unknown. This study aims to identify miRNAs targeting AHR signaling molecules and assess their differential expression in AML and healthy bone marrow plasma and stromal cells. A literature review and reanalysis of publicly available Gene Expression Omnibus (GEO) datasets (GSE84881, GSE142699, GSE283315(in house data)) were performed using Geo2R to determine differentially expressed miRNAs in AML (n = 47). Additionally, 200 miRNAs identified from BM MSC RNA sequencing data from our group were analyzed. miRNAs targeting AHR pathway components (AHR, AHRR, CYP1A1/A2/B11, ARNT, AIP, HSP90AA1, IDO1, NCOA2, EPAS1, TIPARP, NFE2L2) were retrieved from miRTarBase (v10.0). Of the 103 differentially expressed miRNAs in AML, 70 targeted at least one AHR pathway molecule; 44 were upregulated, and 26 were downregulated. miRPathDB 2.0 analysis provided heat maps illustrating pathway enrichment, revealing that upregulated miRNAs were linked to cancer-related pathways, while downregulated miRNAs were associated with key signaling pathways. These findings highlight the role of AHR targeted-miRNAs in AML-associated BM niche remodeling and may help in understanding AML pathogenesis. This study was partially supported by TUBITAK grant number: 220S759.

P-30-092
Casticin – a new perspective for supporting chemotherapy of non-small cell lung cancer

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According to GLOBOCAN, lung cancer is one of the most frequently diagnosed cancers in the world. 5-year survival rate is less than 14% which means that lung cancer, along with pancreatic, liver and brain cancer is the fourth most deadly. At the early stage of the disease, lobectomy (excision of lung) is used. Subsequently, the therapy includes chemotherapy, kinase inhibitors, and immunotherapy. Despite significant progress in lung cancer therapies, patient mortality is still high which determines the need to search for new therapeutic solutions, especially targeted cancer migration to prevent systemic metastasis. Casticin (CST) is a natural compound found in the fruits of *Vitex* sp. Currently, is used to relieve symptoms of menopause, migraine prevention and reducing rheumatic pain. In recent years, anticancer properties of casticin have been evaluated using both *in vitro* and *in vivo* models. Studies indicated that casticin inhibited tumor growth and cancer cell migration by regulation of PI3K/Akt, nuclear factor kappa-B (NF-κB), STAT3 and c-Met signaling pathways. The purpose of this study was to investigate the chemosensitizing properties of casticin in non-small cell lung cancer (A549) cellular model treated with daunorubicin (DNR) and mitoxantrone (MTX). Our results demonstrated that CST chemosensitized A549 cells to DNR and MTX. Cotreatment with CST + DNR/MTX significantly decreased the viability and proliferation of A549 cells in time and dose dependent manner. Furthermore, CST + DNR/MTX inhibited the migration of cancer cells in Transwell model system and wound healing assay and alleviate MPP2 or MPP9 level. CST could be promising candidate for DNR or MTX adjuvant therapy; however, further studies are necessary to establish the exact molecular mechanism of casticin chemosensitizing properties toward A549 cells treated with DNR or MTX.

P-30-093
Comparison of cytotoxic and apoptotic effects of flowering buds, flowers and fruits of *Capparis ovata*

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Breast cancer is a serious health problem affecting millions of women worldwide due to the interaction of genetic and environmental factors. Studies have shown that phenolic and

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flavonoid compounds contained in plant extracts may have potential beneficial effects against breast cancer by regulating various molecular pathways related to tumor initiation, progression and metastasis. It has been proven by studies that extracts of plants belonging to the *Capparis* species can play an important role as a potential treatment agent, especially in the treatment of multiple sclerosis (MS), and also exhibit anticancer, antioxidant, anti-inflammatory, antibacterial, antidiabetic, hypoglycemic and antifungal properties. *Capparis* plants, with their buds, fruits, seeds and roots, are rich in medicinally effective phenolic compounds, flavonoids, tocopherols and glucosinolates. In this context, the aim of this study was to investigate the cytotoxic and apoptotic effects of aqueous extracts obtained from different parts of *Capparis ovata* (flowers, fruits and flowering buds) on breast cancer cells. The effects of *Capparis ovata* flowering buds, flowers and fruits on cell viability in MCF-7 breast cancer cells were evaluated by crystal violet method. In addition, their effects on apoptosis were analyzed by Arthur Image-Based Cytometer using Annexin V/FITC Apoptosis Detection Kit. According to the study results, IC_{50} values of MCF-7 cells after 24 hours of treatment with *Capparis ovata* flowers, fruits and flowering buds were calculated as 14.29 ± 1.38 mg/ml, 11.24 ± 1.12 mg/ml and 10.59 ± 0.95 mg/ml, respectively. In addition, apoptotic cell rates were determined as 35%, 11% and 58% as a result of treatments with these plant parts, respectively. Research findings revealed that *Capparis ovata* flowering buds exhibited stronger cytotoxic and apoptotic effects than other parts, emphasizing that these properties may be important for the pharmaceutical use of the plant.

P-30-094
Targeting the HSF1/SIRT1/FOXO1 signaling axis attenuates glioblastoma cells and spheroids' growth and temozolomide drug resistance

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Glioblastoma multiform (GB) is a primary malignant brain tumor with a poor prognosis and high mortality. Resistance to the drug temozolomide (TMZ) in GB cells limits the effectiveness of the treatment and is closely associated with the induced heat shock response (HSR). There is limited information about the roles of stress/heat shock proteins (Hsp), which play an active role in this process, and their basic transcription factor HSF1 (heat shock factor 1) in the TMZ drug resistance mechanism in GB cells. In this study, the TMZ drug resistance mechanism of gliomas and the roles of HSF1, SIRT1, and FOXO1 proteins in this process were investigated in 2D-monolayer and 3D-spheroid (which reflect tumor physiology better than monolayer culture) U87MG human GB cell cultures. Target gene silencing was achieved with CRISPR/Cas9 method, and protein expression was increased through vector-mediated gene transfer. Protein–protein interactions were examined by co-immunoprecipitation. Cellular localization of transcription factors was evaluated by immunofluorescence labeling. Knockout groups showed increased sensitivity to TMZ, with altered cell signaling (SIRT1, FOXO1, ERK, PI3K, Akt, NF-κB), apoptosis induction (Bax, Bcl-2, caspase-3, PARP1), reduced cellular stress response (Hsp27, Hsp70, Hsp90, HSF1), decreased drug resistance (MRP1, MGMT), and attenuated EMT/stem-like features. Overexpression groups exhibited reversed responses. Overall, the findings indicate the relevance of targeting a cellular pathway involving HSF1, SIRT1, and FOXO1 to potentially mitigate chemotherapy resistance in GB cases. The data obtained from 3D glioma spheroids may offer a new perspective for developing treatment strategies against drug resistance.

P-30-095
Environmental carcinogenic agents activate Epstein-Barr virus Zp promoter in epithelial cells

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Epstein-Barr virus (EBV) infects more than 90% of the human population, establishing latency in B cells with sporadic reactivations. EBV has been detected in head and neck cancers (HNC) and breast cancers, among others, though its etiological role remains unknown in these cancers. EBV-associated malignancies occur only in a restricted subset of subjects, suggesting that cofactors are required. Importantly, lytic cycle activation occurs in EBV-associated tumors which is regulated by the Zp promoter for expression of the BZLF1 gene and the encoded Zta protein. In this study, we evaluated the potential role of cigarette smoke components (CSC, Kentucky University) and the pesticide chlorpyrifos (Merck) in inducing EBV Zp (683 bp, accession number MK540447.1) activation in HNC (CAL27 and SCC143) and breast cancer cells (MCF7). Additionally, HNCs were evaluated for EBV presence and lytic transcript expression. The MTS assay was used to determine non-toxic concentrations of CSC and chlorpyrifos. Both CSC and chlorpyrifos were able to promote an increase in Cytochrome P450 Family 1 Subfamily B Member 1 (CYP1B1) transcripts. To determine Zp activity, the EBV Zp region (nt 91574–90892) was cloned into the pMIR-GLO vector. The construct was transfected into epithelial cells for 24 h, and the cells were exposed to different doses of CSC or chlorpyrifos to evaluate luciferase activity. We found that both CSC (10–50 μg/mL) and chlorpyrifos (5–50 μM) activate Zp in a dose-dependent manner in HNC and breast cancer cells, respectively. Mutant analysis showed that the CCAAT/enhancer-binding protein beta (c/EBPβ) binding region is relevant for Zp activation in HNC cells. Chromatin immunoprecipitation (ChIP) analysis confirmed the recruitment of c/EBPβ to Zp. Additionally, BZLF1 transcripts were detected in EBV-positive HNCs. This study suggests that tobacco smoke and chlorpyrifos modulate the EBV life cycle, favoring lytic activation with potential consequences in carcinogenesis.

P-30-096
Cellular communication network factor 2 regulates cell growth and proliferation in tamoxifen-resistant MCF7 cells via c-MYC

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Tamoxifen resistance remains a major challenge in the treatment of estrogen receptor (ER)-positive breast cancer, affecting approximately one-third of patients. Cellular communication network factor 2 (CCN2), an extracellular matrix-associated protein,

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is involved in tumor development and progression. Although CCN2 has been implicated in tamoxifen resistance, the specific molecular mechanisms underlying its role in cancer cell proliferation remain poorly understood. c-MYC, a transcription factor regulating cellular growth, proliferation, and metabolism, is frequently dysregulated in cancer progression. In this study, we, for the first time, propose the CCN2–c-MYC signaling axis as a key regulator of tamoxifen-resistant breast cancer cell proliferation. CCN2 expression was upregulated in tamoxifen-resistant MCF-7 (TAMR-MCF-7) cells compared to parental MCF-7 cells. To investigate its functional role, we established CCN2-knockdown TAMR-MCF-7 (TAMR-shCCN2) cells. Notably, cell proliferation, colony formation, and spheroid formation abilities were significantly reduced in TAMR-shCCN2 cells. Furthermore, xenograft assays demonstrated significantly decreased tumor growth in mice implanted with TAMR-shCCN2 cells, confirming the critical role of CCN2 in TAMR-MCF-7 cell proliferation. Mechanistically, c-MYC expression was markedly upregulated at both mRNA and protein levels in TAMR-MCF-7 cells but downregulated in TAMR-shCCN2 cells. Subcellular fractionation analysis revealed reduced nuclear localization of c-MYC in TAMR-shCCN2 cells. Importantly, a direct relationship between CCN2 and c-MYC was confirmed, as CCN2 overexpression in MCF-7 cells led to increased c-MYC expression. Collectively, our findings suggest that CCN2 plays a pivotal role in tamoxifen-resistant breast cancer cell growth and proliferation, at least in part, through the upregulation of c-MYC.

P-30-097
Evaluation of prognostic nutritional index (PNI) and hemoglobin-albumin-lymphocyte-platelet (HALP) index in breast cancer

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Systemic inflammation and nutritional status are key to cancer progression and prognosis. The prognostic nutritional index (PNI) and the hemoglobin-albumin-lymphocyte-platelet (HALP) index reflect an impaired immune-nutritional balance and have been shown to be important in various malignancies. The aim of this study was to evaluate the clinical significance of these indices in breast cancer (BC) patients and to investigate their potential prognostic value compared to healthy subjects. A total of 55 BC patients and 50 healthy controls were included in this case-control study. The patients with breast cancer were classified according to the TNM stage and molecular subtypes. Serum hemoglobin, albumin, lymphocyte, and platelet levels were measured, and PNI and HALP indices were calculated with these values. Both patient-control comparisons and comparisons were made in groups formed according to TNM stage and molecular subtypes of BC patients. Preliminary analyses indicate that both the PNI and HALP indices are significantly lower in breast cancer patients compared to healthy controls (both $p < 0.001$). Subsequent analysis of the data in BC patient subgroups revealed that Stage I patients exhibited the highest PNI (62.25 ± 6.74) and HALP (86.41 ± 23.41) values, while Stage IV patients demonstrated the lowest PNI (48.02 ± 3.99) and HALP (26.63 ± 9.59) values. A similar trend was observed when the analysis was restricted to molecular subtypes. Luminal subtype patients exhibited the highest PNI (59.22 ± 4.43) and HALP (63.62 ± 24.87) values, while TNBC patients demonstrated the lowest PNI (46.08 ± 3.23) and HALP (24.34 ± 8.46) values. PNI and HALP decrease with the advancing TNM stage, highlighting their potential as prognostic markers in BC. Their association with molecular subtypes suggests additional clinical relevance in patient stratification and treatment planning. Further research is needed to validate these findings.

P-30-098
Potential role of DNA methyltransferase inhibitors in targeting GSTP1 in drug-resistant cancers

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Glutathione S-transferase P1 (GSTP1) plays a crucial role in cancer drug resistance by detoxifying chemotherapeutic agents and regulating oxidative stress responses. DNA methyltransferases (DNMTs) are key epigenetic regulators in cancer therapy, and their inhibitors can reprogram gene expression to enhance treatment sensitivity. However, increased GSTP1 activity in drug-resistant cancers may reduce the efficacy of DNMT inhibitors. Therefore, this study aims to investigate the potential of DNMT inhibitors to modulate GSTP1 activity. Molecular docking simulations were conducted using the crystal structure of GSTP1 (PDB ID: 2GSS) to evaluate the binding affinity of selected DNMT inhibitors (SGI-110, RG108, zebularine, decitabine, and 5-azacytidine) compared to the reference GSTP1 inhibitor, ethacrynic acid. Binding affinities were calculated using AutoDock Vina, and molecular interactions were analyzed with Discovery Studio. Among the tested compounds, SGI-110 exhibited the highest binding affinity (-8.5 kcal/mol), followed by RG108 (-8.1 kcal/mol), both surpassing ethacrynic acid (-6.6 kcal/mol). Additionally, 5-azacytidine, decitabine, and zebularine showed lower binding affinities (-5.9, -5.7, and -5.5 kcal/mol, respectively). Interaction analysis revealed that SGI-110 and RG108 formed multiple hydrogen bonds and attractive charge interactions with key GSTP1 residues, whereas ethacrynic acid showed fewer interactions, mainly through hydrogen bonding. These findings suggest that DNMT inhibitors may influence GSTP1 activity, highlighting their potential in overcoming drug resistance in cancer therapy. Further *in vitro* and *in vivo* studies are needed to validate these interactions and explore their therapeutic implications. *The authors marked with an asterisk equally contributed to the work.

P-30-099
ATF4 modulates redox homeostasis and ferroptosis sensitivity to sustain tumor progression in tamoxifen-resistant breast cancer

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Breast cancer is the most common malignancy among women worldwide and a leading cause of cancer-related mortality. While

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tamoxifen initially improves outcomes in estrogen receptor (ER)-positive breast cancer, many patients eventually develop resistance, limiting its therapeutic efficacy. This resistance is often associated with dysregulated cellular stress responses. Activating transcription factor 4 (ATF4), a stress-responsive transcription factor, plays a critical role in cellular adaptation to stress, contributing to drug resistance, survival, and metabolic reprogramming in cancer. In this study, we, for the first time, investigated the role of ATF4 in tamoxifen-resistant ER-positive breast cancer cells (TAMR-MCF-7). Our findings reveal that ATF4 is significantly upregulated in TAMR-MCF-7 cells compared to parental MCF-7 cells, along with increased expression of ATF4-regulated target genes, including SLC7A11 and others involved in amino acid metabolism, oxidative stress regulation, sulfur metabolism, and glutathione metabolism. Furthermore, ATF4 knockdown markedly reduced cell growth, colony formation, and migration in TAMR-MCF-7 cells. Xenograft assays demonstrated reduced tumor weight and volume in mice implanted with ATF4-knockdown TAMR-MCF-7 cells, further supporting the critical role of ATF4 in sustaining an aggressive phenotype. Mechanistically, TAMR-MCF-7 cells exhibited lower basal ROS levels, and ATF4 knockdown significantly increased both ROS levels and lipid peroxidation. Moreover, TAMR-MCF-7 cells were more sensitive to ferroptosis inducers such as Erastin and RSL3 compared to MCF-7 cells. Similarly, ATF4 transient overexpression in MCF-7 cells enhanced sensitivity to ferroptosis inducers. In conclusion, our findings demonstrate that ATF4 acts as a key regulator of both the aggressive phenotype and ferroptosis sensitivity in tamoxifen-resistant breast cancer, highlighting its potential as a therapeutic target in overcoming resistance.

P-30-100
Comparative analysis of methodological approaches for the investigation of intratumoral bacteriome profiles: a pilot study

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Intratumoral microbiota is intensively studied, especially in oncological disorders of the gastrointestinal tract such as colorectal cancer (CRC) and pancreatic cancer (PC). The bacteriome of intratumoral tissue is considered an etiopathogenetic factor of CRC, while the role of the pancreatic bacteriome is still questionable due to controversies in the literature. This study aimed to compare two methodological approaches for the investigation of intratumoral bacteriome. Paired samples of tumor and adjacent tissue in a cryopreservation medium from 10 patients with CRC and 10 with PC were analyzed. Each sample was cut in two and DNA was isolated using the Gentra kit from one half and the AllPrep DNA/RNA kit from the other. All 80 resulting samples, along with negative (DNA-free H_2O; NC) and positive (commercial mock community) controls were then analyzed by qPCR for quantification of total bacterial DNA and by 16S rRNA amplicon sequencing. The concentration of total bacterial DNA differed from NCs for every group of sample/kit combinations; there were no significant differences between NCs isolated using the two kits. The alpha diversity of PC samples was comparable with NCs, while CRC samples differed from NCs. However, the median Shannon index as well as the concentration of bacterial DNA in Gentra NCs were significantly higher than in AllPrep NCs, which suggested that Gentra kit might be burdened with bacterial contamination. Multiple bacterial genera, including *Fusobacterium*, were present in Gentra NCs. Relative abundance of *Fusobacterium* was higher in the tumorous tissue from CRC patients than in the adjacent one but this difference was significant only in samples isolated using the AllPrep kit. In matrices with a low abundance of microbial DNA, a proper choice of the isolation kit and a system of internal controls are crucial to ensure the accuracy of the results, especially when bacterial contaminants include potentially disease-associated genera.

P-30-101
Effect of PPAR-gamma agonists and chemotherapy combination on differentiation-related EMT pathway in breast cancer

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Studies have shown that the plasticity associated with EMT (epithelial-mesenchymal transition) can facilitate the differentiation of breast cancer cells into adipocytes, leading to the differentiation of functional adipocytes. However, the effects of combining agents commonly used in breast cancer treatment with this differentiation process remain unknown. This study aims to analyze the effects of rosiglitazone (Rosi), a PPAR-γ agonist, trametinib (Tram), a MEK inhibitor, and their combination with the conventional chemotherapeutic agent paclitaxel (PTX) on the EMT pathway and differentiation in breast cancer. PyMT-1099, primary breast cancer cell line isolated from breast tumor of MMTV-PyMT transgenic mice and pre-adipositic 3T3-L1 cells were used in this study. Six study groups were established: Negative control; TGFβ; PTX; TGFβ + Rosi + Tram; Rosi + Tram; and TGFβ + Rosi + Tram + PTX. Western blot analysis was performed to evaluate the expression of EMT markers, such as E-Cadherin, N-Cadherin, HIF-1α, Snail, Slug, and Twist. The characteristics of the differentiated adipocytes were compared using Oil Red O staining and adiponectin measurement. N-Cadherin, Twist, Slug and HIF-1α expression levels were lowest in the Rosi + Tram group compared to the negative control group. Snail, a key regulatory transcription factor of EMT, was significantly upregulated in the TGFβ group compared to the negative control. E-Cadherin expression was highly increased in the TGFβ + Rosi + Tram + PTX group, while N-Cadherin levels remained unchanged across all groups. As a result, it has been shown that the combination of rosiglitazone and trametinib reduces EMT

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transcription factors, while adding paclitaxel to this combination does not increase EMT transcription factors. However, it significantly increased E-cadherin expression, leading to the suppression of EMT. The research presented in this study was funded by the BAP Project Support of Istanbul Medipol University (Project Number: 2021-05).

P-30-102
Effects of albumin and vitamin K2 combination on human hepatoma cells

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Hepatocellular carcinoma (HCC) is the most frequent type of liver cancer, comprising about 90% of all cases. Its development is affected by lifestyle, environmental, and genetic factors, and the prognosis differs markedly based on the cancer stage and is influenced by various factors, including tumor size, blood levels of α-fetoprotein (AFP), and albumin. Albumin (ALB) is the most abundant blood protein and has multiple functions. Vitamin K2 (VK2) functions in blood coagulation as a prothrombin cofactor, also demonstrates HCC effects, such as promoting cell death and suppressing tumor growth. However, the precise mechanisms underlying these actions are unclear. VK2 and ALB thus have significant HCC growth effects. Although both exhibit individual effects on HCC cell proliferation, their combined impact on HCC progression and metastasis requires investigation. The effects of ALB, VK2, and their combination were investigated through the MTT assay assessed cell proliferation, wound healing assays evaluated migration as a surrogate for invasion, the expression levels of AFP were analyzed via western blotting, apoptosis and cell cycle were examined using flow cytometry, and the expression levels of various EMT markers were quantified using qPCR. We hypothesized that the combination of ALB and VK2 is linked to metastasis, proliferation, and biomarker AFP expression levels in HCC. Combination of ALB and VK2 significantly inhibited the proliferation and migration of human HCC cell lines and caused a reduction in AFP expression levels in these cell lines. ALB and VK2 thus have a potentially important role as inhibitory agents in the progression of HCC, influencing cellular proliferation and metastasis. The combination of ALB and VK2 in HCC cell lines has shown a promising additive effect, inhibiting both tumor growth and migration (a metastasis precursor). Furthermore, this combined treatment led to a significant decrease in the expression levels of AFP in these cell lines.

P-30-103
Silver nanoparticles reveal properties to interfere with oestrogen-signalling in ER+ cancers

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Silver nanoparticles (AgNPs) are the most widely used inorganic nanomaterial in industry. However, the current state of knowledge shows that AgNPs are able to pass through biological barriers and deposit inside human tissues, which raises concerns about the long-term consequences of exposure to AgNPs. In particular, this may include disruption of intracellular oestrogen-dependent signalling. This is attributable to the multiple mechanisms through which ERα/β can be activated and the pivotal role it plays in regulating proliferation or epithelial-mesenchymal transition processes in ER + tumours. The results demonstrate that AgNPs induce an acceleration of proliferation and a transformation of the phenotype of breast cancer towards a mesenchymal-like state. Moreover, AgNPs result in the activation of the transcription of genes regulated by the cytoplasmic oestrogen receptor. This effect is induced only in cells expressing tumour ERα/β, but independently of hormone ligand binding. This indicates an indirect effect on the oestrogen receptor by modifying the activity of oestrogen receptor phosphorylating kinases (i.e. AKT1/2, ERK1/2, CK2 and RSK1). Expression analysis of the proteins responsible for copper metabolism indicates that the interaction of AgNPs with protein kinases is due to the sustained release of silver ions from the nanomaterial, with the silver ion interfering with processes regulated by copper ions due to the similarity of the valence shells of these elements. In conclusion, AgNPs have the potential to activate estrogen-dependent signalling in an ER + tumour model. The molecular mechanism for this effect is an interaction between sustained-release silver ions and kinases whose direct substrate is the oestrogen receptor. The results show that AgNPs can potentially be classified as EDCs (endocrine disrupting chemicals).

P-30-104
A novel approach to selectively increasing the sensitivity of tumor cells to DNA-damage therapy by inhibiting pre-mRNA splicing

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The decreasing efficiency of anti-tumor therapy remain one of the major challenges in oncology. In this context, the strategy of selective increase in sensitivity of tumor cells to chemotherapy appears to be a promising approach. We have previously shown that splicing inhibitors targeting the SF3B1 factor can enhance the sensitivity of tumor cells to DNA-damaging agents. However, the molecular mechanisms underlying the cytotoxic action of pladienolide B (Pl-B) remain to be fully elucidated. To address this knowledge gap, we conducted a comprehensive analysis of the proteomic and phosphoproteomic profiles of ovarian adenocarcinoma SKOV3 cells following Pl-B treatment, as well as the protein partners of SF3B1. The results demonstrated that Pl-B induced a substantial enrichment of the SF3B1 partner protein pool, concurrently attenuating its interaction with splicing factors and histone acetylation regulators. These changes most likely indicate dissociation of

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SF3B1 from chromatin and a change in the splicing pattern from co-transcriptional to post-transcriptional. In addition to these findings, a decrease in the abundance and phosphorylation level of proteins responsible for chromosome organization, cell cycle regulation, and DNA repair processes were detected. This effect may underlie the enhancement of early replicative stress in response to DNA damage following the action of Pl-B on tumor cells. Notably, Pl-B did not increase the sensitivity of normal FT282 fallopian tube epithelial cells and primary human fibroblasts to DNA-damaging agents. A comprehensive analysis, including assessment of cell cycle, proliferation, replicative stress and DNA repair markers, revealed that normal cells are less sensitive to Pl-B at low concentrations and have a greater ability to adapt to the splicing modulation compared to cancer cells, thereby preventing increased sensitivity to DNA damage. The findings underscore the selective potential of splicing inhibitors in cancer treatment.

P-30-105
Effects of progesterone-mediated inhibition of large-conductance voltage- and Ca^{2+}-activated potassium channels on the cell cycle in human glioblastoma cells

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Progesterone (P) is a potent regulator of many cellular processes. In glioblastoma multiforme (GBM) it may affect cell growth and proliferation. Nevertheless, there persists many gaps in understanding the molecular mechanisms behind these effects. In this study, we analyzed the effect of P levels on the activity of large-conductance voltage- and Ca^{2+}-activated potassium channels (gBK) and cell cycle of the human U87-MG cells. To verify the effect of ion channel activity on cell cycle progression, we treated U-87 MG cells with different concentrations of P (0-25 μg/ml) and gBK channel inhibitor paxilline (0-4 μM) for 24 or 48 h. Then we analyzed the cell cycle using flow cytometry. We also examined the gBK channel activity in presence of P and the appropriate controls using single-channel mode of patch-clamp technique at membrane depolarization. The obtained patch-clamp recordings indicate the gBK channel inhibition by P. At +75 mV, the open state probability decreased from pop = 0.59 ± 0.10 (control) to pop = 0.29 ± 0.13 (low P) and pop = 0.29 ± 0.07 (high P). Cell cycle analysis shows that inhibition of gBK activity is accompanied by cycle arrest of U-87 MG cells in the G0/G1 phase. According to the literature, gBK channels can promote glioblastoma growth, therefore, their inhibition may lead to inhibition of tumor growth. Our study supports this hypothesis, as a decrease in the activity of gBK channels is accompanied by a significant increase in the number of U-87 MG cells in the G0/G1 phase. The percentage of U-87 MG cells in the G0/G1 phase is dependent on P concentration suggesting that P concentration is an important determinant of glioblastoma growth.

P-30-106
Effects of 17β-estradiol-mediated inhibition of large-conductance voltage- and Ca^{2+}-activated potassium channels on the cell cycle in human glioblastoma cells

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Glioblastoma (GBM) is the most aggressive primary brain tumor, with incidence, therapy outcomes, and survival rates favoring females over males. To better understand this sex bias in GBM biology, we investigated the effects of 17β-estradiol (E2) on the activity of large-conductance voltage- and Ca^{2+}-activated potassium channels (gBK) and cell cycle progression in human U87-MG glioblastoma cells. Specifically, we explored whether E2-mediated functional changes in these key K channels influence cell-cycle progression. Notably, this aspect of GBM biology remains relatively poorly understood. To verify the effect of ion channel activity on cell cycle progression, we treated U-87 MG cells with different concentrations of E2 (0-1800 ng/ml) and gBK channel inhibitor paxilline (0-4 μM) for 24 or 48 h. We then analyzed the cell cycle using flow cytometry. We also examined the gBK channel activity in presence of E2 and the appropriate controls using single-channel mode of patch-clamp technique at membrane depolarization. Cell cycle analysis showed that the presence of E2 increases the number of U-87 MG cells in the G0/G1 phase. The observed effect is dependent on the concentration of E2. In addition, the obtained patch-clamp recordings indicate the gBK channel inhibition by E2. At +75 mV, the open state probability decreased from pop = 0.59 ± 0.10 (control) to pop = 0.47 ± 0.13 (low E2) and pop = 0.30 ± 0.09 (high E2). Literature data indicate that the effect of channel activity on cell cycle progression is dependent on cell type and origin. Our studies indicate that inhibition of gBK channel activity can lead to cycle arrest of U-87 MG cells in the G0/G1 phase. The observed changes in the behavior of U-87 MG cells are dependent on E2 concentration suggesting that estrogen levels are important for U-87 MG cells.

P-30-107
E-cadherin dysfunction shapes nucleus composition and function determining cell migration

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Hereditary diffuse gastric cancer (HDGC) is an autosomal dominant cancer syndrome caused by germline variants in the *CDH1* gene, which encodes the cell-cell adhesion molecule E-cadherin. Loss or dysfunction of E-cadherin is strongly

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associated with the ability of cancer cells to diffusely invade the gastric wall and disseminate into the peritoneal cavity and adjacent organs, although the molecular mechanisms that drive this invasive behaviour remain poorly understood. We propose that the impairment of E-cadherin activates a mechanotransduction program that leads to nuclear remodelling, ultimately promoting a pro-invasive cellular signature. To address our hypothesis, we have first established an *in vitro* model of transfected cell lines expressing either wild-type E-cadherin or the Y755C variant, identified in a family fulfilling HDGC clinical criteria. Cell fractionation and proteomic analysis of enriched nuclear extracts from wild-type and mutant contexts unveiled significant alterations in nuclear proteins of Y755C mutant cells, when compared to wild-type counterparts. In particular, from the nine nuclear proteins detected as abnormally expressed, four were downregulated and five were upregulated. A multi-step approach, comprising confocal microscopy and western blot, was employed to assess the expression levels and cellular distribution of candidate molecules. Accordingly, ADARB1, THAP4, and PPP1R14B were consistently altered, suggesting their engagement in a pathway triggered by E-cadherin dysfunction. Overall, these findings indicate that loss of E-cadherin function induces changes in the abundance of key nuclear proteins, which can affect nuclear plasticity during cell migration across the extracellular matrix. Understanding these molecular alterations could open new avenues for therapeutic interventions in E-cadherin-mediated gastric cancer.

P-30-108
Targeting KDM6A and KDM6B to regulate tumor progression in IDH1-mutant gliomas

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Gliomas, 80% of malignant brain tumors, are highly aggressive. A subset harbors IDH1 mutation, leading to a hypermethylated phenotype. This study investigates the roles of lysine demethylases KDM6A and KDM6B in glioma progression. Using CRISPR/Cas9, we co-depleted KDM6A and KDM6B in patient-derived IDH1-mutant glioma cells (MGG119, MGG152, TS603) and assessed phenotypic and transcriptomic changes. Loss of KDM6A/6B reduced growth rate and sphere-forming capacity. Glioma cells were arrested at G1, accompanied by apoptosis induction as gauged by Annexin V staining. Western blotting of histone extracts revealed increased H3K27me3 levels upon KDM6A/6B loss. RNA sequencing demonstrated the inhibition of cellular differentiation-related genes and activation of TGF-β signaling. Stress-response genes were upregulated but were insufficient to counteract epigenomic changes exacerbated by IDH1-mutation and KDM6A/6B depletion. Functional experiments with the chemical intervention of TGF-β signaling showed that inhibition partially rescued the phenotype. OLIG1 and OLIG2, whose expression was previously associated with gliomagenesis, were significantly decreased, which was accompanied by increased methylation at those regions as examined by Cut&Run assays. Our current efforts are directed towards further analysis of OLIG1/2 requirements in the KDM6A/6B phenotype through *in vitro* and *in vivo* assays. We show that TS603 cells form readily growing tumors, confirmed via *in vivo* imaging system (IVIS) and histological characterization. Our findings identify KDM6A/6B as key regulators of differentiation in IDH1-mutant gliomas and potential epigenetic therapy targets. Our study aligns with the clinical findings indicating that IDH1-mutant gliomas often become more aggressive after conventional therapies, losing DNA methylation. Therefore, our study highlights epigenetic modulation as a potential therapeutic strategy. Work suported by TUBITAK (219S882) and BIDEB (2211-A).

P-30-109
A set of circular RNAs may modulate S6K2 activity which affects breast cancer cell survival

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S6K2 (S6 Kinase 2), an PI3K/Akt/mTOR signaling effector, contributes to breast cancer cell survival via different routes including positive feedback modulation of Akt [1]. There are still several unknown mechanisms about the post-translational regulation of S6K2 protein. Circular RNAs are a new group of non-coding RNAs which modulate the target proteins either directly by binding them and affecting their activity, stability and subcellular localization or indirectly by sponging microRNAs which regulate the target proteins [2]. Our bioinformatics analysis revealed that a set of 8 circular RNAs (hsa_circ_0023089, hsa_circ_0023090, hsa_circ_0023091, hsa_circ_0023092, hsa_circ_0023093, hsa_circ_0023094, hsa_circ_0023095, hsa_circ_0023096) may target S6K2 (www.circbase.org and circinteractome.irp.nia.nih.gov). To experimentally validate the interaction between S6K2 and circular RNAs, RNA immunoprecipitation will be performed. S6K2-DDK will be overexpressed in MCF-7 cells and will be immunprecipitated using anti-DDK Immunoprecipitation kit. RNAs will be isolated from the immunprecipitates and qRT-PCR will be performed to determine the S6K2-interacting circular RNA(s) using the circRNA-respective primers. Any interacting circular RNA will be overexpressed and the activity of S6K2 will be investigated by western blot using anti-p-S6 antibody. The stability of S6K2 upon respective circRNA overexpression will be tested using cycloheximide (CHX) treatment. Moreover, the ubiquitination pattern of S6K2 upon respective circRNA overexpression will be investigated by western blot by anti-Ub antibody. Also, the effect of respective circRNA overexpression on the proliferation and TNF-induced apoptosis of MCF-7 cells will be tested [3]. References: [1] Sever NI and Cengiz Şahin S (2019) Future Oncol 15, 95-102. [2] Zhou WY et al. (2020) Mol Cancer 19, 172. [3] Tan Y et al. (2019) Mol Carcinog 58, 875–886.

P-30-110
Effects of cannabidiol on lung cancer cell models

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Cannabinoids are naturally occurring compounds found in the *Cannabis sativa* plant. Cannabidiol (CBD) is the second most abundant cannabinoid found in this plant. Clinical research on

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CBD includes studies on anxiety, cognition, movement disorders, chronic pain and cancer, but there is insufficient knowledge on the underlying mechanisms that make cannabidiol a potential treatment for these disorders. In recent years the focus on the studies on CBD is on its profound anti-tumor activity. The objective of the study is to uncover the various complex mechanisms preceding the death (either programmed or not) of the model lung cancer cell lines after CBD treatment. Our first task was to evaluate the cytotoxicity of CBD on two model lung cancer cell lines – A549 (p53+, adenocarcinomic human alveolar basal epithelial cells) and H1299 (p53-, human non-small cell lung carcinoma cell line derived from the lymph node). These preliminary results gave us the different IC_{50} values (Half-maximal inhibitory concentrations) with which we continued our study. Then we proceeded to use flowcytometry to assess the type of cell death after CBD treatment, to obtain data on its effect on the cell cycle and to evaluate whether this treatment affects the mitochondrial membrane potential. After acquiring these results, we used an array of fluorescent and immunofluorescent techniques to analyze the activation of different proteins after treatment and its effect on the proliferation and overall ROS (reactive oxygen species) accumulation followed by DNA/RNA damage. These experiments showed us that CBD has a wide array of effects on our model cell lines. In both cell lines it induces apoptosis, inhibition of proliferation and cell cycle arrest, but its effects differ in terms of ROS accumulation and subsequent DNA/RNA damage. Our data suggests that CBD has a promising potential for usage in adjuvant therapy for lung cancer treatment.

P-30-111
Induction of ferroptosis/apoptosis by dimethyl fumarate in cervical cancer cell models through modulation of redox-related pathways

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Persistent HPV infection is the primary cause of cervical cancer. HPV-mediated transformation relies on viral proteins that by interacting with proteins of the host cells (e.g. p53) dysregulate pathways essential to prevent tumor progression. Failure of conventional treatments (e.g. cisplatin) is frequently observed in cervical cancer, rendering necessary novel therapeutic approach. Dimethyl fumarate (DMF), an FDA-approved anti-inflammatory drug, exhibits potential anti-tumor activity. We explored its role in induction of ferroptosis, a lipid peroxidation-dependent cell death associated to iron accumulation. We tested the effectiveness of canonical ferroptosis inducers [buthionine sulfoximine, erastin, RSL3, and sulfasalazine] and DMF in cervical cancer cell lines (SiHa and C4I). Gene expression analysis, lipid peroxidation, malondialdehyde, and glutathione assays confirmed that DMF induces ferroptosis in a dose-dependent manner. Using SiHa and C4I 3D multicellular tumor spheroid systems, we demonstrated that DMF reduces spheroid size in a concentration-dependent manner. Combined therapy is used to enhance efficacy and reduce adverse effects in clinical practice. Therefore, we evaluated the effects of co-administration of sub-cytotoxic doses of DMF (100 μM) with a dose of cisplatin (CDDP) lower than IC_{50} (20 μM). The results indicated that this combination reduces cell viability and induces both ferroptosis and apoptosis in SiHa and C4I cell lines. As p53 is a key regulator of these processes, we tested the activation of p53 pathway. The results demonstrated that DMF/CDDP combination reactivates p53 that in turn could downregulate SLC7A11 expression, hence fostering ferroptosis and/or apoptosis. In addition, we demonstrated that DMF/CDDP treatments can suppress the growth of SiHa and C4I spheroid models. These findings suggest the possibility to repurpose DMF as a drug to be used alone or in combination with cisplatin to improve the effectiveness of anticancer treatments.

P-30-112
The effectiveness of NLR, PLR, and SII indices in predicting survival for male patients with advanced laryngeal cancer

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Laryngeal cancer is one of the most common malignancies in the head and neck region. Prognosis assessment is done with the tumor-nodes-metastasis (TNM) staging system. The TNM system has some shortcomings. Therefore, additional biomarkers are needed to predict individual patient prognosis. Neutrophil-to-lymphocyte ratio (NLR), platelet-to-lymphocyte ratio (PLR) and systemic immune-inflammatory index (SII) are potential prognostic markers. The aim of this study was to investigate the effect of NLR, PLR and SII indices on survival in patients diagnosed with laryngeal cancer and to determine the prognostic value of these biomarkers. The findings may contribute to the risk stratification of laryngeal cancer patients and to the determination of treatment strategies. SII, NLR and PLR were calculated from preoperative hemogram tests. Cox regression analyses and Kaplan-Meier survival analyses were performed. A total of 35 advanced male patients were included in the study. In multivariate analysis, extralaryngeal invasion (HR = 3.76 p = 0.041) and recurrence (HR = 12.85 $p < 0.001$) were found to be independent prognostic factors for survival. However, no significant relationship was found between NLR, PLR and SII indices and survival (NLR p = 0.323 PLR p = 0.349 SII p = 0.403). This study shows that the most important factors affecting survival in laryngeal cancer patients are lymph node metastasis, perinodal spread, extralaryngeal invasion and recurrence. Recurrence was determined to be the strongest independent risk factor in predicting mortality. These data were consistent with the existing literature. However, the lack of a significant relationship between NLR, PLR, SII and survival may be due to factors such as inconsistency with the literature, small sample size, the stages of the patients, the pathological characteristics of the tumor and whether the patients received preoperative treatment or not, and the different autoanalyzer used for hemogram parameters. *The authors marked with an asterisk equally contributed to the work.

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P-30-113
The effectiveness of NLR, PLR, and SII indices in predicting survival for non-laryngeal head and neck tumors

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Head and neck cancers are a major cause of morbidity and mortality. In recent years, the role of systemic inflammation in tumor development and metastasis has received increasing attention, and hematological inflammatory markers have been considered as prognostic factors in head and neck cancers. The neutrophil-to-lymphocyte ratio (NLR), platelet-to-lymphocyte ratio (PLR), and systemic immune-inflammation index (SII) are simple indicators of systemic inflammation and have prognostic significance in many solid tumors In this study, the efficacy of NLR, PLR, and SII in predicting survival in patients with non-laryngeal head and neck tumors was evaluated. SII, NLR and PLR were calculated from preoperative hemogram tests. Cox regression analyses and Kaplan-Meier survival analyses were performed. A total of 45 patients were included in the study. The distribution of tumor localization was as follows: oral cavity (48.5%), lips (21.2%), skin (15.2%), oropharynx (9.1%) and parotid (6.1%). In multivariate analysis, the only variable independently associated with survival was perinodal spread ($HR = 14.037$; $p < 0.001$). The effect of NLR, PLR and SII values on survival was not statistically significant. This study evaluated prognostic factors related to survival in non-laryngeal head and neck tumors. The findings suggest that perinodal spread is an independent prognostic factor for survival. NLR, PLR and SII did not have a significant effect on survival, which may be due to the small number of patients, the stage of the patients, the pathological characteristics of the tumor and the lack of knowledge about whether the patients received preoperative treatment. In conclusion, it is suggested that treatment strategies for patients with perinodal spread should be planned more aggressively. *The authors marked with an asterisk equally contributed to the work.

P-30-114
Analysis of the anti-cancer effects and alkaloid content of an *Amanita muscaria* extract – preliminary results

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In recent years, increasing attention has been directed toward the evaluation of various plant and fungal extracts promoted for their health-enhancing and potential medicinal properties. Fungi are receiving more and more attention for their various actions, including against very serious conditions such as cancer, and a lot of species' potential medicinal qualities and the compounds behind them are just now being described. Despite its reputation for toxicity due to the presence of the neuroactive alkaloids ibotenic acid and muscimol, the fungus *Amanita muscaria* has garnered anecdotal interest for its possible therapeutic effects. Prompted by reports of patients consuming *A. muscaria* water-ethanol extracts (tinctures) in attempts to combat lung cancer, we investigated the cytotoxic activity of such an extract on the human lung cell lines MRC-5, H1299 and A549 using the MTT cytotoxicity assay. Results showed IC_{50} values for *in vitro* cytotoxicity of or below 0.5% (*v*/*v*) across all tested cell lines. Further analysis through flow cytometry and EdU incorporation assays revealed a significant inhibition on cell proliferation and cell cycle arrest in the treated cells. Additionally, using an EU incorporation assay, we observed the formation of nucleoprotein stress granules in the cytoplasm of the treated cells. In light of these preliminary findings, we performed a number of analytical chemistry techniques – HPLC, CZE/CCD, and UHPLC-MS/MS – to evaluate the content of ibotenic acid and muscimol in the *A. muscaria* water-ethanol extract. Our results indicate that these neuroactive alkaloids are present only in negligible amounts in the tincture. Our findings suggest that the *A. muscaria* water-ethanol extract exhibits promising anticancer activity against lung cancer cells *in vitro* while containing minimal levels of neurotoxic compounds. These results support further investigation into its potential therapeutic applications.

P-30-115
Establishment of an orthotopic patient-derived meningioma model for interrogation of the tumor microenvironment

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Meningiomas are the most common primary intracranial tumors, with the majority being WHO grade 1 tumors. WHO grades 2 and 3 tumors are increasingly aggressive and characterized by invasiveness, recurrence, and poor clinical outcomes. The tumor microenvironment most likely plays an important role in aggressiveness but is poorly understood in meningiomas. This study aims to establish an orthotopic patient-derived (PD) meningioma model to explore how the tumor microenvironment influences aggressiveness and identify potential therapeutic targets. Driven by this goal, primary tumor cells from meningiomas of different WHO grades were purified and implanted intracranially into NMRI nude mice to study tumor formation and the tumor microenvironment. The Grade 3 IOMM-Lee cell line was used as a control. Mice were closely monitored for the onset of illness, and brains were subsequently collected, sectioned, and analyzed using immunohistochemistry. Additionally, tissue samples are being collected for single-nucleus RNA sequencing (snRNA-seq) and spatial transcriptomics (ST)

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to further investigate molecular profiles. Preliminary analysis of brains implanted with the IOMM-Lee cell line revealed a 100% tumor take rate, with high Ki67 expression, indicative of significant proliferation. A significant presence of microglia and macrophages (IBA1, CD204) was also observed. Current efforts are focused on establishing PD orthotopic xenografts through the implantation of tumor cells from patients with WHO grade 1-2 meningiomas. This model will provide valuable insights into the cellular composition, heterogeneity, and tumor-immune cell interactions, including receptor-ligand dynamics. Ultimately, it will uncover potential therapeutic targets for meningioma patients. This project is supported by the European Union's Horizon 2020 research and innovation programme under the Marie Sklodowska-Curie grant agreement No. 945322 (LEAD) and Lundbeck Foundation.

P-30-116
Interaction of ZEB1 with VEGFA, and MAGE-D2 in uveal melanoma

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Uveal melanoma (UM) develops from melanocytes of the uveal tract and makes up about 5% of all melanoma cases The spread of UM cells is hematogenous and the largest number of UM metastases is detected in the liver. There is a poor understanding of the role of epithelial–mesenchymal transition (EMT) in UM progression. By analogy with other tumours, it can be assumed that targeting of regulators of this process may reduce the growth of the tumour node and metastasis spread. Since hypoxia is a major stimulator of EMT, we investigated the interaction of proteins under the control of the hypoxia-inducible factor 1α (HIF-1α): ZEB1, VEGFA and melanoma antigen family gene D2 (MAGE-D2). Ten UM cell lines were used as models. ZEB1-hyperexpression cell line was derived from ZEB1-deficient cell line using lentiviral transduction. We found that hyperexpression of ZEB1 mRNA in transgenic cells stimulated a 30-fold increase in mRNA VEGF receptor level relative to parental cells. We constructed correlation curves between ZEB1, MAGE-D2 expression and VEGFA secretion under normoxia and hypoxia. Interestingly, all cultures examined had the phenotype of E-cadlow/N-cadlow, and ZEB1 mRNA levels differed significantly. All UM cells expressed MAGE-D2 mRNA under normoxia conditions, and MAGE-D2 mRNA was also increased under hypoxia. Human UM cells are more typical by the expression of MAGE-D2 mRNA than ZEB1. MAGE-D2 may be a target for anti-UM therapy. ZEB1 may be involved in the development of UM cell resistance to anti-VEGF drugs. The study was supported by the grant of the Russian Science Foundation 23-14-00285.

P-30-117
Silencing NLRP1 promotes ferroptotic cell death in triple negative breast cancer cells

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The overexpression of inflammasome components has been observed in cancer. Therefore, it remains an open question whether inflammasome activation contributes to the ability of cancer cells to evade different types of cell death. In this study, we aimed to investigate the potential link between nucleotide-binding and oligomerization domain (NOD)-like receptor protein-1 (NLRP1) and ferroptotic cell death in human triple-negative breast cancer cells. To this end, NLRP1 was silenced using small interfering RNA (siRNA) in MDA-MB-231 cells. The efficacy of silencing was confirmed by western blot analysis. Cell viability was assessed using the MTS/PMS assay. Ferroptosis was evaluated by measuring lipid peroxide levels and glutathione peroxidase 4 (GPX4)/glutathione (GSH) levels in NLRP1-silenced cells in the presence or absence of the ferroptosis inhibitor, ferrostatin-1. The responses of NLRP1-silenced cells were compared to those of cells treated with the ferroptosis activator, erastin. Suppression of NLRP1 expression induced cell death in MDA-MB-231 cells. Additionally, GPX4 activity and GSH levels were significantly decreased in NLRP1-silenced cells ($p < 0.05$). However, treatment with ferrostatin-1 reversed the effects of NLRP1 silencing by reducing lipid peroxide levels and restoring GPX4 activity. Our findings demonstrate that NLRP1 may play a crucial role in regulating cell death mechanisms, including ferroptosis. These results highlight the need of novel agents targeting NLRP1 inflammasome activation in cancer therapy. This study was supported by the TÜBITAK 1001-Scientific and Technological Research Projects Support Program (Project No. 122S667).

P-30-118
Development of innovative multifunctional nanocarriers for doxorubicin-resistant breast cancer

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Fucoidan (FUC), produced from brown algae, has anti-tumor effects. Tariquidar (TRQ) is used as a p-glycoprotein inhibitor to overcome multidrug resistance. In this study, we aimed to develop multifunctional nanocarriers containing fucoidan for the treatment of doxorubicin (DOX)-resistant breast cancer and to demonstrate their efficacy *in vitro*. DOX was loaded inside the liposomes and TRQ was loaded on the hydrophobic part of the liposomes. FUC was coated on the surface of the liposomes by ionic binding. Resistance development in cells was evaluated by RT-PCR and western blot analysis of P-glycoprotein (P-gp) and multidrug resistance-associated protein-1 (MRP-1). The effect of

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liposomal agents on apoptotic cell death in MDA-MB-231-R cells was determined flow cytometry. MDA-MB-231 cells developed resistance to DOX at increasing concentrations based on the IC_{50} dose. In MDA-MB-231-R cells, application of liposome constructs composed of DOX, FUC and TRQ decreased cell viability in a time-dependent manner in all groups. Resistance development in the cells was mediated by an increase in P-gp and MRP1in gene expression ($p < 0.005$). In liposomal agents applied to MDA-MB-231-R cells, P-gp protein expression level increased in TRQ-FUC and DOX-FUC groups compared to the control. MRP-1 protein showed higher expression levels in LIPO-DOX, TRQ-DOX-FUC and DOX-FUC groups compared to the control. As a result of LIPO-FUC treatment of MDA-MB-231-R cells, it was determined that there was a 1-fold increase in apoptosis and a 7-fold increase in necrosis compared to the control. In the LIPO-DOX group, there was a 50-fold increase in necrosis. While there was no significant increase in apoptosis in the DOX-FUC group compared to the control, there was a significant 50-fold increase in necrosis. Doxorubicin resistance was developed in triple negative breast cancer and a multifunctional nanocarrier was developed and its *in vitro* cytotoxic activity was demonstrated. This study is financially supported by TUBITAK 1001 (Grant No: 121M625).

P-30-119
siRNA-mediated SIRT1 silencing attenuates glioblastoma cells and spheroids' growth, EMT state, and migratory ability by inducing oxidative, ER stress, and apoptotic responses

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Glioblastoma (GB) is a highly malignant primary brain tumor with a poor prognosis. Sirtuin 1 (SIRT1), a NAD^+-dependent deacetylase, plays a crucial role in tumor growth, metastasis, and response to cancer treatments. This study aimed to examine the effects of SIRT1 silencing on glioblastoma cells and spheroids as a therapeutic approach. Herein, small interfering (si)RNA transfection was used to block SIRT1 expression in U-87MG human GB cells. The knockdown efficiency for 2D and 3D cell culture was quantified as 79.7% and 57.6%, respectively. Silencing SIRT1 reduced glioma cell viability and colony formation ability. The silencing of SIRT1 led to a notable decrease in phosphorylated HSF1 levels, as well as Hsp90, Hsp70, Hsp60, and Hsp27 levels. It induced intracellular reactive oxygen species (ROS) levels and ER stress, downregulated the SIRT1/FOXO1/NF-κB axis, and lowered p-ERK1/2 and p-AKT levels. Immunofluorescence labeling showed decreased SIRT1 and NF-κB levels but increased FOXO1 in nuclear localization. Moreover, SIRT1 silencing suppressed EMT, migration, and angiogenesis, potentially causing autophagy and apoptosis, and slowed GB spheroid growth. This study first evaluated the anticancer mechanisms of SIRT1 silencing in both 2D-monolayer cells and 3D-spheroids, highlighting SIRT1 as a promising therapeutic target for GB and suggesting the need for further clinical investigation.

P-30-120
A new role for ZRF1 in breast cancer metastasis via the S100A9-tumor-associated neutrophil axis

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The tumor microenvironment (TME) plays a critical role in breast cancer metastasis and therapy resistance. Tumor-associated neutrophils (TANs) have recently emerged as key players in this process, yet the factors shaping their function remain unclear. S100A9, a well-known regulator of inflammation, has been implicated in metastatic progression, potentially linking TANs to the metastatic cascade. However, how S100A9 regulates TAN behavior and influences breast cancer progression remains largely unexplored. Here, we demonstrate that ZRF1 (Zuotin-related factor 1), a chromatin-associated protein involved in epigenetic gene regulation, controls S100A9 expression in breast cancer cells in a context-dependent manner. ZRF1 depletion alters S100A9 expression based on HER2 and TNBC status, impacting neutrophil adhesion and transendothelial migration. Conditioned medium (CM) collected from ZRF1-depleted cells affects neutrophil function by reducing TAN1 (anti-tumor) markers while leaving TAN2 (pro-tumor) markers unchanged. In sequential co-culture experiments, control neutrophils negatively affect cancer cell viability, whereas those primed by ZRF1-depleted cells lose this effect. Furthermore, breast cancer cell adhesion is significantly altered after co-culture with primed neutrophils, an effect that can be reversed by using neutrophils primed under opposite conditions. These results highlight a previously uncharacterized ZRF1-S100A9-TAN axis in breast cancer metastasis. Whether ZRF1-driven S100A9 expression is sufficient to dictate TAN polarization and metastatic potential requires further investigation.

P-30-121
Dual impact of MAPK inhibition and nutrient stress on translational control and stress adaptation in colorectal cancer cells

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The MAPK-ERK-RSK pathway is a critical regulator of cancer cell adaptation, influencing ribosomal activity and stress responses, while mTORC1 is a well-characterized modulator of translation and also functions as a nutrient sensor. Colorectal cancer cells incubated in a nutrient-deficient (ND) medium consisting of 10% of the nutrients found in complete medium (nutrient rich, NR) activated nutrient-sensing pathways such as inhibition of mTOR, activation of AMPK, and autophagy. Interestingly, we also observed the activation of ERK1/2 signaling in the ND cells. Therefore, we examined how MAPK inhibition of MEK via U0126 affects ribosome function, translation dynamics, and stress adaptation in cancer cells. Polysome profiling revealed that U0126 treatment led to an accumulation of stalled 80S ribosomes and reduced polysome formation, indicating impaired translation initiation. This was reflected in a luciferase assay showing a decrease in 5'CAP-dependent translation. Interestingly, phosphorylated RPS6

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persisted in the 80S fraction despite ERK1/2 inhibition and decreased mTOR signaling, suggesting an alternative mechanism maintaining its activation. LoVo cells exhibited better polysomal integrity compared to HCT-116, implying a differential reliance on ERK-RPS6 signaling. Further analysis showed that U0126 treatment triggered an integrated stress response (ISR), marked by increased ATF4 expression, but not translation through an internal ribosomal entry site (IRES), indicating a shift toward stress adaptation rather than complete translational shutdown. Additionally, ND conditions alone induced nucleolar stress, highlighting the impact of metabolic constraints on ribosome homeostasis. These findings reveal a distinct role for ERK-RPS6 signaling in ribosome regulation and stress adaptation.

P-30-122
The role of miR-27b-5p in the head and neck squamous cell carcinoma

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Head and neck squamous cell carcinoma (HNSCC) is one of the most prevalent and therapeutically challenging cancers worldwide. Recently, many reports indicated that non-coding RNAs might constitute a chance for a breakthrough in cancer therapy and diagnosis. In particular, microRNAs are frequently aberrantly expressed and implicated in processes pivotal for cancer development, e.g., miR-27b, a known epithelial-to-mesenchymal transition (EMT) regulator. The Cancer Genome Atlas (TCGA) expression and patients' clinicopathological data were downloaded from the UCSC Xena and the cBioPortal database to study survival curves, gene enrichment, and potential targets of both miR-27b mature strands using online tools. Cell line models with induced miR-27b expression were subjected to functional tests and transcriptome analysis using RNA-seq. Expression and protein levels of chosen potential targets were assessed via qRT-PCR and/or the western blot method, and the most promising interactions were evaluated using the luciferase assay. In TCGA patients, *miR-27b-5p* had a higher expression level in cancer compared to the normal margin. *In silico* analyses of clinicopathological parameters indicated that elevated 5' strand expression predisposed individuals to more favorable outcomes and better overall survival. Increased *miR-27b-5p* levels were associated with reduced proliferation and negative regulation of the cell cycle, and its' low levels were linked with overexpression of genes implicated in processes promoting cancer growth, e.g., EMT and TGF-β signaling pathway, based on both – TCGA data and cell line experiments. The above emphasizes the significant impact of *miR-27b-5p* on cancer biology. Elucidating its vast regulatory potential might provide a better understanding of HNSCC and new therapeutic approaches. This research was supported by the National Science Centre, Poland, grant: 2021/41/N/NZ5/02966.

P-30-123
Interrogating the essentiality of RBBP7 chromatin modifier in IDH1-mutant glioma

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Gliomas are the most prevalent incurable malignant tumors of the central nervous system, affecting around six per 100 000 individuals. IDH1-mutant gliomas are a distinct subtype of diffuse gliomas characterized by mutations in the isocitrate dehydrogenase 1 (*IDH1*) gene, leading to the accumulation of the oncometabolite *D*-2-hydroxyglutarate. This metabolic reprogramming results in hypermethylated DNA and histone profiles, promoting tumorigenesis and progression while simultaneously creating unique therapeutic vulnerabilities. To explore these epigenetic vulnerabilities, we established parallel Cas9-stable A172 cell lines overexpressing either IDH1-wildtype (WT) or IDH1-R132H mutant (MUT) proteins via lentiviral transduction. Next, using our targeted CRISPR/Cas9-based epigenetic knock-out library (EPIKOL) screen in IDH1-WT and MUT A172 cell lines, we identified retinoblastoma-binding protein 7 (*RBBP7*) – a nuclear chromatin remodeling factor – as a novel epigenetic regulator of IDH1-MUT glioma cell viability *in vitro*. While RBBP7 has been reported to be upregulated in various cancer types, its role in IDH1-MUT glioma remains unexplored. To validate its functional relevance, we assessed the selective vulnerability of RBBP7 knockout (KO) in IDH1-WT and MUT A172 cells, together with primary MGG119 and TS603 glioma cell lines. Loss of RBBP7 resulted in a significant reduction in proliferation and clonogenicity in IDH1-mutant cells. Ongoing studies aim to investigate the impact of RBBP7 KO on cell cycle progression, apoptosis, transcriptomic alterations, and *in vivo* tumor growth. The data obtained so far support further exploration of this chromatin modifier as a potential therapeutic target. *The authors marked with an asterisk equally contributed to the work.

P-30-124
Functional analysis of MSH2 A733T variant of unknown significant mutation effect on human MutS homolog 2 (hMSH2) protein

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Lynch Syndrome is a genetic disease that increases the risk of cancers, including colon cancer, due to loss-of-function mutations in the DNA mismatch repair (MMR) genes: MLH1, MSH2, PMS2, and MSH6. MSH2 and MSH6 form the MutSα complex, which recognizes and repairs DNA mismatches through binding MutLα. When this repair process is disrupted, mutations accumulate by increasing the cancer risk. Our previous study that screened 371 colon cancer patients and 914 healthy individuals in the Turkish population identified multiple variants of uncertain significance (VUS) in MSH2/6 genes, including an Ala733Thr missense mutation in MSH2 [Previously published in: Akcay IM et al. (2021) Int J Cancer 148, 285–295]. Here, we aim to characterize MSH2 A733T and its functional effects at the

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protein level. We initiated our experiments by checking the wild-type and mutant MSH2 and MSH6 transcript levels using qPCR, revealing a direct correlation that suggests transcriptional regulation. After transient transfection to MSH2-deficient LoVo cells, protein levels were analyzed following proteasome inhibitor treatment, to assess the effect of the mutation at the translational level. Since MSH2 mediates DNA damage-induced apoptosis, we examined the functional role of mutant MSH2 protein in apoptosis by treating cells with cisplatin to induce excessive DNA damage, and further analyzed key signaling proteins. We found that mutant MSH2 overexpression resulted in reduced levels of cell death proteins compared to wild type. We also report that the mutant does not affect heterodimerization as determined by a pull-down assay. However, functional studies showed that, the mutant fails to modulate apoptosis as compared to wild type. In conclusion, these findings contribute to the reclassification of MSH2 A733T as pathogenic rather than a VUS. We propose that MSH2 A733T leads to MutSα dysfunction beyond its observed effects, suggesting further investigation into its impact on downstream partner binding.

P-30-125
The effect of postbiotics obtained from *Bifidobacterium bifidum* and *Bifidobacterium breve* strains against oxidative stress developing in colorectal cancer cells

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It is thought that dysbiosis developing in the intestinal microbiota plays an important role in the development of colorectal cancer and oxidative damage. Studies have shown that bifidobacteria can reduce the risk of colorectal cancer and have positive effects on oxidative stress. However, the biotic properties of bacteria called probiotics are strain specific. Postbiotics produced by bifidobacteria also make important contributions to this process. Short-chain fatty acids (SCFAs) are among the most important postbiotics produced by bifidobacteria and it is thought that they may help reduce the risk of colorectal cancer. In our study, the effect of butyrate, one of these SCFAs, on colorectal cancer cells was investigated *in vitro*. In our study, the cytotoxic activity of butyrate (6.25-500 mM) obtained from *B. bifidum* and *B. breve* strains at different concentrations in $CaCO_2$ and HT29 cell lines was investigated by the MTT method. Malondialdehyde, glutathione, total oxidant and total antioxidant levels were evaluated spectrophotometrically as an indicator of oxidative balance. In our study, it was determined that butyrate obtained from *B. breve* strains was more cytotoxic on cancer cells, while it was also observed that butyrate obtained from these strains, especially at 125 and 250 mM concentrations, had positive effects on oxidative stress markers. The data obtained indicate that further research is needed on the effects of bifidobacteria on colorectal cancer. *The authors marked with an asterisk equally contributed to the work.

P-30-126
Targeting ceramide metabolism as a new strategy to overcome drug resistance in myeloid leukemia cells with dysregulated GATA-1 expression

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GATA-1 is a key transcriptional factor in hematopoiesis. Two GATA-1 isoforms, GATA-1_{FL} and a shorter isoform (GATA-1_S), are described. A balanced GATA-1_{FL}/GATA-1_S ratio contributes to control normal hematopoiesis, whereas GATA-1_S dysregulation has a pro-leukemic role. Based on the evidence that several metabolic pathways are dysregulated in myeloid leukemia (AML), we questioned whether GATA-1_S over-expression could contribute to these processes. With the aim to address this issue we performed lipidomic analysis that revealed a reduced PUFA content in K562 cells over-expressing GATA-1_S (GATA-1_S cells). These findings allowed us to gain insights into mechanisms of ferroptosis resistance triggered by GATA-1_S in these cells [Trombetti et al., (2023) Antioxidants 12, 537]. Lipidomic analysis had also revealed a reduced ceramide content in these cells. Given the role played by sphingolipid species in the regulation of proliferative and apoptotic pathways, these findings prompted us to investigate the expression levels of enzymes involved in the ceramide metabolism in GATA-1_S cells. In line with the pro-leukemic role exerted by GATA-1_S we found an increase in the expression levels of ceramide and sphingosine kinase enzymes (CERK1 and SPHK1), which are responsible for the synthesis of ceramide-1 phosphate and sphingosine-1 phosphate, respectively, two sphingolipid species which exert pro-proliferative and anti-apoptotic roles. A siRNA-mediated silencing of CERK1 and SPHK1 was then performed in GATA-1_S cells. As expected, treatment with cisplatin in these cells resulted in an increased apoptotic rate with respect to non-silenced cells. Overall, our results indicate that alterations in the ceramide metabolism play a key role in the pro-leukemic mechanisms triggered by GATA-1_S and suggest novel promising therapeutic targets to overcome drug resistance in myeloid leukemia.

P-30-127
The cytotoxic and oxidative stress effects of *Bifidobacterium breve* paraprobiotics in breast cancer cell (MCF7)

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Breast cancer is a disease caused by uncontrolled growth of cells in breast tissue. MCF-7, which is widely used in breast cancer research, is valuable for hormone-receptor-positive breast cancer research. The relationship between probiotics and breast cancer has been a subject of increasing interest in recent years. Some studies suggest that probiotics may affect estrogen metabolism. In our study, paraprobiotics obtained by heat inactivation were used. The effects of paraprobiotics on breast cancer cells and

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their effects on oxidative stress were investigated *in vitro*. The time-dependent cytotoxic effects (24 h, 48 h and 72 h) of 5 different concentrations (50-250 mM) of paraprobiotics obtained from *Bifidobacterium breve* strains on the MCF7 cell line were determined by the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) method. Total oxidant and total antioxidant levels at the cellular level were evaluated by spectrophotometric methods. In our study, paraprobiotics obtained from *B. breve* strains were determined to have time-dependent cytotoxicity (maximum cytotoxic effect was obtained at 48 h) on MCF7 cancer cells at a certain concentration (125 mM). The lowest IC_{50} dose was found at 72 hours. It was observed that total oxidative status decreased, and antioxidant status increased at the cellular level. This change in total oxidative stress parameters was determined to be statistically significant ($p < 0.05$, $p = 0.032$). Conclusion, probiotics may provide potential benefits in reducing the risk of breast cancer. However, more research is needed on this topic. *The authors marked with an asterisk equally contributed to the work.

P-30-128
Investigation of long non-coding RNA-ATB and miR-200b-3p expressions in prostate cancer cell lines

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Prostate carcinoma remains a leading male malignancy, necessitating novel therapies. Long non-coding RNAs (lncRNAs) play key roles in cancer development. Long non-coding RNA-ATB (lncRNA-ATB), activated by TGF-β, is upregulated in various cancers except pancreatic cancer. The miR-200 family, particularly miR-200b, functions in tumor suppression. This study examines lncRNA-ATB and miR-200b-3p expression in prostate cancer cell lines: non-malignant PNT1A, androgen-sensitive LNCaP, moderately metastatic DU145, and highly metastatic PC-3. Total RNA was isolated from the prostate cancer cell lines PC-3, LNCaP and DU-145 and the normal prostate epithelial cell line PNT1A using the miRNeasy Tissue/Cells Advanced Kit. Expression levels were determined using quantitative real-time reverse transcription polymerase chain reaction (qRT-PCR). The relative expression levels of lncRNA-ATB and miR-200b-3p were calculated using the $2-\Delta\Delta Ct$ method, with GAPDH and U6 serving as endogenous controls. Statistical analyses were conducted using Student's t-test. The expression levels of lncRNA-ATB were found to be significantly increased ($p < 0.05$) in the prostate cancer cell lines DU-145, PC-3, and LNCaP compared to the normal PNT1A cell line. Conversely, in the LNCaP and PC-3 cell lines, miR-200b-3p expression was significantly lower than in PNT1A ($p < 0.05$). However, miR-200b-3p expression was significantly upregulated in DU-145 compared to PNT1A ($p < 0.05$). These findings suggest that there is a relationship between lncRNA-ATB and miR-200b-3p expressions and prostate cancer and its behavior.

P-30-129
Lipid profiling of primary tumors and peritoneal metastases in ovarian cancer: insights into metastatic progression

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Ovarian cancer exhibits high lethality due to late-stage diagnosis and aggressive transcoelomic spread, resulting in peritoneal dissemination and poor prognosis. While primary and metastatic tumors share a similar genomic landscape, metabolic reprogramming particularly dysregulated lipid metabolism plays a critical role in tumor survival, invasion, and metastasis, presenting a potential therapeutic target. This study utilizes lipidomics analysis to profile lipid alterations in primary ovarian cancer (POC) and peritoneal metastases (PM), providing insights into their role in disease progression and dissemination. A comprehensive whole lipidomics analysis from POC and PM tissues, was performed using a direct infusion mass spectrometry approach. This high-throughput method allows simultaneous detection and quantification of diverse lipid species. It enables profiling of up to 30 lipid classes and ~600 lipids from POC and PM, offering insights into lipid metabolic alterations in OC progression. As a result of the study, significant alterations were observed in various ceramide-based sphingolipids, including HexCer 34:2;3, 40:2;2, 18:0;3-24:0;0; diHexCer 18:1;2-18:0;0, 16:1;2-24:1;0, triHexCers 18:1;2/16:0;0, 18:1;2/24:0;0, 16:0;2/26:2;0, 18:1;2/24:1;0 between POC and PM tissues. The observed changes in ceramide-based sphingolipids, suggest a dynamic remodeling of sphingolipid metabolism during tumor dissemination. These findings provide new insights into the lipidomic adaptations associated with peritoneal metastasis and may serve as a foundation for future investigations into sphingolipid-targeted therapeutic strategies (This project was supported by The Scientific and Technological Research Council of Turkey-TÜBITAK, Project No: 222S520, and Hasan Kurter was supported by the Council of Higher Education 100/2000 Scholarship Programs.)

P-30-130
Silencing of EZH2 in hepatocellular carcinoma alters cancer stemness, drug resistance, and survival pathways

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Hepatocellular carcinoma (HCC) is the second leading cause of cancer-related mortality worldwide, characterized by poor prognosis, high recurrence rates, and therapeutic resistance. The Polycomb group (PcG) complex, particularly its catalytic subunit EZH2, is a key epigenetic regulator that maintains cellular identity and tumor progression through histone H3K27 trimethylation (H3K27me3). High EZH2 expression is strongly linked to tumor invasion, metastasis, and poor clinical outcomes

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in HCC, making it a critical therapeutic target. However, its precise role in cancer stem cell (CSC) regulation and drug resistance remains incompletely understood. To investigate its functional impact, EZH2 was stably silenced in Huh7 cells using lentiviral shRNA, followed by qPCR-based gene expression analysis. Successful EZH2 knockdown was confirmed by decreased mRNA levels. Silencing EZH2 led to reduced expression of CSC markers (SOX2, ABCG2) and FGF3, suggesting loss of self-renewal capacity and drug resistance. However, ERK1/2 and AKT1 were upregulated, indicating a compensatory activation of pro-survival and mitogenic pathways. While EZH2 suppression diminishes CSC properties, the activation of ERK1/2 and AKT1 signaling presents a potential adaptive survival mechanism, which could limit the efficacy of EZH2-targeted therapies. Additionally, TP53 downregulation suggests altered cellular growth regulation, potentially through indirect epigenetic or signaling pathway interactions. These findings demonstrate that EZH2 is crucial for maintaining cancer stemness and drug resistance, but its suppression triggers compensatory proliferative mechanisms that may diminish the therapeutic benefits of EZH2 inhibition. This highlights the need for a dual-targeted approach, combining EZH2 inhibitors with ERK1/2 and AKT1 pathway suppression, to overcome adaptive resistance and improve treatment outcomes in HCC. *The authors marked with an asterisk equally contributed to the work.

P-30-131
Adipose-derived mesenchymal stem cells drive endometrial cancer progression by establishing pro-tumorigenic metabolic crosstalk with tumor cells

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Obesity prevalence among women, especially those under 45, has significantly increased, correlating with a rise in endometrial cancer (EC), the most common gynecologic cancer in developed countries. This evidence highlights the need to better characterize the relations between obesity, aging, and EC progression. The molecular and metabolic interaction between adipose-derived mesenchymal stem cells (AD-MSCs), adipocytes, and EC cells remain underexplored. In this study, we characterized AD-MSCs and patient-derived organoids (PDOs) from patients undergoing EC surgery, along with commercial cell lines, setting up a co-culture system to study paracrine interactions between AD-MSCs and EC cells. Estrogen-dependent (Ishikawa) and estrogen-independent (HEC1A) EC cells were co-cultured with AD-MSCs. Co-cultured EC cells showed a 1.5-fold increase in cell proliferation compared to controls, supporting the role of AD-MSCs in promoting EC cell growth. Spheroid formation and migration assays revealed enhanced 3D growth and migration potential in co-cultured EC cells. Additionally, HEC1A cells exhibited a 3-fold increase in chemoresistance after 20 hours of exposure to paclitaxel, further highlighting the pro-tumorigenic role of AD-MSCs. Morphological analysis of co-cultures revealed significant lipid droplet accumulation in AD-MSCs, suggesting adipogenic differentiation. HEC1A cells exhibited increased expression of lipid metabolism-related genes, including diacylglycerol O-acyltransferase 2 and CD36, both linked to poor EC prognosis. Also, increased gene expression of peroxisome proliferator-activated receptor γ coactivator 1α (PGC-1α) and elevated mitochondrial membrane potential suggested a link between lipid metabolism and mitochondrial function in EC progression. Our findings confirm that AD-MSCs significantly contribute to EC progression, validating the co-culture system's efficacy and supporting the ongoing development of a AD-MSCs and organoid biobank at UPO.

P-30-132
Deciphering the role of estrogen receptor alpha and its E3 ubiquitin ligase in breast cancer

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Estrogen receptor alpha (ERa) is involved in various pathophysiological conditions, including breast cancer. The interaction of ERa with its ubiquitin ligases, TRIM56 and CUL4B-DDB1-AhR, has been reported to be key to breast cancer cell progression and inhibition, respectively. We aimed to investigate the role of ERα and its E3 ubiquitin ligases in the expression of proteins downstream, i.e., NF-κB and Bcl-2. First, we examined the basal expression of the protein involved in the MCF-7 cell line. We confirmed that all proteins were expressed. We then investigate the downstream protein expression under ERα, TRIM56, and AhR knockdown using siRNA. We found that the NF-kB and Bcl-2 were altered. The knockdown of ERα decreased the expression of NF-κB p65 and Bcl-2. Under TRIM56 knockdown, the expression of ERα was slightly decreased, as also demonstrated by NF-κB p65 and Bcl-2. AhR knockdown resulted in a slight increase in ERα expression, which can also be observed in NF-κB p65 and Bcl-2 expression. We are confirming the stabilization or degradation by TRIM56 and CUL4B-DDB1-AhR, and analyzing the expression of proteins downstream after MG132 treatment. We also tested several compounds to demonstrate that ERa and its E3 ubiquitin ligase could be potential targets for developing breast cancer drugs. An isolate from the *Acanthus ilicifolius* plant reduced ERa expression in MFC-7 WT compared to the control. This decrease was also observed in TRIM56, NF-κB p65, and Bcl-2 expression following siRNA ERa treatment. *In silico* analyses were employed to support the evidence of the interaction between ERα-TRIM56 and ERα-CUL4B-DDB1-AhR. *The authors marked with an asterisk equally contributed to the work.

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P-30-133
Anti-PTK7 monoclonal antibodies suppress tumor growth and angiogenesis in PTK7-positive cancers

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Protein tyrosine kinase 7 (PTK7) is a catalytically inactive receptor tyrosine kinase frequently upregulated in multiple cancers, including esophageal squamous cell carcinoma (ESCC), and associated with poor clinical outcomes. To evaluate its therapeutic potential, we developed anti-PTK7 monoclonal antibodies (mAbs) that target distinct extracellular immunoglobulin (Ig) domains of PTK7. *In vitro*, PTK7 mAbs (32-m and 43-m) significantly suppressed three-dimensional proliferation, adhesion, wound healing, and chemotactic migration and invasion of ESCC KYSE-30 cells. These mAbs also reduced MMP-9 secretion, disrupted actin cytoskeletal polymerization, and inhibited phosphorylation of key oncogenic signaling mediators, including ERK, SRC, and FAK. *In vivo*, PTK7 mAbs significantly inhibited tumor growth in KYSE-30 xenografts, reducing tumor volume to 68.8 ± 5.3% (mAb-32) and 61 ± 7.7% (mAb-43) of controls, along with decreased Ki-67 expression, indicating reduced proliferation. Mechanistically, PTK7 enhanced angiogenesis by directly interacting with KDR (VEGFR2), promoting receptor oligomerization and downstream signaling. PTK7 mAbs disrupted this interaction and significantly inhibited VEGF-induced capillary-like tube formation in HUVECs. These anti-angiogenic effects were further validated in *ex vivo* aortic ring assays. *In vivo*, co-treatment with VEGF and mAb-32 at 3 or 10 μg/mL reduced hemoglobin (Hb) levels in Matrigel plugs to 1.73 ± 0.36 g/dL (54.7% of control) and 1.15 ± 0.49 g/dL (36.4%), respectively. Similarly, mAb-43 reduced Hb to 1.44 ± 0.19 g/dL (45.5%) and 1.13 ± 0.06 g/dL (35.7%). These findings demonstrate robust, non-cytotoxic inhibition of angiogenesis. Collectively, these results establish PTK7 as a promising therapeutic target in PTK7-overexpressing cancers and support the development of PTK7-neutralizing antibodies and antibody–drug conjugates for clinical applications. *The authors marked with an asterisk equally contributed to the work.

P-30-134
Dual-signature role of INHBA and DDIT4 as biomarkers and therapeutic targets in colorectal cancer

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Colorectal cancer (CRC) ranks third in incidence and second in cancer deaths globally. Despite advancements in conventional diagnostic methods and therapies, CRC's molecular heterogeneity limits effective management and prevention, keeping it among the leading causes of cancer mortality. This study aimed to find prognostic/diagnostic biomarkers and therapeutic targets for CRC using bioinformatics, clinicopathological data, and experimental analyses. We analyzed three GEO CRC datasets, identifying 388 differentially expressed genes (DEGs) between tumor and normal samples (-log2FC- ≥ 1, adj $p < 0.01$), with 139 consistently upregulated. Enrichr pathway analysis revealed oncogenic pathways linked to these genes. We then performed Cox regression to identify key genes associated with CRC prognosis and found INHBA (HR = 2.240, p = 0.002) and DDIT4 (HR = 2.279, p = 0.001) to be significant independent prognostic markers. A positive correlation between INHBA and DDIT4 was observed, with high expression linked to shorter survival outcomes ($p < 0.05$), advanced stages ($p < 0.05$), and recurrence ($p < 0.05$). Expression rose from normal tissue to polyps to cancer across five cohorts ($p < 0.001$), aiding early diagnosis insights. GSEA analyses suggested these genes are involved in CRC carcinogenesis via hypoxia and epithelial-mesenchymal transition. A prognostic risk score model based on these two genes and cancer stage was developed and validated in external datasets, stratifying patients into high/low-risk groups with distinct survival ($p < 0.001$). Experimentally, INHBA and DDIT4 overexpression was confirmed in CRC tissues and cells; dual siRNA knockdown reduced proliferation and boosted apoptosis, proving their roles in progression. These findings highlight INHBA and DDIT4 as promising biomarkers and therapeutic targets, with the risk score model offering potential for CRC management. Dual inhibition of these genes presents a novel therapeutic strategy. Supported by TUBITAK (grant number: 122S340).

LB-R-30-01
Expression patterns and potential clinical relevance of ASAH1 in rectal cancer

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Acid ceramidase (ASAH1) is a central enzyme in sphingolipid metabolism with a key role in maintaining the balance between cell survival and apoptosis. Its elevated activity may contribute to reduced sensitivity to therapy in rectal cancer. This study aimed to evaluate the gene and protein expression of ASAH1 in rectal cancer patients and investigate their associations with the apoptotic status of tumor tissue and response to therapy. Primary tumor tissue, adjacent healthy mucosa, and serum samples were collected from 27 patients with locally advanced rectal cancer before undergoing neoadjuvant chemoradiotherapy (nCRT). The relative mRNA expression of ASAH1, pro-apoptotic BAX, and anti-apoptotic BCL2 in tissue samples was assessed by quantitative real-time polymerase chain reaction, while serum ASAH1 levels were measured using enzyme-linked immunosorbent assay. ASAH1 gene expression significantly differed between tumor and non-tumor tissues (p = 0.022). In 67% of patients, the expression of ASAH1 was 1.1-35-fold

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decreased in tumor tissues compared to non-tumor tissues. While ASAH1 expression showed no correlation with apoptotic markers, serum ASAH1 levels were negatively correlated with the BAX/BCL2 ratio in tumor tissue (rs = -0.537, p = 0.026). We found no association of analyzed parameters with response to nCRT. Receiver operating characteristic (ROC) analysis demonstrated limited predictive value, with ASAH1 gene expression in tumor tissue showing an AUC of 0.608 and serum ASAH1 an AUC of 0.558. Our results suggest potential association of serum ASAH1 levels with an anti-apoptotic status of tumor tissues. However, ASAH1 expression in tissue and serum showed no significant correlation with therapy response. Larger and more comprehensive studies are needed to further evaluate the role of ASAH1 in predicting treatment outcomes.

LB-R-30-02
SLPI overexpression in A549 lung adenocarcinoma cells promotes migration and metastasis

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Lung cancer is the leading cause of cancer-related deaths globally. Due to its molecular diversity, understanding the biology of lung cancer is crucial for developing effective treatments. The secretory leukocyte protease inhibitor (SLPI) is known for its roles in cell proliferation, immune responses, and repair mechanisms, along with its antimicrobial properties. It may also function as a tumor regulator, influencing the progression and prognosis of various cancers. Data analysis from the GEPIA and UALCAN databases revealed that SLPI gene expression is significantly lower in lung adenocarcinoma patients compared to healthy individuals. This study investigates the effects of SLPI overexpression on the migration, proliferation, and metastasis of A549 LUAD cells *in vitro*. A549 cells were stably transfected with either N-terminal p3XFLAG-CMV or N-terminal p3XFLAG-CMV-SLPI. SLPI mRNA levels were confirmed via reverse transcription polymerase chain reaction (RT-PCR). To assess the impact of SLPI overexpression, wound healing, CCK8, and Matrigel invasion assays were performed. Although SLPI overexpression did not affect A549 cell proliferation, the wound area significantly decreased in the SLPI-overexpressing group after 48 hours, indicating enhanced migration and metastasis compared to control groups. *The authors marked with an asterisk equally contributed to the work.

LB-R-30-03
Profile of oxylipins in the blood of children with primary malignant bone tumors

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Although malignant bone tumors in children are rare, they represent an important problem in pediatric oncology: they are characterized by an extremely aggressive clinical course, early metastasis, low sensitivity to chemotherapy and high mortality rates. The aggressive nature of these diseases urgently requires expanded research into their pathogenesis, identification of new targets for drug therapy and modern methods for assessing prognosis. It is known that oxidized derivatives of polyunsaturated fatty acids (PUFA) oxylipins play an important role in the development, invasion, metastasis and immune response of malignant tumors, however, their changes in bone sarcomas have not been previously studied. The aim of this study is to study the oxylipin profile using UPLC-MS/MS in the blood plasma of children with osteosarcoma and Ewing's tumor. Plasma samples from 20 healthy children in the control group, 29 patients with osteosarcoma, and 23 patients with Ewing tumor (average age of study participants ~12 years) were studied. Analysis of blood plasma samples revealed 32 lipid compounds. The greatest differences with the control group were found in children with Ewing tumors. Concentrations of 4 compounds, oxylipins (lipoxygenase pathway): 9-HODE, 9-HpODE, 13-KODE (derivatives of linoleic acid) and 15-HETE (metabolite of arachidonic acid) were increased in patients with Ewing tumors. Thus, only in Ewing's tumor was a significant increase in the synthesis of lipoxygenase metabolites observed. In contrast to other types of malignant tumors that we have previously studied, where we observed pronounced changes in various proinflammatory oxylipins, the data obtained in bone sarcomas in children may indicate a weakly expressed inflammatory response to the tumor and, probably, a less active activation of the body's defense mechanisms. The work was supported by the System of Grants for Scientific Projects of the Peoples' Friendship University of Russia No. 214853-2-000.

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LB-R-30-04
Chou-Talalay analysis: investigating pharmacological effects of cisplatin and resveratrol in gastric cancer cells

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Cisplatin (cis-diamminedichloroplatinum-II) is a platinum-containing cytostatic drug used in cancer treatment. It forms DNA cross-links and disrupts DNA strands, and therefore is used for the treatment of various cancers, such as bladder, lung, and ovarian cancers. Resveratrol (3,5,4-trihydroxytrans-stilbene), a natural polyphenol, exhibits anticancer properties and might act as a potential adjuvant to reduce chemotherapy-related toxicity. Gastric cancer continues to pose a considerable global health challenge due to late detection and restricted treatment alternatives; thus, development of innovative combination therapy is essential. This study aimed to investigate the pharmacodynamic interaction of cisplatin and resveratrol in AGS cells using the Chou-Talalay method to assess their synergistical effects. AGS cells were treated with various concentrations of cisplatin (0.5–100 μg/mL) and resveratrol (0–1000 μM), separately, for 24, 48 and 72 hours. Cell viability was assessed using MTT assay, and it was observed that cisplatin induced a dose- and time-dependent decrease in cell viability. Resveratrol monotherapy also showed significant reductions in viability at concentrations above 70 μM. Drug interactions were analyzed using Compusyn Software to calculate the combination index (CI) and dose reduction index (DRI). Combination treatment analysis revealed synergistic effects with the use of 10 μg/mL CIS and 20 μM resveratrol, achieving over 90% cell death with CI values < 1. Following the analysis, we also confirmed through the MTT assay that the combined therapy has a greater effect on cells. These findings suggest enhanced apoptosis and reduced chemoresistance in AGS cells with combination therapy. The combination therapy of cisplatin – resveratrol offers a promising approach against gastric cancer, improving efficacy while minimizing toxicity. Further studies on molecular mechanisms and *in vivo* models are needed to support clinical translation of these results.

LB-R-30-05
Role of ATGL in the sensitivity of hepatocellular carcinoma to chemotherapy

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Liver cancer is one of the most common cancers worldwide; hepatocellular carcinoma (HCC) accounts for about 90% of cases. Among available treatments, surgery is the most efficient, however, it is not applicable for patients with advanced stages, for which systemic therapy is the only option. Based on this, research efforts have been addressed to understand the molecular mechanisms of HCC better in order to both improve cancer cells' sensitivity to drugs and avoid the development of resistance. Even if genotoxic drugs have been used to treat HCC, the problem of resistance and low efficacy occurred. Our results demonstrated that the levels of ATGL, which is down-regulated in HCC, are involved in the DNA damage degree upon genotoxic stimuli. In our experiments, two different genotoxic drugs (etoposide and doxorubicin) were tested on HCC cells, producing DNA damage. A potential mechanism explaining the association between ATGL expression and the sensitivity of HCC cells was suggested by our data highlighting an ATGL-mediated modulation of p53 functions. The relation between ATGL and p53 has already been demonstrated in HCC, indeed ATGL increase the acetylation level of p53 determining a metabolic rewiring toward oxidative phosphorylation (1). Here, we define that ATGL catalytic activity modulates p53 in order to induce pro-apoptotic genes expression (e.g., Puma). Therefore, a clear definition of the role of ATGL in HCC's response to therapy will clarify whether 1) the modulation of this lipase is a mechanism used by the tumor to reduce drug-induced damage; 2) ATGL level can be exploited as a predictive marker of HCC sensitivity to the genotoxic insults. 1. Di Leo L, Vegliante R, Ciccarone F, Salvatori I, Scimeca M, Bonanno E, et al. Forcing ATGL expression in hepatocarcinoma cells imposes glycolytic rewiring through PPAR-α/p300-mediated acetylation of p53. Oncogene. 2019 Mar; 38(11):1860–75.

LB-R-30-06
TRAIL-R2 and Abl are key mediators of sodium orthovanadate toxicity in K562 cells

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Sodium orthovanadate (Na_3VO_4) is a potent phosphatase inhibitor with recognized cytotoxic effects, but its precise mechanisms remain to be fully elucidated. In this study, we investigated Na_3VO_4-induced toxicity in K562 chronic myeloid leukemia cells and identified critical molecular mediators. Treatment of K562 cells with Na_3VO_4 (100 μM, 72 hours) reduced proliferation by approximately 70% as measured by the alamarBlue (resazurin) assay. Co-treatment with TRAIL or imatinib further enhanced cytotoxicity, reducing viable cells to 10% and 5%, respectively. To investigate the role of TRAIL-R2, we generated TRAIL-R2 knockout K562 cells by CRISPR-Cas9 plasmid transfection using Turbofect. In these knockout cells, Na_3VO_4 toxicity was significantly reduced with only ~35% reduction in proliferation. Strikingly, co-treatment with imatinib (Abl inhibitor) completely rescued TRAIL-R2-deficient cells from Na_3VO_4-induced toxicity and restored proliferation to control levels. Our results demonstrate that TRAIL-R2 and ABL are key mediators of Na_3VO_4 cytotoxicity in K562 cells. This suggests that the toxic effects of sodium orthovanadate are driven by pathways involving death receptor signaling and ABL kinase activity. These findings provide mechanistic insight into the toxicity of vanadium compounds and point to potential strategies for modulating their effects in leukemia therapy and potentially in other settings where vanadium compounds are used. *The authors marked with an asterisk equally contributed to the work.

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LB-R-30-07
Novel insights to evaluate pharmacological RTKs targeting with 3D cell culture models: toward next-generation preclinical testing

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The development and progression of cancer are directly influenced by microenvironmental signals, including those from the extracellular matrix (ECM). Breast cancer remains a leading cause of cancer-related mortality in women worldwide, with its progression shaped by both complex downstream signaling pathways and the tumor microenvironment (TME). Receptor tyrosine kinases (RTKs), such as EGFR and IGF-IR are key mediators in breast cancer, as they play key roles in cell proliferation and differentiation. In this study, we investigated the effects of EGFR and IGF-IR pathways inhibitions (both individually and in combination) on two breast cancer cell lines: Hs578T (triple-negative) and shERβ MDA-MB-231 [estrogen receptor beta (ERβ)-suppressed], using both conventional 2D and spheroid-based 3D cell culture models. While traditional 2D models offer a simple, cost-effective platform that is commonly used in early preclinical studies, they lack the ability to mimic tumor architecture and its complex cell-cell and cell-matrix interactions. By incorporating 3D cultures, we aimed to more accurately assess functional changes in tumor behavior, including growth, dissemination, and migration. Our results showed that 3D models were also responsive to RTK inhibition, revealing distinct patterns in cell behavior that highlight the influence of EGFR/IGF-IR crosstalk on aggressive tumor traits. Additionally, we explored the gene expression of key ECM effectors and epithelial-mesenchymal transition (EMT) markers under treatment conditions. This dual-model approach underscores the importance of 3D systems in cancer research and provides new insights into RTK signaling in breast cancer, potentially guiding the development of novel pharmacological strategies targeting ECM molecules.

LB-R-30-08
Serglycin is a novel regulator of the unfolded protein response (UPR) and ubiquitin-proteasome system (UPS) in malignant cells

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Serglycin (SRGN) is the only intracellular proteoglycan, participating in the formation and maturation of secretory vesicles, as well as in the regulation of their cargo bioavailability. Recent findings have established that extracellular SRGN promotes oncogenic signaling and the aggressive properties of malignancies, including multiple myeloma (MM) and glioblastoma multiforme (GBM). Upon malignant transformation, the endoplasmic reticulum (ER) experiences stress and activates adaptive proteostasis mechanisms, such as the unfolded protein response (UPR) and the ubiquitin-proteasome system (UPS). We have shown that MM and GBM cell lines express high levels of SRGN and exhibit activated UPR/UPS to relieve ER stress. In this study we utilized aggressive MM and GBM cells and developed stably transduced LN-18^{shSCR} and LN-18^{shSRGN} GBM cell lines, with the latter having depleted SRGN expression. Loss of SRGN diminished UPR and UPS markers expression and attenuated UPR/UPS activity in LN-18^{shSRGN} cells. Treatment with tunicamycin (TM), a N-glycosylation inhibitor, revealed that LN-18^{shSRGN} cells are non-responsive to ER stress and predisposed to ER stress-induced cell death. Furthermore, we highlighted the implication of SRGN in NF-kB-related pro-survival inflammatory responses and suppression of caspase-dependent apoptotic cascades. Additionally, we utilized the chemotherapeutic agent and proteasome inhibitor, bortezomib (BTZ), reaffirming the essential role of SRGN in UPS activity, and we proceeded with combined BTZ/TM treatment of LN-18 cells. Both TM and BTZ treatment alone provoked UPR signaling in LN-18^{shSCR} cells, whereas co-treatment led to apparent cell death and downregulation of UPR activity. Altogether, our results underline a novel role of SRGN as a modulator of UPR/UPS mechanisms, operating in synergy for cellular adaptation and survival under prolonged ER stress.
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LB-R-30-09
Redundant roles of Tcf1 and Lef1 in the context of Notch1-driven T-cell acute lymphoblastic leukemia (T-ALL)

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NOTCH1, a critical lineage specifier in T-cell development, ranks among the most frequently mutated oncogenes across all subtypes of T-cell acute lymphoblastic leukemia (T-ALL). Yet the mechanism by which aberrant NOTCH1 signaling establishes a leukemia-permissive chromatin landscape during disease initiation remains poorly defined. The high-mobility-group transcription factor T-cell factor-1 (Tcf1; encoded by Tcf7) is essential for remodeling chromatin accessibility and three-dimensional topology at distal regulatory elements, thereby licensing oncogenic NOTCH1 to drive leukemogenesis. We reveal that Lymphoid Enhancer Factor-1 (Lef1) collaborates redundantly with Tcf1: deletion of either Tcf7 or Lef1 in pre-leukemic double-positive (DP; CD4 CD8) progenitors modestly prolongs survival in mice, whereas simultaneous ablation markedly delays leukemia onset and progression. These findings establish that Tcf1/Lef1-dependent epigenetic priming at the earliest T-lineage progenitor stage is a prerequisite for NOTCH1-driven T-ALL, identifying the Tcf1/Lef1 axis as a potential therapeutic vulnerability. Future studies will employ inducible Cre-reporter mouse models to trace the progeny of Tcf1/Lef1-deficient DP cells, defining how loss of epigenetic priming shifts the balance between normal T-cell development and leukemic transformation *in vivo*. *The authors marked with an asterisk equally contributed to the work.

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LB-R-30-10
A targeted nanotherapeutic approach against CD44 prostate cancer stem cells

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Prostate cancer stem cells are a subpopulation of cells characterized by self-renewal capacity, the expression of surface markers such as CD44 and CD133, and the expression of pluripotency genes including NANOG, SOX2, and OCT4. These cells are resistant to conventional chemotherapy and hormonal therapies, thereby limiting the success of treatment strategies. In this study, a multicomponent nanoparticle formulation composed of nanoselenium (NS), apigenin (Api) and hyaluronic acid (HA) was developed to selectively target CD44 PCSCs. The synthesized NS-Api-HA structure was evaluated in LNCaP CD44 stem cells for cytotoxicity, apoptosis, cell cycle distribution and gene expression. The NS-Api-HA combination reduced cell viability to below 16%, exhibiting a significantly stronger antiproliferative effect compared to individual treatments. Flow cytometry analysis with Annexin V/PI staining demonstrated a high proportion of late apoptotic cells and a marked reduction in viable cells. At the molecular level, the combination therapy upregulated the expression of pro-apoptotic genes BAX, CASP3, CASP8 and P21, while downregulating the anti-apoptotic gene BCL2 and pluripotency-related genes. Moreover, selective delivery via CD44 targeting by HA, oxidative stress induced by NS and the pro-apoptotic mechanisms of apigenin collectively generated a synergistic therapeutic response. Cell cycle analysis showed an accumulation of cells in the G2/M phase and a decrease in the S phase following NS-Api-HA treatment. The 16-fold increase in P21 expression further supported p53-mediated cell cycle arrest. Additionally, the suppression of pluripotency genes NANOG, SOX2 and OCT4 indicated effective targeting of stem cells and a reduced risk of tumor recurrence. In conclusion, the NS-Api-HA formulation exerts a potent and selective therapeutic effect on CD44 prostate cancer stem cells by simultaneously targeting apoptotic, proliferative, and pluripotency-related pathways.

LB-R-30-11
Evaluation of EGFR and IGF-IR signaling in the expression of matrix effectors and cell functional properties in conventional 2D cell cultures and 3D spheroids

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The extracellular matrix (ECM) is a 3D highly dynamic macromolecular network that constantly changes in normal and pathological conditions. The action of the various matrix effectors may alter ECM macromolecular composition, a process associated with cancer progression. On the other hand, receptor tyrosine kinases (RTKs) are critical signaling effectors for gene expression and cell functional properties. Among RTKs, epidermal growth factor receptor (EGFR) and insulin-like growth factor receptor (IGF-IR) are considered important players in breast cancer diagnosis and treatment since they are implicated in tumor growth and invasiveness potential. Notably, crosstalk between EGFR and IGF-IR plays a significant role in regulating the behavior and morphology of breast cancer cells, including MDA-MB-231 (highly aggressive) and MCF-7 (low invasive hormone-dependent) cell lines. Conventional 2D cell culture models have been used for many years to study the net effects of signaling molecules and receptors. However, these models fail to mimic the dynamics of the tumor microenvironment in respect to true 3D tumor development, growth, and spreading. To this end, the recently developed 3D cell culture models are considered as a more closely related condition regarding the biochemical properties and cell-cell interactions of tumors than traditional 2D cultures. For that reason, this study aims to utilize 3D cell platforms in comparison to 2D ones to evaluate the effect of agents inhibiting the EGFR and IGF-IR pathways (alone or in combination) on the expression of ECM key components and the functional properties of breast cancer cells. Our results are indeed promising, both in advancing the understanding of RTKs signaling, with particular emphasis on the intricate crosstalk between EGFR and IGF-IR pathways, and in demonstrating the utilization of 3D models as effective tools for unraveling the biological processes governing the distinct breast cancer phenotypes.

LB-R-30-12
Helixor A promotes natural killer cell function via PAI-1-mediated p38/MAPK activation

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Helixor A, a mistletoe-derived anticancer agent, exerts immunostimulatory effects by enhancing T cell and natural killer (NK) cell activity in cancer and endometriosis patients. However, its mechanisms of action on NK cells remain unclear. Here, we demonstrate that Helixor A enhances NK cell cytotoxicity in a dose-dependent manner by upregulating activating receptors NKp44 and NKp30 and increasing the expression of granzyme B, perforin, and the degranulation marker CD107a. Helixor A also elevated the production of interferon gamma (IFN-γ) and tumor necrosis factor alpha (TNF-α). Notably, Helixor A did not affect NK cell differentiation from CD34 hematopoietic progenitors. Mechanistically, Helixor A activated the p38 mitogen-activated protein kinase (MAPK) pathway and induced plasminogen activator inhibitor-1 (PAI-1) expression. Inhibition of p38 abrogated Helixor A-induced cytotoxicity and PAI-1 expression, while overexpression of PAI-1 further enhanced NK cell cytotoxicity and p38 phosphorylation. A PAI-1 promoter assay confirmed that p38 MAPK activation increases PAI-1 transcriptional activity, and co-immunoprecipitation revealed a direct interaction between p38 and PAI-1, which was intensified by Helixor A. These findings identify Helixor A as a potent enhancer of NK cell cytotoxicity via the p38/PAI-1 axis and suggest PAI-1 overexpression as a novel strategy to augment NK cell-based gene therapy.

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LB-R-30-13
Regulation of glioblastoma multiforme progression and response to therapy by platelet-derived extracellular vesicles

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Glioblastoma multiforme (GBM) is one of the most aggressive primary brain tumors and is characterized by high mortality. The interactions between cancer and host cells are critical for tumor progression. Circulating platelets interacts with different types of cancers and alter the phenotype of cancer cells though different strategies, including the release of highly reactive platelet-derived extracellular vesicles (PEVs). PEVs are very efficient in transferring platelet-derived molecules to cancer cells and can induce pro-tumoral responses. The functional consequences of PEVs interaction with GBM cells remain unexplored. In this study we evaluated the ability of PEVs to modulate GBM phenotype and response to the chemotherapeutic agent temozolomide (TMZ). Three different GBM cell lines (U251, T98G and U87) characterized by a different sensitivity to TMZ, were used to investigate interaction with purified PEVs as well as the impact of PEVs, alone or in combination with TMZ, on cancer cell viability, duplication and apoptosis. Our results indicate that GBM cells were differently able to bind and internalize PEVs, with U87 cells displaying the strongest interaction. PEVs uptake led to the transfer of platelet-specific biomarkers, such as integrin αIIb, to target cells. TMZ treatment induced apoptosis and a strong alteration of cell cycle in U87 and U251, but not in TMZ-resistant T98G. Exposure of GMB cells to PEVs, alone or in combination with TMZ, resulted in minimal changes in cell cycle progression. Interestingly, PEVs strongly decreased mitochondrial reductase activity, without potentiating TMZ-induced apoptosis. In conclusion, PEVs interact with GBM cells but cause a modest alteration of cell viability. The ability to be internalized by GBM cells without manifesting pro-tumoral effects suggest the possibility to exploit PEVs as possible carriers for a drug delivery strategy. This work was support by Italian Ministry of Health, Project Immuno-HUB (T4-CN-02).

LB-R-30-14
Capsaicin prevents obesity-induced PD-L1 upregulation and tumor progression in a prostate cancer mouse model

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Prostate cancer (PCa) remains a major health burden, particularly in Western countries, where obesity has been identified as a risk factor for both incidence and progression. Immunotherapy has had limited success in PCa, partly due to its immunosuppressive tumor microenvironment and low mutational burden. Notably, immune checkpoint proteins such as PD-L1 contribute to immune evasion. Capsaicin, the active component of chili peppers, is known for its anti-obesity and antitumoral effects, including AMPK activation and modulation of lipid metabolism. In this work, we investigated whether dietary capsaicin could modulate the obesity-associated tumor microenvironment and PD-L1 expression in a prostate cancer model. TRAMP and C57BL/6 mice were fed for 6 months with either standard or high-fat diets (HFD), supplemented or not with capsaicin. Body weight, metabolic parameters, PSA levels, and tumor burden were assessed. PD-L1 protein expression in prostate tissues was analyzed by western blot. We found that HFD-fed mice exhibited increased body weight, higher PSA levels, and elevated PD-L1 expression. Capsaicin supplementation significantly reduced adiposity, cholesterol, and triglyceride levels. Moreover, capsaicin partially reversed the HFD-induced upregulation of PD-L1 and decreased prostate tumor burden and metastasis. In conclusion, capsaicin prevents obesity-induced PD-L1 upregulation and prostate tumor progression. These findings suggest that capsaicin may enhance the efficacy of immunotherapy in obese PCa patients by converting "cold" tumors into "hot" immunologically active ones. Acknowledgement: This work has been supported by University of Alcalá Grant GP2024-03.

LB-R-30-15
Role of the ascitic fluid in the acquisition of a malignant phenotype in ovarian cancer

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Ovarian cancer is the leading cause of death in women with gynaecological diseases. There are multiple subcategories of this tumour type, and high-grade serous ovarian cancer is the most aggressive type. In advanced stages, it is often associated with the presence of ascite, a pathological accumulation of fluid in the intraperitoneal cavity. The presence of ascite is not simply a consequence of the tumour, but represents a factor that negatively influences the prognosis, contributing to reduced progression-free survival. In this regard, we conducted a preliminary study aimed at investigating the influence of ascitic fluid on two fundamental aspects of tumor progression, namely the development of chemoresistance and the ability to undergo epithelial-mesenchymal transition (EMT), thus sustaining increased invasive potential. We therefore decided to evaluate how a different concentration of ascitic fluid (from 10% to 50%) and/or different exposure times could impact the two phenotypes under study. We highlighted that both the above-mentioned conditions can induce an increase in chemoresistance, assessed both with equimolar concentrations of cisplatin and by estimating the IC_{50}. Therefore, we observed that incubation with ascitic fluid increased the ability of ovarian cancer cells to invade a basement membrane-like. Accordingly, the expression levels of several genes implicated in EMT were modulated in a pro-malignant manner. Furthermore, incubation of tumor cells with ascitic fluid at different concentrations led to an increase of expression of the transcription factors nuclear factor erythroid 2-related factor 2 (Nrf2) and ETS proto-oncogene 1 (ETS1), a key drivers of cancer invasion, along with a diminution of intracellular reactive oxygen species (ROS) production. These results strongly highlight the contribution of ascitic fluid to the acquisition of a more aggressive phenotype of ovarian cancer.

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LB-R-30-16
Investigation of potassium channel modulation by flavonoids in a prostate cancer cell line: functional role of BKCa channels

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Prostate cancer is one of the most common malignancies in men, and its incidence increases with age. Ongoing research aims to elucidate the mechanisms of prostate cancer progression and develop more effective therapeutic strategies. Baicalein and quercetin are flavonoids known to have anticancer effects. In this study, we investigated the effects of these flavonoids on potassium ion currents in the prostate cancer cell line Du145 using whole-cell patch clamp electrophysiology and examined the effects of specific ion channel inhibitors. The expression of calcium-activated (KCa) and voltage-dependent (Kv) potassium channels was confirmed by RT-PCR. Electrophysiological recordings showed that baicalein and quercetin activated outward potassium currents with a flashing pattern. These currents were significantly inhibited by iberiotoxin (IBX), a selective blocker of large-conductance calcium-activated potassium (BKCa) channels, and tetraethylammonium (TEA), a broad-spectrum potassium channel inhibitor. Interestingly, combined treatment with flavonoids and ion channel inhibitors had minimal effects on flavonoid-induced apoptosis, mitochondrial depolarization, or inhibition of cell migration. Moreover, flavonoid treatment alone had limited effects on cell proliferation. These results suggest that BKCa channels are functionally expressed in Du145 prostate cancer cells and respond to flavonoid modulation, but may play only a minor role in regulating key cancer-related cellular processes under the conditions tested. Further studies are needed to clarify the therapeutic potential of targeting BKCa channels in prostate cancer.

LB-R-30-17
A natural therapeutic approach to fibrocystic breast disease: antiproliferative effects of phenolic cocktail and metformin on a fibrocystic breast cell line model

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Fibrocystic breast disease is the most prevalent benign breast condition in women of reproductive age, triggered by hormonal fluctuations and characterised by increased breast density, lumps, dimpled skin, or nipple discharge. Severe cases may require hormone therapy or surgery. Without appropriate preventive measures, affected women are at risk of developing malignant tumours. As a natural approach, polyphenols are recognised as non-toxic to healthy cells, exhibiting minimal or no side effects, antioxidant and antiproliferative properties. Antidiabetics are of interest due to their potential to affect cellular energy homeostasis in hormone-sensitive tissues, such as breast tissue, by maintaining glycemic control. Metformin, the most common antidiabetic drug, has been observed to indirectly activate the PPARγ pathway and has been hypothesised to be able to affect malignancies by inducing apoptotic or antiproliferative signals. In this study, the cytotoxic effects of metformin hydrochloride (MetHCl), a patented polyphenolic cocktail (PFK^{5120}), and their combination (MIX) on a human fibrocystic breast epithelial cell line (MCF10A) were investigated. The potential of these substances to induce cell death in fibrocystic breast model was evaluated using the WST8 assay. Statistical analysis was performed using a two-way ANOVA test. MCF10A cells demonstrated a time-dependent cytotoxic response to MetHCl, with the highest toxicity observed at 96 h. The cytotoxic effect of PFK^{5120} was significant at all time points, even at low doses. Furthermore, the combination of both compounds significantly increased toxicity to MCF10A cells in a dose-dependent manner, similar to the effects of MetHCl alone ($p < 0.0001$). The findings of this study underscore the synergistic impacts of the combined utilization of polyphenols and Metformin in impeding cellular proliferation within fibrocystic breast models. Further exploration into the therapeutic potential of these compounds is warranted.

LB-R-30-18
Role of circadian rhythm disruption by clock gene mutations in lynch syndrome-associated colorectal cancer

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Circadian rhythm, the internal clock of living beings, regulates 24-hour biological cycles, and physiological activities like DNA repair. Little is known about the role of circadian rhythm disruptions in Lynch syndrome-related cancers. This study evaluates how CLOCK and MMR mutations interact to affect Lynch syndrome-related cancers. It discusses how chronotherapy, which times treatments according to circadian rhythm, can improve outcomes. The sample consists of 24 Lynch syndrome patients diagnosed with cancer at Marmara University Prof. Dr. Asaf Ataseven Hospital. After Ethics Committee Approval, a retrospective, anonymous study was conducted using medical records and Clinical Exome Sequencing (CES) panel. CLOCK, PER1, PER2, MLH1, PMS2, MSH2, and MSH6 genes were screened in CES. SOPHIA DDM was used for genomic analysis, and IBM SPSS for statistics. Chi-square test, independent t-test, and Pearson correlation were utilized ($p < 0.05$) 19 CLOCK gene variants were detected (8 PER1, 8 PER2, 3 CLOCK). PER2 c.1995G>A synonymous variant significantly increased in younger patients (mean = 50) compared to noncarriers (mean = 60; $p = 0.003$). No significant gender differences were observed among variants ($p > 0.05$). Among variant trends, PER2 c.1992G>A and CLOCK c.623C>G were the most commonly detected; each was present in colorectal, gastric, and endometrial cancers. PER2 c.3600C>T variant was only seen in colorectal cancers. Colorectal cancers were evaluated as the highest variant

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burden cancer type. Distinct CLOCK variant patterns were identified in different cancer types; they were enriched in colorectal, endometrial cancers. PER2 c.3600C>T variant was thought to cause colorectal cancer predisposition. PER2 c.1995G>A associated with younger age was thought to have a potential role in early-onset tumorigenesis (mean = 50). CLOCK variants may serve as biomarkers for cancer susceptibility and subtyping. Further mechanistic studies are needed. *The authors marked with an asterisk equally contributed to the work.

LB-R-30-19
Proteomic analysis reveals TRPV1 as a key regulator of cell cycle and DNA replication in prostate cancer cells

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The transient receptor potential vanilloid 1 (TRPV1) channel is a polymodal sensor of noxious stimuli involved in pain, inflammation, and cellular proliferation. While aberrant TRPV1 expression has been reported in multiple cancers, its molecular role in prostate cancer (PCa) remains insufficiently understood. Here, we applied quantitative mass spectrometry-based proteomics to investigate signaling alterations induced by TRPV1 silencing in metastatic PC3 prostate cancer cells. Cells were transduced with either an empty vector (EV) or TRPV1-targeting shRNA (shTRPV1). Principal component and Pearson correlation analyses revealed distinct global proteomic profiles between conditions, indicating a robust impact of TRPV1 knockdown. Differential expression analysis identified 543 significantly altered proteins ($\log_2 FC \geq |1|$, $p < 0.05$), with clear separation between EV and shTRPV1 groups in hierarchical clustering. TRPV1 knockdown resulted in marked downregulation of the proliferation markers Ki-67 and PCNA, suggesting reduced proliferative capacity. Gene Ontology (GO) and KEGG pathway enrichment analyses highlighted significant changes in DNA-templated replication, mitotic nuclear division, and sister chromatid segregation. Disruption of multiple DNA repair pathways, including nucleotide excision, base excision, and mismatch repair, was also observed. These results uncover TRPV1 as a key modulator of cell cycle regulation and genome maintenance in PCa, underscoring its potential as a therapeutic target in prostate cancer.

LB-R-30-20
Uncovering the proteomic impact of DPP4 in prostate cancer cells

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Prostate cancer (PCa) remains one of the most prevalent malignancies among men, with progression to castration-resistant prostate cancer (CRPC) representing a major therapeutic challenge. Dipeptidyl peptidase-4 (DPP4), a multifunctional serine protease regulated by androgen receptor (AR) signaling, has been implicated as a tumor suppressor in PCa. Although DPP4 inhibition is associated with a reduced risk of PCa in diabetic patients, decreased DPP4 expression correlates with increased tumor aggressiveness, suggesting a dual and context-dependent role. To investigate the functional impact of DPP4 overexpression, we conducted quantitative proteomic profiling of metastatic PC3 prostate cancer cells transduced with DPP4. Principal component and correlation analyses revealed distinct proteomic signatures between wild-type and DPP4-overexpressing cells. Volcano plot and heatmap analyses identified numerous differentially expressed proteins (DEPs), highlighting significant alterations in the proteome driven by DPP4. Gene Ontology (GO) enrichment analysis of DEPs indicated modulation of biological processes such as cell-substrate adhesion, wound healing, epidermis development, and cell–cell junction organization. These proteomic alterations may reflect the tumor-suppressive activity of DPP4 and support its dual enzymatic and scaffolding roles in PCa pathogenesis but more research is needed to clarify its role.

LB-R-30-21
The role of TCTP (translationally controlled tumor protein) in cancerous and non-transformed cells

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Translationally controlled tumor protein (TCTP) is one of the proteins that play a crucial role in cellular pathophysiological processes, including cell proliferation, survival, and cell cycle

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regulation. The downregulation of TCTP expression in cells by RNAi silencing the TPT1 gene, which encodes this protein, activates apoptosis and decreases proliferative potential. The functions of the TCTP protein are particularly important in the context of cancer development. Further investigation into the role of TCTP in cancerous and non-cancerous cells may help uncover the differences between these cell types. Moreover, TPT1 silencing has been shown to induce a tumor reversal phenotype in specific cancerous cells. Our study aims to examine the impact of manipulating TCTP protein levels on cancerous HepG2 and non-transformed Thle2 hepatic cells cultured under normoxic conditions (19% oxygen) as well as hypoxia (1% oxygen), which is the natural oxygen condition in solid tumors. To determine whether oxygen conditions affect TCTP levels, we measured TCTP levels in cells collected at different time points during *in vitro* cell culture. TCTP knockdown in HepG2 and Thle2 cells was achieved via RNAi transfection with Lipofectamine under normoxia and hypoxia. Effects were evaluated using viability and proliferation assays, fluorescence microscopy, and western blot analysis of protein expression. Analysis of TCTP protein levels at various time points under normoxic and hypoxic conditions showed fluctuations over time, but no significant differences related to oxygen levels. Reduced viability and proliferation in non-transformed Thle2 cells with silenced TPT1 suggest higher sensitivity to decreased TCTP compared to HepG2 cancer cells. The ongoing research focuses on molecular pathways through which TCTP affects cell proliferation and apoptosis in cancerous and non-cancerous cells.

LB-R-30-22
Indolamine 2,3 dioxygenase-1 (IDO-1) activity in the sera/tissues of non-small cell lung cancer (NSCLC) patients and the effects of IDO-1 silencing on cell proliferation in NSCLC cell lines and an animal model

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Our project proposes a candidate IDO-1-targeted treatment approach for NSCLC by evaluating data from patients, cell lines and animal models. Tryptophan (TRP), kynurenine (KYN), kynurenic-acid (KA) levels were measured by HPLC in serum and autophagy, apoptosis and proliferation-related proteins were examined by western blot (WB) in matched tissues. The transfection with 2 different IDO-1 gene-specific siRNAs was used to silence A549, HCC-827, and Beas-2B cells. IDO-1 protein levels were checked by WB. The proliferation/viability, apoptosis/autophagy rates, clonogenic clone formation and migration were analysed by MTS, annexin-V, and acridine orange assays. For the development of NSCLC, NCr nude mice were injected with A549 cell. After 2 weeks, mice were divided into 2 groups: treated with nanoliposome encapsulated IDO-1 siRNA or control siRNA. Tumor volumes were measured during treatment. In NSCLC patients, KA level was significantly lower in advanced staged group vs early staged group ($p = 0.043$). The discrimination power of KA in these stages was confirmed by ROC analysis (AUC:0.962; $p < 0.001$). Cell culture studies showed that proliferation, clone formation, viability and cell migration were significantly depressed ($p < 0.001$) and the number of autophagic vesicles and the rate of apoptosis induced by IDO-1 siRNAs transfected cells vs control siRNA group ($p < 0.001$). In mice bearing A549 tumors with systemic injections of IDO-1 siRNA, it was found that IDO-1 inhibition delayed A549 tumor growth, suggesting IDO-1 promotes tumor growth. Our findings showed that *IDO1* gene silencing by IDO-1 siRNA led to an increase in autophagy and apoptosis/necroptosis and a significant decrease in proliferation, colony formation and migration properties. To conclude, IDO-1 is a strong target molecule that can be used as a therapeutic agent in NSCLC treatment. This study was funded by Scientific Research Projects Coordination Unit of Istanbul University. Project Number: "TUA-2020-34163".

LB-R-30-23
Relationship between squamous cell carcinoma and paraoxonase-1

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Paraoxonase-1 (PON1) is an antioxidant enzyme found in the membranes of most cells and also in the circulation bound to high-density lipoproteins (HDL). Low PON1 activity is associated with high oxidative stress in various diseases involving inflammation. There are studies showing that low PON1 activity is associated with a worse prognosis in cancer patients. Our study aimed to compare and evaluate PON1 levels in squamous cell carcinoma (SCC) patients and healthy volunteers. Plasma was obtained by centrifugation from blood samples taken into EDTA tubes. PON1 levels in the obtained plasma samples were measured by the kinetic method in accordance with the ReL Assay Diagnostic kit protocol. 15 squamous cell carcinoma (SCC) patients and 10 healthy volunteers were included in the study. Data were analyzed using SigmaStat version 3.5; normality was assessed with Shapiro-Wilk, variance homogeneity was assessed with Brown-Forsythe tests, and independent samples t test was used for group comparisons ($P < 0.05$). No statistically significant difference was observed in PON1 levels in plasma obtained from SCC patients compared to healthy volunteers ($P = 0.332$). This study included a total of 25 individuals, 15 squamous cell carcinoma (SCC) patients and 10 healthy volunteers. No significant statistical difference was found between the two groups in our study. The variability in PON1 activity in various cancer cases is quite high in the literature. The fact that no significant difference was obtained in our study may

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be related to the limited number of samples or the fact that the enzyme activity does not play a significant role in the pathogenesis of SCC. It is important to take into account the type of cancer, ethnicity, gene interactions and various factors contributing to cancer development in enzyme activity. Increasing the number of samples in our future study and studying ROS parameters will reveal the diagnostic or prognostic value of PON1 levels in SCC more clearly.

LB-R-30-24
Can HMGB1 and defensins be evaluated as biomarkers in liquid biopsy for patients with locally advanced and metastatic squamous cell carcinoma?

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High mobility group box 1(HMGB1) is a nuclear protein involved in inflammation. It is hypothesized that increased HMGB1 may be associated with tumorigenesis. β-Defensins (hBDs) are classes of cationic antimicrobial peptides. Some studies have shown that hBDs are expressed in cancer cells. Our study aimed to compare HMGB, hBD1-2-3 levels in squamous cell carcinoma (SCC) patients with those in healthy volunteers and to evaluate their potential as a biomarker. Plasmas were obtained from blood samples. In order to determine the levels of HMGB1, hBD1-2-3 in plasma samples, they were analyzed by ELISA method according to the kit procedure. Statistical analyses were performed with SigmaStat program. Normal distribution was evaluated with Kolmogorov-Smirnov/Shapiro-Wilk, and variance homogeneity was evaluated with Brown-Forsythe test. Differences between groups were analyzed with Mann-Whitney U test ($P < 0.05$). A statistically significant difference was found in HMGB1 levels between the SCC group and the healthy control group (n = 13, median: 89 250) ($p = 0.002$) a statistically significant difference was found in HBD1 levels between the healthy control group (n = 13, median: 88 370) ($p = 0.001$); a statistically significant difference was found in HBD2 levels between the healthy control group (n = 13, median: 7420) ($p; < 0.001$). The use of HMGB1 as a cancer biomarker is complex. Since it plays a role in various physiological and pathophysiological processes other than tumor progression, its levels may not always show a significant increase compared to healthy individuals. Therefore, HMGB1 results shouldn't be interpreted independently of the context. In head and neck SCC cases, differences in hBD subtype levels examined in the tissue may be related to mechanisms such as induction of some subtypes (hBD-3) by growth factors or post-transcriptional suppression (hBD-1,-2). In our study, all proteins examined were found to be statistically significantly low in SCC patients and it was planned to clarify the relationships by expanding the sample.

Cancer diagnostics

P-31-001
Predictive value of hematology analyzers according to flagging performance in cancer patients

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Complete blood count (CBC) systems use flags to alert operators to abnormal cells. The digitization of hematology has made CBC device flagged data interpretation more important for advanced studies. Our investigation examined flag data from two commercial devices (DXH900, Beckman Coulter and XN1000, Sysmex) for consistency. At Gazi University Hospital Core Laboratory, 251 patients participated in our preliminary study. CBC tests were evaluated in both devices. Flag outcomes and predictive value of both devices were assessed. Statistics utilized MedCalc's Diagnostics free statistical calculator. Blast flag was detected in 16 samples with the Sysmex analyzer (n = 8, hematological cancer; n = 4, non-hematological cancer), whereas blast result was identified in 12 samples using the Beckman analyzer (n = 6, hematological cancer; n = 2, non-hematological cancer). A total of 31 samples exhibited immature granulocyte (IG) flag on the Sysmex analyzer (n = 9, hematological cancer; n = 4, non-hematological cancer). Additionally, 18 IG flags were identified on the Beckman analyzer (n = 5, hematological cancer; n = 5, non-hematological cancer). Upon the inclusion of additional flags associated with WBC alongside the blast and IG flags in both devices, the sensitivity of the Sysmex analyzer for malignancies was assessed at 53% (40-66%) and the specificity at 70% (62-77%). The positive predictive value was 42% (33-50%), while the negative predictive value was 79% (73-83%). In the Beckman analyzer, the observed values were 38% (24-53%), 80% (72-86%), 40% (28-52%), and 79% (74-82%); respectively. The predictive value of both analyzers was shown to be agreement. These results suggest that additional investigations about any flags in hematology analyzers are crucial for early diagnosis. Additional research with an expanded focus, a larger sample size, and more comprehensive screenings is required.

P-31-002
MiRNAs as potential biomarkers in lung adenocarcinoma associated brain metastasis

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Brain metastases (BM) are the most prevalent type of intracranial malignant tumor and are associated with high mortality. More than 50% of BM cases arise from lung cancer, and lung adenocarcinoma (LUAD) is most associated with the development of BM (25%).

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Differentiating solitary BM from glioblastoma (GBM) – one of the most aggressive and fatal brain tumors – presents a significant clinical challenge. miRNAs play a critical role in gene expression regulation, and their potential as biomarkers for tumor diagnosis and prognosis is promising. We used next generation RNA sequencing (RNA-seq) to map miRNA expression profiles in 6 LUAD-BM, 6 GBM, and 6 control human brain tissue samples. Upregulated miRNAs (miR-200c-5p, miR-141-5p, miR-200a-5p, miR-375-3p, miR-196b-5p, miR-130b-3p, miR-200c-3p, miR-210-3p, miR-503-5p) and downregulated miRNAs (miR-383-5p, miR-129-2-3p, miR-410-3p, miR-9-5p, miR-195-5p, miR-138-2-3p) were selected for RT-qPCR validation. RNA-seq analysis revealed 244 differentially expressed miRNAs between LUAD-BM and control samples, and 99 between LUAD-BM and GBM. miRNA-centered target and functional enrichment analyses were performed using the miRNet tool. The Mann-Whitney U test confirmed significant differences in the expression levels of 15 selected miRNAs between LUAD-BM and controls. We identified that miRNAs such as hsa-miR-200c-5p, hsa-miR-141-5p, hsa-miR-200a-5p, hsa-miR-375-3p, hsa-miR-383-5p, hsa-miR-129-2-3p, hsa-miR-410-3p, and hsa-miR-9-5p can reliably distinguish LUAD-BM samples from GBM. ROC curve analysis further evaluated the diagnostic potential of these miRNAs. Conclusions: We identified a panel of 15 miRNAs that can effectively distinguish LUAD-BM from control samples with high diagnostic accuracy. Furthermore, 8 of these miRNAs also enable differentiation between LUAD-BM and GBM, highlighting their potential as reliable biomarkers for distinguishing between different types of brain metastases.

P-31-003
The prognostic potential of mathematically derived markers of inflammation in different types of breast cancer

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Breast cancer is a disease characterized by various subtypes, which exhibit distinct biological behaviors and clinical outcomes. Recent studies have investigated the role of inflammatory markers; Systemic inflammation response index (SIRI), Systemic immune-inflammation index (SII) – neutrophil-to-lymphocyte ratio (NLR), lymphocyte-to-C-reactive protein ratio (LCR), and monocyte-to-neutrophil ratio (MNR) – in understanding the prognosis of different breast cancer types. In our sample (Canton Sarajevo, Bosnia and Herzegovina), invasive ductal carcinoma (IDC) is the most common type of breast cancer, accounting for about 80% of cases, followed by invasive lobular carcinoma (ILC), which comprises 10-15% of cases. Our research indicates that patients with IDC exhibit elevated levels of inflammatory indices, particularly NLR, SIRI and SII. High NLR values are associated with larger tumor size and lymph node involvement, suggesting that systemic inflammation contributes to tumor aggressiveness and poorer outcomes. Monitoring these markers can provide valuable prognostic information, for patients with IDC, aiding in treatment decisions and patient stratification. Patients with ILC display elevated NLR, SIRI and SII, but the correlations are generally less pronounced, likely due to ILC's distinct biological behavior and growth patterns. LCR and MNR were investigated for their prognostic potential in breast cancer, and they were significant in both cases ($p < 0.05$). Elevated MNR has been linked to poorer outcomes in these types of breast cancer. Higher LCR may be associated with better prognostic outcomes, suggesting that a robust lymphocytic response might counteract tumor progression. These statistically significant relationships ($p < 0.05$) underscore the clinical relevance of inflammatory indices in breast cancer management. Incorporating inflammatory indices into routine clinical practice can enhance breast cancer management.

P-31-004
Characterization and optimization of Y5 receptor selective agonists for therapeutic application

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The neuropeptide Y (NPY) Y_5 receptor is a member of the NPY multi-ligand/multi-receptor system. It regulates food intake in the central nervous system, while in the periphery it is overexpressed in various cancer types [1]. As a result, the Y_5 receptor has emerged as a target for diagnostic and therapeutic applications. A selective Y_5 receptor agonist, the chimeric peptide [cPP(1–7), NPY(19–23), Ala^{31}, Aib^{32}, Gln^{34}]hPP (sY_5ago) was previously identified by Cabrele *et al.* [2]. Our goal is to modify the peptide by incorporating a DOTA (1,4,7,10-tetraazacyclododecane-1,4,7,10-tetraacetate) chelator to facilitate radiolabeling [3]. The modified peptide was characterized regarding receptor binding and activation as well as metabolic stability to assess its suitability for diagnostic applications. Receptor activation was measured by calcium flux and IP_1 accumulation assays, both showing little to no loss of activity compared to unmodified sY_5ago and NPY. To evaluate receptor subtype selectivity, all assays were performed additionally at the Y_1, Y_2 and Y_4 receptor. The results indicate high selectivity of the compound for the Y_5 receptor. For determination of receptor binding a nanoBRET-based binding assay was used, which relies on a nanoluciferase at the N-terminus of the receptor interacting with a fluorophore coupled to the peptide ligand. In a displacement approach binding was measured by displacement of a TAMRA-labeled control peptide by the unlabeled peptide of interest. The sY_5ago-variants show high displacement of TAMRA-NPY indicating specific receptor binding. Comparing experiments with the closely related receptors confirmed the high selectivity of sY_5ago for the Y_5 receptor. The modification of the sY_5ago peptide does not interfere with receptor binding, activation or selectivity and could be used for future diagnostic or therapeutic applications.

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P-31-005
Altered glycosylation of PSA in prostate cancer

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Changes in protein glycosylation have been observed in several cancers, where they affect cellular growth behavior, invasiveness and acquisition of metastatic potential. Such changes are not random and have been found to associate with cancer aggressiveness. Thus, detection of protein glycosylation may provide valuable diagnostic and prognostic information. We recently established a novel *in situ* proximity ligation-based method for the detection of different protein-glycoforms in tissue sections. This method utilizes protein-specific antibody and glycan-binding lectins. We optimized this method for the detection of different glycoforms of a well-known prostate cancer marker, prostate-specific antigen (PSA), in formalin-fixed paraffin-embedded prostatic tumor samples. With this method we first showed, using 25 different lectins and a small tissue microarray (TMA), that glycosylation of PSA in cancerous tissues is different from that in benign prostate. In a larger TMA with samples from 162 patients, PSA-glycoforms reacting with two of the lectins were enriched in cancerous tissues as compared to adjacent benign tissues from the same patients ($p < 10^{-5}$ for both), although total PSA staining was stronger in benign tissues ($2.3*10^{-14}$). In conclusion, we showed here, using tissue-based approach, that glycosylation in PSA is changed in prostate cancer. The identified lectins showing preferential binding to cancer-associated PSA-glycoform should be useful for setting-up of an assay for diagnostic test using serum samples. Such an assay has potential to revolutionize prostate cancer diagnostics.

P-31-006
Investigation of the effects of PEGylated carbon quantum dot (CQD) – peptide complex on the cell viability, apoptosis and cellular localization

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Breast cancer is the most common cancer among women worldwide, and its early detection is crucial for better prognosis. Carbon quantum dots (CQDs) which are semiconductor-biocompatible, easily modifiable nanocrystals are used as bioimaging agents thanks to their unique properties such as photostability. The effects of their modifications on cell death at molecular level have not yet been examined. In this study, it was aimed to investigate the effects of PEGylation and cell penetrating peptide (CPP) physical adsorption to the CQDs on cell viability, apoptosis, and cellular localization of the material, and investigate the potential of the bare or modified CQDs in breast cancer theranostics. To achieve this aim, CQDs were synthesized with a new method, hot bubble synthesis, developed by our group, coated with PEG polymer and a CPP, Pep-1, via non-covalent interactions. The most optimized PEG coating was found to be stirred at 300 rpm by drop-wise addition of the PEG, and PEGylated CQDs displayed ≈154 nm hydrodynamic diameter, had spherical morphology, and were negatively charged (≈-28.3 mV) but had higher charge than bare CQDs (≈-41.5 mV). Pep-1 physical absorption to bare or PEGylated CQDs was found to be the most optimum at 16:1 (peptide:CQD) (w/w) ratio and Pep-1 absorbed bare or PEGylated CQDs had ≈284.8 and ≈263.8 nm hydrodynamic diameter and had ≈-1.70 and -2.89 mV surface charge respectively. Bare or modified CQDs showed fluorescent property under 385, and 475 nm. The effects of the PEGylation and Pep-1 addition to the CQDs on cell viability, cell localization, and apoptosis of the MCF-7 and HEK293 cells are still being investigated with CCK-8 assay, confocal microscope, and western blotting for checking certain proteins' levels (total Caspase 3, cleaved Caspase 3, Bcl-2 and Bax). Thus, it is expected the bare or modified CQDs to have a huge potential for breast cancer theranostics, and this will be stated with the study.

P-31-007
Blood plasma miRNA levels as predictors of prognosis in malignant melanoma

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Malignant melanoma is an aggressive skin cancer with a high potential for metastasis. Prognostic assessment is critical for improving patient outcomes, because new modalities of adjuvant treatment (immunotherapy, targeted therapy) are most beneficial for early-stage patients with poor prognosis. Circulating tumour markers, such as microRNAs (miRNAs) detected by liquid biopsy, offer a promising tool for personalising treatment strategies. This study aimed to evaluate the prognostic significance of selected circulating miRNAs in patients undergoing curative resection for melanoma building on our previous identification of candidate miRNAs in a pilot study on 22 patients [published in Bagheri MS et al. (2023) Anticancer Res 43, 695-706.] A total of 112 patients with stage I–III melanoma were enrolled into the validation study. Blood plasma samples were taken from the peripheral blood collected both preoperatively and in the early postoperative period. Quantitative estimation of the selected circulating miRNAs was performed by RT real-time PCR using TaqMan miRNA Assays (Thermo Fisher Scientific, Foster City, CA, USA). Cel-miR-39 was used as an exogenous spike-in control. We observed that elevated

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preoperative plasma levels of miR-99a, miR-320, miR-494, and miR-1908 were significantly correlated with both, a shorter disease-free interval (DFI) and with a reduced overall survival (OS). The findings suggest that miR-99a, miR-320, miR-494, and miR-1908 hold potential as prognostic biomarkers for early-stage malignant melanoma. Identification of patients at higher risk of recurrence or poor survival outcomes could facilitate more personalised clinical decision making, including the timely administration of modern adjuvant therapies. Further validation in independent cohorts is warranted to confirm the clinical utility of these biomarkers. This work was supported by the Cooperation Program, research area SURG, Charles University.

P-31-008
Circulating tumour DNA (ctDNA) analysis as a diagnostic tool in personalised medicine for patients with advanced melanoma

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Melanoma is characterised by the highest number of somatic mutations, with common variants occurring in the *BRAF*, *NRAS*, and *TERT* promoter. Identification of the mutated *BRAF* oncogene is crucial for the selection of targeted therapy. In *BRAF* detection, tumour DNA is the gold standard, but liquid biopsy (LB) is gaining popularity for its low invasiveness, multiple sampling, and representativeness. This study evaluated the usefulness of plasma circulating tumour DNA (ctDNA) in the detection of mutations and the prediction of treatment responses in patients with advanced stage III and IV melanoma undergoing targeted therapy or immunotherapy. The 40 patients provided blood samples before treatment (LB1) and during the 3rd to 12th week of therapy (LB2), from which cell-free DNA (cfDNA) was isolated. The amount of ctDNA was measured with digital PCR (dPCR) for the following variants: *BRAF* V600E, *BRAF* V600K, *NRAS* Q61K, *NRAS* Q61L, *NRAS* Q61R and *TERT* C250T. Furthermore, in 25 cases, selected variants were analysed in both ctDNA (LB1) and tumour using next-generation sequencing (NGS). Concordance analysis revealed that 88% and 84% of variants detected in tumour DNA were also detected in ctDNA using NGS and dPCR, respectively. Based on the status of ctDNA in LB2, patients were grouped by detectable or undetectable ctDNA. The correlation between ctDNA status and response to treatment was evaluated. Patients with detectable ctDNA showed a higher progression rate (81%) compared to patients with undetectable ctDNA (21%). Kaplan-Meier survival analysis confirmed that detectable ctDNA in LB2 significantly increases the risk of progression within 12 months of follow-up. In conclusion, monitoring of ctDNA can predict disease progression, making it a potential predictive and prognostic biomarker in patients with advanced melanoma. Analysis of ctDNA provides additional value in personalised medicine as a companion diagnostic tool. National Science Center, Grant No. 2021/43/B/NZ7/01812.

P-31-009
MLDx-α: a machine learning-based methylation and clinical feature assay for early hepatocellular carcinoma detection

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Hepatocellular carcinoma (HCC) is a leading cause of cancer-related mortality, and its early detection remains a critical challenge, particularly in patients with diverse underlying liver diseases. Current surveillance strategies rely on ultrasound and alpha-fetoprotein (AFP), but these methods lack sufficient sensitivity and specificity, especially in non-viral etiologies. To address this limitation, we developed MethyLiverDx-alpha (MLDx-α), a machine learning-based blood assay that integrates PCR-derived methylation biomarkers with clinical features to improve detection accuracy. A total of 1688 clinical plasma samples were analyzed, with the model trained on a subset and validated on an independent test set. MLDx-α demonstrated superior performance in detecting early-stage HCC, achieving an area under the curve (AUC) of 0.85, outperforming AFP (AUC = 0.74). Unlike AFP, which exhibited reduced accuracy in non-viral etiologies, MLDx-α maintained consistent performance across diverse patient populations. Additionally, high-risk samples identified by MLDx-α exhibited biomarker distributions indicative of liver dysfunction, aligning with patterns observed in HCC patients. The assay also demonstrated stability across different sample collection methods, including EDTA and cfDNA preservative tubes. These findings highlight MLDx-α as a highly sensitive and robust blood-based assay for HCC detection, providing a promising alternative to conventional biomarkers for surveillance and early diagnosis.

P-31-010
Investigation of differentially expressed genes and identification of potential biomarker candidates in TP53 mutation positive high-grade serous ovarian cancer (HGSOC) patients

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Ovarian cancer has types such as [high-grade serous (HGSOCs) (60–80% of women), low-grade serous (LGSOC) and low malignant potential (LMP)] [1,2]. Patients are diagnosed at an advanced stage due to the lack of effective diagnostic methods. In HGSOC, TP53 gene is mutated at early stages in 96.2% of patients [2,3]. We aimed to determine the differentially expressed genes (DEGs) specifically upregulated in HGSOC patients with TP53 mutation. DEG analyses were performed by LIMMA package in R with gene intensity data of 75 patients with and 15 patients without TP53 mutation (from GSE63885), and 10 healthy individuals (from GSE69428). Using GSE6008 dataset, 15 patients with and 22 patients without TP53 mutation and 8 healthy individuals were used. DEGs were determined using gene

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intensity values of 22 HGSOC patients and 21 LGSOC or LMP patients and 6 healthy individuals (from GSE27651). Finally, survival analyses were performed with 25 common DEGs to determine how 25 common genes affect the survival rate of patients by multivariate COX analyses with R by using GSE63885 dataset. TP53 mutation was found to be significantly ($p < 0.05$) affecting the survival rate of patients (hazard ratio (HR) = 5.01181). Among 25 genes, H2BC11/H2BC8 (HR = 0.62113), EGFL6 (HR = 1.48248) and ECT2 (HR = 0.19323) genes were identified as potential biomarkers for HGSOC with TP53 mutation. We will further determine potential drugs with the help of docking studies. References 1. Webb, P. M., & Jordan, S. J. (2024). Global epidemiology of epithelial ovarian cancer. Nature Reviews Clinical Oncology, 21(5), 389-400. 2. Wang, Y., Duval, A. J., Adli, M., & Matei, D. (2024). Biology-driven therapy advances in high-grade serous ovarian cancer. The Journal of Clinical Investigation, 134(1), e174013. 3. Mittempergher L. (2016). Genomic Characterization of High-Grade Serous Ovarian Cancer: Dissecting Its Molecular Heterogeneity as a Road Towards Effective Therapeutic Strategies. Current Oncology Reports, 18(7), 44. *The authors marked with an asterisk equally contributed to the work.

P-31-011
Evaluation of trimethylamine N-oxide and its precursors in bladder cancer using LC-MS/MS: a preliminary study

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Bladder cancer is a common malignancy characterized by significant heterogeneity in tumor stages. Recent studies suggest that gut microbiota-derived metabolites, particularly trimethylamine N-oxide (TMAO) and its precursors (carnitine, choline, and betaine), may play a role in cancer development. However, their association with bladder cancer remains unclear. This preliminary study aims to evaluate the serum levels of TMAO and its precursors in different stages of bladder cancer compared to healthy controls. Serum samples from bladder cancer patients at different stages (n = 25) and healthy controls (n = 22) were analyzed by liquid chromatography-tandem mass spectrometry (LC-MS/MS). Kruskal-Wallis tests, followed by Bonferroni correction were applied for statistical comparisons between groups. All metabolites (carnitine, choline, betaine, TMAO) levels were increased in bladder cancer patients compared to controls, with the most statistically significant differences observed in carnitine ($p < 0.001$), followed by choline ($p = 0.003$) and betaine ($p = 0.008$), while the increase in TMAO was less significant ($p = 0.04$). The most pronounced changes were observed particularly in the T2 stage. No statistically significant differences were observed among the cancer subgroup. Our preliminary findings indicate that serum levels of TMAO and its precursors are elevated in bladder cancer patients, with betaine, carnitine, and choline showing the most significant differences. The observed differences suggest a potential link, but further research with larger cohorts and additional validation experiments is necessary to confirm these findings. Increasing the sample size and integrating complementary analyses will be crucial for understanding the biological significance of these metabolites in bladder cancer progression and their potential role in disease characterization.

P-31-012
Unmasking and shooting brain tumours metastases by targeting ALDH1A3 enzyme with selective and potent gadolinium and boron probes

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Of all brain tumors, over 30% are metastatic lesions originating from different primary sites. Early unmasking and shooting of tumour metastases are crucial to prevent recurrence and to develop effective therapies by understanding metastases molecular mechanisms. Our studies link aldehyde dehydrogenase 1A3 (ALDH1A3) to the radio- and chemotherapy resistant mesenchymal (MES) subtype of glioblastoma multiforme (GBM)[1] and to other difficult-to-treat tumours. The enzymatic function of ALDH1A3 is the oxidation of retinaldehyde to retinoic acid, strongly involved in cell differentiation and proliferation. Based on the structural and *in-silico* analyses of human ALDH1A3 enzyme, we developed two pharmacological tools: a selective inhibitor (NR6)[2] and a fluorescent probe (Probe10)[3], enabling precise detection and elimination of ALDH1A3-positive cancer cells. The aim is to functionalize them with gadolinium for early diagnosis with MRI, and with boron for boron neutron capture therapy (BNCT). We have characterized a BNCT probe derived from Probe10, which shows selectivity for ALDH1A3 with suitable affinity and potency. We evaluated its fluorescence properties in murine GBM cells, confirming its efficient uptake and its potential as early diagnostic tool. These findings underline the advantages of specific targeting to overcome the current limitations of BNCT probes. Precision medicine, imaging, and adrotherapy are tangled to enhance modern cancer care. Validating ALDH1A3 as a key player in brain metastasis is a significant step in unveiling its role and in the mechanistic understanding of this phenomenon. Our results will aid in developing new strategies for early diagnosis of metastatic lesions using innovative molecules for effective and individualized cancer treatments. References: [1]Mao P et al. (2013) Proc. Natl. Acad. Sci. U.S.A. 110 (21) 8644-8649. [2]Gelardi ELM et al. (2021) Cancers (Basel). 2021;13(2):356. [3]Gelardi ELM et al. (2022) Commun Biol. 2022;5(1):895.

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P-31-013
Influence of eGFR on plasma and urinary metanephrine levels and their correlation in patients with adrenal mass

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Plasma and urine metanephrine tests are valuable for diagnosing pheochromocytoma and other neuroendocrine tumors that produce catecholamines. The enzyme-linked immunosorbent assay (ELISA) is commonly used for metanephrine determination in laboratories. While the plasma metanephrine test (PMET) offers higher sensitivity, the urine test (UMET) serves as a confirmatory tool, particularly when PMET results are inconclusive, although it can be affected by renal function. This study aims to examine the relationship between plasma and urinary metanephrine levels in patients with adrenal masses and the influence of estimated glomerular filtration rate (eGFR) on these parameters. A retrospective study was conducted at the Clinical Center University of Sarajevo, involving 76 patients with confirmed adrenal masses of unknown origin. The study analyzed PMET (pg/mL), UMET (μg/day), and eGFR (ml/min/1.73 m^2). Nonparametric tests were applied, considering $p < 0.05$ statistically significant. The study included 18 male and 58 female patients, with a mean age of 53 ± 13.67 years. Thirty-seven patients had elevated PMET values (> 65 pg/mL), while only ten had UMET values outside the reference range (< 350 μg/day). A statistically significant correlation between plasma and urine metanephrine levels was observed (Spearman's rho = 0.240, p = 0.045). In the eGFR > 90 ml/min/1.73 m^2 group (N = 32), a strong positive correlation was noted with plasma metanephrine levels (rho = 0.788, $p < 0.001$). The eGFR < 90 ml/min/1.73 m^2 group (n = 32) showed a weaker, but still significant, positive correlation (rho = 0.326, p = 0.031). In patients with renal impairment, UMET had a strong positive correlation with eGFR (rho = 0.744, $p < 0.001$). The study demonstrates that plasma and urine metanephrine levels are positively correlated, with renal function playing a significant role in this relationship.

P-31-014
The role of CA-125 and inhibin B in excluding malignancies in women with ovarian cysts

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Cancer antigen 125 (CA-125) and inhibin B are biomarkers that provide information related to ovarian function, helping to discriminate between benign and malignant cysts. While CA-125 is useful for diagnosing ovarian tumors, its effectiveness is limited due to elevated levels in benign conditions. Inhibin B can help differentiate between types of tumors, but its levels in women with ovarian cysts are generally not specific for determining cyst benignity or malignancy. This study aims to evaluate the values of CA-125 and inhibin B in women with benign ovarian cysts and the significance of their combined use. Retrospective data were collected from the Clinical Center of the University of Sarajevo, analyzing laboratory results from 40 women with benign ovarian cysts, where malignancies were excluded. CA-125 levels (U/mL) were measured by electrochemiluminescence immunoassay (Cobas PRO, Roche), while Inhibin B levels (pg/mL) were assessed using enzyme-linked immunosorbent assay (DRG Diagnostics GmbH). The mean age of women with benign cysts was 35.4 ± 7.4 years. 34 (85%) women had CA-125 values within the reference range (< 35 U/mL) with a mean of 12.20 ± 3.20 U/mL. 38 (95%) had inhibin B levels within the reference range, with a mean of 44.80 ± 41.60 pg/mL. 6 (15%) women exhibited elevated CA-125 values, and 2 (5%) had elevated inhibin B values. CA-125 showed a specificity of 85% for confirming benign cysts, while inhibin B had a specificity of 95%. The combined use of both biomarkers yielded a specificity of 80.75%. Sensitivity of the tests was not applicable in our research. CA-125 did not show a significant correlation with Inhibin B (rho = 0.93, p = 0.567). These findings indicate that CA-125 and inhibin B can enhance the assessment of benign ovarian cysts. While they can potentially be used together to enhance the assessment of benign ovarian cysts, their interpretation should be made within a clinical context, alongside imaging studies and other diagnostic tools.

P-31-015
Development and testing a novel molecular technique, SHARD-PCR, for detecting point mutations in the promoter of the TERT gene

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Telomerase reverse transcriptase promoter mutations (TERTpms) are frequently targeted tumor markers. However, they are located in a GC-rich region, that makes them difficult to examine by simple molecular techniques or even next-generation sequencing (NGS). Bladder cancer (BC) is characterized by a high frequency of TERTpms, yet none of the available tools in clinical practice have proven effective for detecting TERTpms by a simple, noninvasive method. Therefore, we developed a novel PCR-based technique for detecting the two most prevalent TERTpms. Selective high-sensitivity amplification of restricted DNA by PCR (SHARD-PCR) involves PCR and restriction digestion steps that are easy to implement even in less-equipped laboratories. Here we demonstrate the application of SHARD-PCR in analyzing BC samples. The method was tested on paired urine and tumor tissue samples from BC patients and the results were also validated through NGS. Our data obtained from 18 urine and corresponding tumor tissue samples by SHARD-PCR resulted identical mutation pattern between sample pairs, which were also perfectly consistent with the NGS results. Following this initial study, we tested a group of urine samples from BC patients and control individuals to evaluate the method's performance in a blinded manner. Our results demonstrate that SHARD-PCR is a simple, cost-effective, and reliable method for detecting TERTpms, which are common in various cancer types. In the context of bladder cancer, SHARD-PCR can be applied to noninvasive urine samples and has the potential to either replace or complement current techniques.

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P-31-016
Comparing targeted NGS panels and whole-exome sequencing for hereditary and somatic variant detection in oncology diagnostics

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Next-generation sequencing (NGS) plays a crucial role in detecting both hereditary and somatic variants, but the choice between targeted panels and whole-exome sequencing (WES) remains a subject of debate. This study compared the performance of two targeted Illumina NGS panels – TruSight Hereditary Cancer Panel and a custom-designed Myeloid panel – sequenced on the MiniSeq (Illumina, San Diego, CA, USA) platform with WES results obtained on the DNBSEQ G400 (MGI Tech, Shenzhen, China) platform. We analyzed samples from 32 patients, including EDTA whole blood for hereditary oncology cases and bone marrow for somatic hematological neoplasms. Variant detection accuracy, reading depth and variant allele frequency (VAF) were the primary comparison criteria. Hereditary variants detected by both targeted panels and WES were identical, with BRCA1 and BRCA2 variants confirmed using Sanger sequencing (for positive cases) and MLPA (for negative cases). However, inconsistencies were observed in somatic variants, particularly in cases of leukemia and myeloproliferative neoplasms, where WES failed to detect some low-VAF variants captured by targeted panels. Additionally, WES data were examined for pathogenic and likely pathogenic variants potentially linked to disease but not covered by the targeted panels. No significant additional findings were identified, indicating that WES did not provide clinically relevant variants beyond those detected by targeted panels. These results suggest that while WES offers broader genomic coverage, targeted panels may be more effective for detecting relevant variants in hereditary and hematologic malignancies, balancing sensitivity, specificity, and clinical utility.

P-31-017
Biclonal B-cell chronic lymphoproliferative disorder: a case report

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Chronic B-cell lymphoproliferative disorders (B-CLPD) are common hematological malignancies which are classified by exhibiting CD5, CD10, CD19, CD20, CD23, FMC7, ROR, and kappa-lambda surface immunoglobulin expression in flow cytometry for differential diagnosis. A 69-year-old patient with hypertension, hyperlipidemia, and diabetes mellitus had a complete blood count (CBC) on February 25, revealing WBC: 15.7 × 10/L, lymphocytes: 7.38 × 10/L (47.1%), hemoglobin: 11.5 g/dL, and PLT: 291 × 10/L. Persistent lymphocytosis was confirmed in May 2024. A peripheral smear showed 72.5% lymphocytes, atypical lymphocytes, and smudge cells. No lymphadenopathy, hepatomegaly, or splenomegaly was detected. Flow cytometry identified two B-cell clones, both CD5 + CD19 +, yet differing in other markers. Clone 1 (CLL-like) was CD5 +, CD23 +, CD5 + CD19 + (703 cells), CD19 + (1500 cells), FMC7-, ROR +, CD81dim, CD200 +, and kappa-lambda negative. Clone 2 (Non-CLL, unclassified) was CD19 +, CD5 +, CD22 +, CD23-, CD81 +, FMC7 +, ROR-, CD43 +, CD25-, CD103- and lambda monoclonal. While B-CLPD typically originates from a single B-cell clone, biclonal cases are rare. In this case we have seen this rare, atypic biclonal B lenfoproliferative disease immunophenotype findings. As in our case; a study of 5523 CLL cases reported 3.4% biclonality, linked to shorter treatment duration [Previously published in: Kern W et al. (2014) Br J Haematol 164, 565–569]. Another study of 507 patients identified 53 biclonal cases, including 24 with typical CLL, 4 with hairy cell leukemia, and 2 unclassified. They found biclonality associated with increased splenomegaly and early treatment requirements, lower WBC counts, and in CLL-Non-CLL biclonal cases presents higher incidence of B symptoms and diffuse bone marrow infiltration [previously published in Sanchez et al.(2003) Blood 102, 2994-3002]. Our patient was diagnosed with Biclonal B-Cell Chronic Lymphoproliferative Disorder (CLL-Non-CLL, Unclassified), pending genetic, clinical and immunophenotypic confirmation. *The authors marked with an asterisk equally contributed to the work.

P-31-018
Risk evaluation of indeterminate thyroid nodules: mass spectrometry and machine learning-based proteomics for biomarker discovery

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Thyroid carcinoma is the most common endocrine malignancy, with diagnostic challenges, particularly in distinguishing between benign and malignant thyroid nodules (TN) within the indeterminate category (25% of TN), leading to unnecessary surgery in 80% of cases. In this context, the identification of new biomarkers and the advancement of liquid biopsy are gaining interest. Mass spectrometry (MS)-based proteomics coupled with machine learning (ML) represents a suitable approach offering high specificity and sensitivity for exploring biological matrices. We included 16 patients with indeterminate TN, who, after histological assessment, were classified into 7 malignant carcinomas (M) and 9 benign adenomas (B). For each patient, 3 samples were collected: tissue from the pathological nodule, tissue from the healthy region of the thyroid (C), and plasma. All samples underwent a shotgun proteomic approach. Tryptic peptides were analysed by nano-RP-HPLC high resolution-ESI-MS/MS followed by label-free quantification and ML for statistical analysis. Random Forest analysis of the tissue proteome showed a high classification accuracy (93%) of M and B-TN. The Mann-Whitney test revealed 128 significant protein abundance variations ($pV < 0.05$, FDR < 5%) among the three groups, with the most prevalent changes showing increased levels in M (n. 41) and B (n. 50) TN compared to C. In the comparison between M-TN and B-TN, 14 proteins showed

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increased levels, while 6 were decreased in malignant nodules. These proteins are involved in metabolic processes and cellular motility. Plasma analysis highlighted 2 proteins with higher abundances in B compared to M: the complement system factors CFI and CFB, which have protease activity. CFI was also found to be altered in the tissue, showing increased levels in B compared to C. This pilot study found proteomic variations between M and B in both tissue and plasma, underscoring the project's potential and the possibility for further analysis.

P-31-019
Spectral biomarker detection for diagnosis of bladder cancer from urine using ATR-FTIR spectroscopy

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Bladder cancer is a common urological malignancy with a very high recurrence rate (up to 70% of cases). Cystoscopic examination is the gold standard for the diagnosis of bladder cancer, but it is still not considered satisfactory due to its subjectivity, low sensitivity, and time-consuming nature. Therefore, fast, cheap, non-invasive, and objective techniques are needed for early diagnosis of bladder cancer. Herein, we applied Fourier transform infrared spectroscopy coupled with multivariate analysis to identify spectral biomarkers of bladder cancer in human urine. The detailed spectral characterization studies showed significant spectroscopic differences between normal and bladder cancer tissues in the 1800-450 cm^{-1} region, which harbors urine, protein, creatinine, and nucleic acid in the malignant urine samples. The potential diagnostic markers were identified between the 1200-800 cm^{-1} region. Furthermore, the chemometric analysis demonstrated that healthy and high-grade cancer samples were successfully separated from each other. This study is a part of uCAIR Project, which is funded by the European Union's Horizon Europe research and innovation programme under Grant Agreement no. 101135175.

P-31-020
Evaluation of pseudogenes potential as biomarkers in head and neck cancer: insights into expression patterns and clinical implications

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Head and neck squamous cell carcinomas (HNSCC) present considerable challenges due to their aggressive nature and poor clinical outcomes. Recent research has highlighted the potential role of pseudogenes in cancer, particularly as biomarkers for diagnosis and prognosis. In our analysis, we focused on the expression patterns of pseudogenes ANXA2P2, PA2G4P4, AURKAPS1, HERC2P2, and SDHAP1 in HNSCC, applying data from The Cancer Genome Atlas. Findings revealed that ANXA2P2 and PA2G4P4 were significantly upregulated in tumor tissues compared to normal samples, with their expression correlating negatively with overall survival and varying by HPV status. Similarly, AURKAPS1 exhibited substantial upregulation linked to aggressive cancer phenotypes, while HERC2P2 and SDHAP1 levels depended on tumor localization. Furthermore, high expressions of AURKAPS1 and HERC2P2 were associated with improved survival. These pseudogenes are implicated in critical oncogenic pathways and present potential for future biomarker development in HNSCC, warranting further investigation into their roles in DNA repair and therapeutic response.

P-31-021
EGOT as a prognostic biomarker in cancer: insights from long non-coding RNA expression in HPV-related head and neck squamous cell carcinoma

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Long non-coding RNAs (lncRNAs) play significant roles in the epigenetic regulation of cellular phenotypes, with eosinophil granule ontogeny transcript (EGOT) being a notable example. Differences in EGOT expression have been linked to various pathological conditions, including tumorigenesis and viral infections. However, its biological function and potential diagnostic value remain poorly understood. We performed an analysis of EGOT using The Cancer Genome Atlas (TCGA) focusing on pathological and clinical features, cellular pathways, and genomic alterations. Our findings indicate that elevated EGOT expression is associated with improved survival in invasive breast carcinoma (BRCA), head and neck squamous cell carcinoma (HNSC), and kidney renal clear cell carcinoma (KIRC), while it correlates with poorer outcomes in liver hepatocellular carcinoma (LIHC). Expression patterns vary significantly among these cancers, influencing key cellular pathways, proliferation rates, wound healing capabilities, and intratumor heterogeneity, particularly in BRCA and KIRC. Notably, high EGOT levels were linked to better overall survival in HPV-positive HNSCC, suggesting its utility in distinguishing between HPV-positive and HPV-negative tumors. EGOT may serve as a promising prognostic biomarker, with potential implications for patient stratification and therapeutic decision-making in HNSCC, especially in relation to viral activity and immune response dynamics. This work was supported by Greater Poland Cancer Centre—grant no.: 22/02/2024/BAK/WCO/002 to ZC.

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P-31-022
Integrating circadian rhythms, redox biology, and proteomics for PDAC detection

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Pancreatic ductal adenocarcinoma (PDAC) remains one of the deadliest malignancies due to its asymptomatic progression and lack of reliable early detection strategies. Circadian rhythms regulate key cancer hallmarks, including oxidative stress and metabolic reprogramming, yet their role in biomarker discovery is poorly understood. This study employed a mass spectrometry-based label-free quantitative proteomics approach to investigate circadian-regulated protein expression in PDAC cell lines. Secretome profiling across a 24-hour cycle revealed rhythmic protein fluctuations associated with tumour metabolism, immune evasion, and redox homeostasis. Concurrent oxidative stress assays demonstrated a time-dependent imbalance in reactive oxygen species production and antioxidant activity, with PDAC cells exhibiting elevated oxidative stress and reduced antioxidant capacity compared to normal pancreatic cells. Subtype-specific differences were also evident. The quasi-mesenchymal subtype showed enhanced extracellular matrix (ECM) remodelling and metabolic reprogramming, while the classical subtype exhibited increased ECM degradation. These findings highlight the dynamic interplay between circadian rhythms and tumour progression, offering novel insights into PDAC pathophysiology. Our results underscore the potential of circadian-based biomarker panels for early PDAC detection and suggest that integrating temporal biology into oncology could improve diagnostic precision and therapeutic timing. Future work aims to validate these biomarkers in clinical samples and explore chronotherapy-driven treatment strategies.

LB-R-31-01
The emerging role of pax6 in pathogenesis and progression of acute myeloid leukemia: a bioinformatic study

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Acute myeloid leukemia (AML) is a heterogeneous and aggressive hematologic malignancy characterized by the clonal expansion of myeloid precursor cells in the bone marrow, peripheral blood, and other tissues. Recent advancements in targeted therapies, such as FLT3 inhibitors, IDH1/2 inhibitors, and monoclonal antibodies, have demonstrated efficacy in improving outcomes, particularly in specific molecular subgroups. PAX6, recognized as a master regulator, plays a critical role in cell differentiation, proliferation, and tissue patterning during embryogenesis. Consequently, we aimed to examine the potential prognostic features of the *PAX6* gene in AML utilizing various bioinformatics databases. We utilized the GEPIA2, UALCAN, KMplotter, Cancer Hallmark, and STRING databases to evaluate the prognostic properties of the *PAX6* gene. The *PAX6* gene was downregulated in AML compared to normal controls ($p < 0.05$). Furthermore, individuals with FTL3 mutation exhibited statistically significant upregulation compared to those without ($p < 0.05$). Low *PAX6* gene expression levels were associated with shorter overall survival status ($p = 0.036$). According to Cancer Hallmarks analysis, the *PAX6* gene was associated with replicative immortality ($p = 0.036$). Additionally, the *PAX6* gene demonstrated strong associations with DCAF7, SOX2, BANF1, SIX3, and TBR1 genes. Our findings suggest that PAX6 is downregulated in AML and associated with poor prognosis, particularly in patients with low expression levels. Its correlation with replicative immortality and key regulatory genes underscores its potential role in AML progression. Further investigations are warranted to elucidate its clinical and therapeutic implications.

LB-R-31-02
Monitoring of chromogranin A concentration in spinal schwannoma treatment outcome – a case report

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Chromogranin A is a glycoprotein that is released by neuroendocrine tumors in large quantities. Its greatest value is in monitoring and assessing the response to therapy. A decrease in the initial value by 50% is a good prognostic indicator. A schwannoma is a benign, slow-growing nerve sheath tumor composed of Schwann cells. They are most commonly seen in the lumbar region and comprise more than 20% of all primary tumors of the spinal cord. Spinal schwannomas arise on the roots of spinal nerves. The objective of this study was to show how monitoring serum concentrations of chromogranin A can serve as a valuable tool in monitoring neuroendocrine tumors. Serum and urine samples from a patient with L4 spinal nerve root Schwannoma were analyzed on the Dynex DSX® analyzer at Department of Clinical Biochemistry and Laboratory Medicine Clinical Center University of Sarajevo. A 67-year-old woman, has been observed at the Clinic for Nuclear Medicine and Endocrinology under the diagnosis of Diabetes mellitus type 2, Hyperinsulinemia and Hypoglycemia reactiva. The patient was observed for hypoglycemia in terms of a neuroendocrine tumor. For this reason, the level of 5-HIAA in the patient's urine is measured. The finding showed elevated values: 18.4 mg/day (ref range: 0-15 mg/day). It is indicated to perform a serum chromogranin A level test. The finding showed elevated values: 1986 ug/L (ref range: 0-100 ug/L). The patient was referred for histopathological diagnosis which showed that the tumorous tissue corresponds to a neuroendocrine tumor L4 spinal nerve root Schwannoma. The tumor was surgically removed. Two months later reanalysis showed a significantly lower value for chromogranin: 139.57 ug/L and for 5-HIAA: 1 mg/day. In conclusion, determining the level of chromogranin A can be used as a powerful tool for monitoring the effectiveness of treatment and detecting the recurrence of neuroendocrine tumors.

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LB-R-31-03
Unique untargeted lipidomics profiles along with transcriptomics data as a predictive and prognostic model of the incidence of inflammatory bowel disease-associated colorectal cancer

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The incidence of inflammatory bowel disease-associated colorectal cancer (IBD-CRC) is declining, but its prognosis remains poorer compared to sporadic colorectal cancer. Several distinct features of IBD-CRC may contribute to this difference in clinical outcomes. These include younger age at diagnosis, diagnosis under emergency or critical conditions, a higher likelihood of right-sided colonic involvement, and specific histological characteristics such as multifocal tumors, poorly differentiated or undifferentiated histology, and mucinous carcinomas. Therefore, precise risk stratification is essential for effective, personalized cancer surveillance by distinguishing high-risk individuals from those at lower risk. Both types of CRC – IBD-associated CRC (IBD-CRC) and sporadic CRC not associated with IBD – share common genetic factors such as APC, P53, MYC, KRAS, PIK3CA, SMAD4, and ARID1A. Moreover, specifically APC1A, APC2, SFRP1, and SFRP2 are characteristic of the progression from IBD to IBD-CRC. However, unlike sporadic CRC, IBD-CRC does not follow the conventional adenoma–carcinoma sequence; instead, it may progress from low-grade dysplasia to high-grade dysplasia, and ultimately to colorectal cancer. Untargeted lipidomics provides useful information about changes in signaling processes, especially in the tumor microenvironment. Lipidomics was performed with ultra-high performance liquid chromatography (UHPLC); Mass spectra were obtained using a IMS-QTOF; both systems from Waters (Milford, MA, USA). The untargeted lipidomics profiles were obtained in MSe mode. Analysis of gene expression (RT-qPCR) was conducted using real-time PCR supermix on Real-Time PCR thermocycler (Bio-Rad CFX1000) all primers were synthesized by Genomed (Warsaw, Poland). Untargeted lipidomics profiles along with targeted transcriptomics have high potential as machine learning model parameters to evaluate the risk of progression of IBD patients to colorectal cancer. *The authors marked with an asterisk equally contributed to the work.

Cancer therapy

P-32-001
Synergistic and resistance-modulating effects of U-359 ON 5-fluorouracil in breast cancer

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Breast cancer remains a leading cause of cancer-related mortality in women, underscoring the urgent need for innovative therapeutic strategies. Traditional chemotherapy, often limited by high toxicity and drug resistance, highlights the necessity for novel approaches to enhance treatment outcomes. This study focuses on the synergistic potential of the synthetic uracil analog, 3-p-bromophenyl-1-ethyl-5-methylidenedihydrouracil (U-359), in combination with 5-fluorouracil (5-FU) in MCF-7 breast cancer cells. 5-FU, a nucleoside analog, disrupts DNA synthesis by inhibiting thymidylate synthase and induces apoptosis through modulation of apoptotic regulators, including Bax/Bcl-2 and Bcl-xl. However, its clinical efficacy is often compromised by resistance and severe side effects. Our findings demonstrate that U-359 significantly enhances 5-FU-induced apoptosis, as evidenced by increased Caspase 3/9 activity and cleaved PARP1 levels. Furthermore, U-359 modulates key drug resistance proteins, including ABCB1, ABCG2, and NF-κB, which are associated with 5-FU resistance. Notably, the combination of U-359 and 5-FU resulted in robust synergistic effects, validated using the Bliss Independence Surface Model, particularly in reducing ABCG2 and NF-κB levels. This modulation not only sensitizes breast cancer cells to 5-FU but also optimizes dosing strategies, potentially reducing side effects. The selective cytotoxicity of U-359 towards cancer cells further underscores its therapeutic potential. In summary, our results highlight U-359 as a promising adjunct to 5-FU therapy, providing a compelling strategy to overcome chemotherapy resistance and improve breast cancer treatment outcomes. References: 1. Pieta M, et al. Tetrahedron. 2019; 75:2495-250. 2. Sedeta ET, et al. J Clin Oncol. 2023; 10528. 3. Długosz-Pokorska A, et al. Biologics. 2023; 69-83. 4. Sethy C, Kundu CN. Biomed Pharmacother. 2021; 137:111285.

P-32-002
Evaluation of *in vitro* photodynamic therapy properties and of pyridine-containing silicon (IV) phthalocyanines on lung cancer cells

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Lung cancer (LC) is the leading cause of cancer-related deaths worldwide according to World Health Organization reports. The limitations of most common treatments (surgery, chemotherapy, and radiotherapy) for LC have increased interest in innovative approaches like photodynamic therapy (PDT) which involves a photosensitizer (Pc), light, and oxygen. Once the Pc accumulates in the target tissue, it is activated by light of an appropriate

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wavelength, producing singlet oxygen that irreversibly destroys cancer cells. Phthalocyanines are promising Pcs due to their photochemical and photophysical properties. The aim of this study is to investigate the *in vitro* PDT activity of silicon (IV) phthalocyanine (P1SiQ and P2SiQ) containing water-soluble pyridine side group. The DNA/BSA interaction of compounds were determined using UV-Vis spectrophotometer and agarose gel/SDS-PAGE electrophoresis. Their cytotoxic/phototoxic properties were evaluated on human lung carcinoma (A549) cells using the MTT cell viability assay (white, 17.5 mW/cm^2, 15 and 30 min). The interactions of the compounds with ct-DNA were identified as partial intercalation based on the binding studies. DNA nuclease studies revealed that both compounds caused DNA and BSA damage by generating singlet oxygen. Also, both compounds exhibited phototoxic activity than MB, which used as a positive control ($*p < 0.001$). All these studies claims that P1SiQ and P2SiQ have significant potential for the treatment of LC, and further studies are recommended to support this hypothesis. This work was supported by Office of Scientific Research Projects of Karadeniz Technical University. Project number: TDI-2024-15932.

P-32-003
A ketogenic diet boosts neuroblastoma chemo- and immunotherapy

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Previous studies demonstrated that a ketogenic diet (KD) effectively targets neuroblastoma (NB) as a standalone treatment or in combination with chemotherapy in xenograft models. Emerging evidence suggests that the anticancer effects of a KD are partially mediated through its immune-modulatory properties. This study aimed to evaluate the efficacy of a KD in supporting chemo- and immunotherapy to reduce tumor growth and improve survival rates in a fully immunocompetent mouse model of NB. An immunocompetent NB model was established using allografts derived from the transgenic TH-MYCN mouse. A suspension of 2.5×10 cells, isolated from a TH-MYCN tumor, was mixed with 10% Matrigel and subcutaneously injected into the flanks of 6-week-old female wild-type 129X1/SvJ mice. When tumors reached ~75 mm^3, mice were randomized to receive either a KD with an 8:1 ketogenic ratio of fat to carbohydrates + protein or a control diet, alone or in combination with temozolomide chemotherapy (2.5 mg/kg, intraperitoneally, three times per week) and/or anti-GD2 immunotherapy (2 mg/kg, intraperitoneally, twice per week). Tumor sizes were measured twice weekly using calipers, and mice were euthanized when tumors reached 1300 mm^3. A KD significantly slowed the growth of MYCN-overexpressing NB allografts when combined with temozolomide and anti-GD2 treatment. The combination of a KD, chemotherapy, and immunotherapy (triple therapy) achieved the most potent anti-tumor effects, leading to a pronounced increase in survival in tumor-bearing mice. These findings revealed that a KD slows tumor growth in immunocompetent models of NB and synergizes with chemo- and immunotherapy to enhance therapeutic efficacy. This indicates a KD as a promising adjunct treatment strategy for neuroblastoma. Metabolomics, single cell transcriptomics and multiplexed immunofluorescence imaging will unravel potential underlying mechanisms of the KD-induced antitumor effects.

P-32-004
RNAvolution in CAR-T cell therapy – optimizing RNA-based generation of CAR-T cells

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Chimeric antigen receptor (CAR)-T cell therapy has emerged as one of the most prominent cancer immunotherapies. However, current commercial CAR-T cell products are generated by gene insertion using viral vectors, with a risk of insertional mutagenesis and an expensive and complex manufacturing process. Using RNA to transiently modify T cells addresses these shortcomings. Nevertheless, the rapid degradation and dilution of transfected CAR-mRNA limits CAR-T cell efficacy. Therefore, we aim to establish and optimize circular RNA (circRNA), self-amplifying RNA (saRNA) and trans-amplifying RNA (taRNA) to enhance RNA-based CAR-T cell therapy. These RNA formats extend transgene expression compared to mRNA by a higher stability (circRNA), or intracellular RNA amplification (saRNA & taRNA). Jurkat or primary T cells were electroporated with *in vitro* produced RNA to assess transgene expression and cell viability. Initial optimization studies with GFP revealed, for example, that the percentage of modified nucleotide incorporation inversely correlates with circRNA and saRNA expression. Furthermore, taRNA cytotoxicity was substantially improved by optimizing the RNA replicase complex. In a first head-to-head comparison of CAR-circRNA and CAR-mRNA in primary T cells, circRNA-CAR-T cells outperformed mRNA-CAR-T cells, demonstrating prolonged CAR expression. Future work will finalize RNA optimization and refine T cell transfection using lipid nanoparticles (LNPs) instead of electroporation. Engineering RNAs for CAR-T cell therapy, we ultimately aim to pave the way for a safer, more cost-effective and more widely accessible alternative to current CAR-T cell therapies.

P-32-005
Phytochemical profile of *Rheum ribes* and modulation of invasion, metastasis, and angiogenesis in breast cancer cell line

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Breast cancer is one of the most common malignancies among women worldwide. Research is ongoing to develop new therapeutic agents that are non-toxic or have lower toxicity, in order to improve prognosis and reduce mortality rates. This study aimed to investigate the antitumorigenic effects of two different extracts (ethanol and $EtOH{\cdot}H_2O$ (70:30)) obtained from three different parts (seed (S), rhizome (R) and shoot (Sh)) of the

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plant *Rheum ribes* (RR) by determining their total phenolic and flavonoid contents and antioxidant capacities on the breast cancer cell line MCF-7. RR was collected from the plateaus of Van by Prof. Dr. Ilhan Kaya Tekbudak in the spring. Ethanol and EtOH·H_2O (70:30) extracts were obtained from S, R and Sh fractions in the laboratory of Prof. Dr. Figen Zihnioglu from Ege University. The phenolic content of the samples was determined using the Folin-Ciocalteau method, while flavonoid content was assessed through aluminum chloride analysis. The antioxidant potential of the samples was quantified through DPPH analysis. After determining the IC_{50} concentrations of RR and docetaxel (DTX) in the MCF-7 cell line, a colony formation assay was performed to evaluate the effects of RR and DTX on clonal proliferation, and a soft agarose colony formation assay (SACFA) was conducted to assess the tumorigenic potential. The extracts prepared with EtOH·H O (70:30) were found to have statistically significantly higher total phenolic-flavonoid contents and antioxidant capacities ($p < 0.05$). The SACFA revealed a significant difference in spheroid sizes between the control group and the treatment groups ($p < 0.001$). It was observed that the treatments applied to the MCF-7 cell line inhibited cell proliferation. Based on quantitative analyses, we suggest that extracts with high phenolic and flavonoid content may have antitumorogenic effects in breast cancer cell lines by managing oxygen radicals. *The authors marked with an asterisk equally contributed to the work.

P-32-006
Targeting tissue transglutaminase to enhance ovarian cancer cells response to different therapeutic strategies

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Ovarian cancer (OC) is the seventh most common cancer in women worldwide and the eighth leading cause of cancer-related deaths in females. Late-stage diagnosis caused by lack of early symptoms results in poor prognosis and limited treatment options. Tissue transglutaminase (TG2) is upregulated in ovarian tumours and promotes cancer dissemination by forming a complex with fibronectin (FN) and integrins, thereby enhancing cell adhesion to the extracellular matrix. More recently, we started investigating the potential of TG2 targeting to sensitize OC cells to new FLASH radiation sources, such as electrons generated with a high-intensity laser plasma accelerator system[1] or pulsed X-rays. Our previous work identified MT-4 as a promising small molecule inhibitor of the TG2-FN protein-protein interaction sensitizing OC cells to paclitaxel[2]. We then characterized five new MT-4 analogues (#2997, #2998, #3002, #3010, #3011) for their effects on OC cells. TG2KO cells were used as controls for drug target specificity. Out of these 5 compounds, #3011 abrogated sphere formation, while #3002 reduced OC cell adhesion. Despite the potential of TG2-FN targeting, some OC cells evaded this inhibition and attached to fibronectin. To address this resistance, we used SILAC-based phosphoproteomics to explore adaptive signalling pathways in OC cells treated with MT-4 and identified sirtuins and p21-activated kinases (PAKs) as upregulated proteins. Co-targeting SIRT1 with TG2-FN inhibitors reduced spheroid formation, colony formation, and induced cytotoxicity in OVCAR5 cells in 2D as well as 3D culture conditions, while co-targeting PAK1 and TG2-FN interaction enhanced inhibition of cell adhesion. These findings highlight promising molecular co-targets to overcome resistance to TG2-FN inhibitors. Further studies are needed to validate their role in preventing OC progression and metastasis. References: 1. Orobeti S et al. (2024), Sci Rep 14, 14866 2. Sima LE et al. (2019), Mol Cancer Ther 18(6), 1057-1068 *The authors marked with an asterisk equally contributed to the work.

P-32-007
Imipramine regulates cellular survival by inducing endoplasmic reticulum stress in cervical cancer cells

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Cervical cancer is the fourth most prevalent cancer affecting women worldwide. *SLC12A5* has been identified as an oncogene, playing a role in cancer progression. This study aims to investigate the inhibitory effects of imipramine on endoplasmic reticulum (ER) stress and apoptosis mediated by SLC12A5 in HeLa cervical cancer cells. Initially, the cytotoxicity of imipramine was assessed in the HeLa cell line. Next, changes in cellular morphology following imipramine treatment were examined. Furthermore, the study analyzed the impact of imipramine on the expression levels of various proteins, including SLC12A5, C/EBP homologous protein (CHOP), glucose-regulated protein-78 (GRP78), activating transcription factor-6 (ATF6), caspase-3 (CASP3), and cytochrome c (CYC), in these cells. The half-maximal inhibitory concentration (IC_{50}) value of imipramine for HeLa cells was determined to be 260 μM. Treatment with this concentration, as well as an intermediate concentration of 150 μM, resulted in significant morphological and nuclear abnormalities in HeLa cells. Following 24 hours of imipramine exposure, the levels of SLC12A5, ATF6, CHOP, GRP78, CASP3, and CYC were altered in comparison to untreated control cells. Notably, imipramine treatment led to a reduction in SLC12A5 expression, while the levels of ATF6, CHOP, GRP78, CASP3, and CYC significantly increased. In conclusion, these findings underscore the therapeutic potential of imipramine in regulating ER stress and inducing apoptosis in HeLa cells by targeting SLC12A5.

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P-32-008
A new reporter system for the identification of eIF4F inhibitors: validation of the compounds identified in the high-throughput screening

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The eIF4F translation initiation complex plays a critical role in cancer. The complex's enhanced activity was identified as a nexus of therapy resistance and a promising target in melanoma. However, the spectrum of available eIF4F inhibitors is limited, and none is in clinical use. One reason could lie in the relative complexity of techniques used to identify such inhibitors. Based on our previous finding that eIF4F functions in the negative regulation of the RAS/RAF/MEK/ERK mitogen-activated protein kinase (MAPK) signaling pathway, we were able to build a unique cell-based reporter system suitable for the high-throughput identification of novel eIF4F inhibitors in small-molecule compound libraries. The system responds to eIF4F inhibition by changes in luciferase expression in a dose-dependent manner. The reporter system was tested in a panel of cell lines, determining the impact of eIF4F inhibition on luciferase activity. Its applicability for the high-throughput screening (HTS) of bioactive compounds was verified in 384- and 1536-well format. Several highly diverse libraries comprising over 80 thousand compounds were assessed in the HTS. Surprisingly, apart from compounds potentially targeting eIF4F, the screening revealed possible new ways of ERK pathway regulation. We are currently validating the most potent compounds in orthogonal assays.

P-32-009
Role of the AKT1 and CTNNB1 mutations in paclitaxel resistance

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Drug resistance is a major limiting factor in cancer treatment. Tumour cells exhibit high adaptability and develop resistance through various molecular mechanisms. Paclitaxel (PTX) is a taxane-based chemotherapy drug used to treat breast cancer (BC), and many molecular alterations, including mutations, are closely associated with PTX resistance. Therefore, identifying mutations responsible for PTX resistance is crucial. The PI3K/AKT and WNT/β-catenin signaling pathways are two important pathways involved in essential cellular processes such as proliferation, apoptosis, and maintenance of cellular homeostasis. Abnormal activation of these pathways is frequently observed in BC. In the present study, the impact of missense mutations in the *AKT1* and *CTNNB1* genes, which are key components of the PI3K/AKT and WNT/β-catenin signaling pathways, on PTX resistance in BC cells (MCF-7 and MDA-MB-231) was investigated for the first time *in vitro*. The effects of *AKT1* (E17K/E49K/L52R) and *CTNNB1* (S33P/T41A/S45F) mutations on PTX resistance were analyzed using site-directed mutagenesis, transfection, MTS, and western blot methods. The results demonstrated that *AKT1* mutations were associated with PTX resistance in both cell lines, whereas *CTNNB1* mutations were only associated with PTX resistance in MCF-7 cells. Our findings suggest that *AKT1* and *CTNNB1* mutations, which contribute to PTX resistance in BC cells, could potentially serve as biomarkers for the development of personalized cancer treatment protocols, when supported by further *in vivo* and clinical studies.

P-32-010
Combining colchicine with autophagy modulators to overcome drug resistance in 2D and 3D models of lung cancer cells

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Lung adenocarcinomas are characterized by inherent drug resistance and the further development of resistance during long-term treatment, presenting a significant challenge in lung cancer therapy. Colchicine (Col), a mitotic poison with promising antitumor effects, is limited in clinical application for lung cancer due to its ability to induce cytoprotective autophagy, a process that contributes to chemoresistance. Here, we investigate strategies to enhance the therapeutic efficacy of Col by combining it either with (i) a quinoline autophagy inhibitor (QIH) to block Col-induced autophagy, or (ii) autophagy inducers based on quinoline (QID) or statin (SID) structures to promote cytotoxic autophagy. The cytotoxic effect of each combination was evaluated in both 2D and 3D models (spheroids) of inherently Col-resistant lung adenocarcinoma cells (A549) and in A549-R cells, the in-house-developed cells with further increased Col resistance after long-term treatment. Fluorescent labeling of the autophagosomes confirmed that Col/QID acted synergistically via the induction of cytotoxic autophagy. In contrast, stimulated emission depletion microscopy revealed that Col/SID stabilized and condensed microtubules (MT). Among the compound combinations tested, Col/QIH proved the most promising for inducing synergistic cytotoxicity via MT stabilization and the inhibition of Col-induced cytoprotective autophagy in both A549 and A549-R cells. Thus, the combination of Col with autophagy modulators represents a potent strategy for overcoming drug resistance, improving the clinical applicability of Col in treating lung adenocarcinomas.

P-32-011
P-glycoprotein inhibitors reverse colchicine resistance in both 2D and 3D *in vitro* breast cancer models

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Colchicine, a mitotic poison targeting rapidly dividing cells, has shown promise in cancer therapy. However, its clinical use faces challenges, including possible cellular resistance caused by the overexpression of P-glycoprotein (P-gp). In this study, we evaluated the ability of colchicine combined with two P-gp inhibitors, based on aryl sulfide and dicarboxylic acid diamide structural motif, to resensitize resistant breast cancer cells. First, the combined effect was determined in MCF-7 and UFH-001 cell growing in monolayer by WST-1 assay. The results showed that the inhibitors exhibited stronger synergistic cytotoxic effects in resistant cancer cells compared to non-resistant ones. Next, to confirm that the effect was caused by P-gp inhibition, P-gp

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expression and activity were analysed using immunoblot and fluorometric multidrug resistance assay respectively. Both compounds inhibited P-gp activity in a concentration-dependent manner and significantly inhibited the expression of P-gp. Finally, 3D models of the same cell lines in form of spheroids were used to better mimic the *in vivo* conditions. Colchicine demonstrated strong cytotoxic effect in combinations with P-gp inhibitors in the MCF-7 and UFH-001 spheroids; indeed, their synergistic effects were comparable to those observed in 2D cultures. Our results indicate that the combination of colchicine and P-gp inhibitors has considerable potential for the treatment of colchicine-resistant cancers.

P-32-012
Synergistic potential of colchicine combined with selected anticancer drugs for enhanced anticancer effect

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Cancer remains a leading cause of death worldwide, driving the need for more effective and selective therapeutic strategies. Colchicine, a potent microtubule-disrupting agent, has demonstrated promising antiproliferative and systemic antitumor activity. However, its clinical application is limited by a narrow therapeutic window, toxicity, and resistance development. To overcome these limitations, this study explores the synergistic potential of colchicine in combination with various anticancer agents to enhance its therapeutic efficacy while reducing systemic toxicity. The half-maximal inhibitory concentrations (IC_{50}) of colchicine and selected drugs were determined in human breast cancer cell line (MCF-7) at 24- and 72-hour time points. Synergistic effects were assessed using the SICoDEA Bliss independence model at both time points. Our findings reveal significant synergy between colchicine and specific anticancer agents, leading to enhanced cancer cell death, reduced effective drug concentrations, and improved selectivity for malignant cells. These results underscore the potential of colchicine-based combination therapies as a promising strategy for overcoming drug resistance and optimizing cancer treatment. Future studies will focus on uncovering the molecular mechanisms and validating these discoveries through alternative approaches, ultimately contributing to more effective and personalized cancer therapy.

P-32-013
Targeting HSF1 reduces growth and increases the efficiency of 4′hydroxytamoxifen treatment in ER+-positive breast cancer cells

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Breast cancer is the most common malignancy in women. Approximately 70% of all cases are ER-positive (ER+; expressing estrogen receptors), and their growth is estrogen-dependent. They are first treated with hormonal therapy based on ER antagonists (i.e. tamoxifen) or inhibitors of estrogen synthesis. About 20-40% of patients acquire resistance to therapy, which is clinically manifested with relapse or metastases. Our recent studies have shown the potential role of heat shock transcription factor 1 (HSF1) in ER+ breast cancer cell growth and its resistance to hormonal therapy. HSF1 plays a key role in the cell response to stress, and also supports many aspects of cell metabolism enabling tumor growth. Our study was directed to demonstrate the utility of HSF1 targeting in the inhibition of ER+ breast cancer cell growth and enhancing the efficiency of tamoxifen-based therapy. We tested the effects of several HSF1 inhibitors (CCT251236, KRIBB, and DTHIB) on the proliferation of ER+ breast cancer cells (MCF7, T47D, and BT-474) and their response to 4′hydroxytamoxifen (4′OHT, the active metabolite of tamoxifen). We showed that the tested compounds inhibited the expression of HSP genes (molecular targets of HSF1). They also reduced breast cancer cell growth at 2D and 3D conditions. HSF1 inhibitors, except for KRIBB, increased the sensitivity of cells to 4′OHT. Interestingly, the inhibitory concentration of the DTHIB is lower for breast cancer cells compared to its doses necessary to reduce the proliferation of non-tumorigenic mammary epithelial MCF10A cells. No such effects were demonstrated for the CCT251236 compound. Our results indicate that pharmacological inhibition of HSF1 may affect the growth of ER+ breast cancer cells and increase their sensitivity to tamoxifen. It is worth noting that ER+ breast cancer cells were more sensitive to the DTHIB than non-tumorigenic breast cells. The work was supported by the National Science Center, grant no. 2021/43/B/NZ5/01850.

P-32-014
Targeting ferroptosis: a lipophilic ruthenium(III) complex as a novel weapon against triple-negative breast cancer

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In recent years, studies have shown hard-to-treat triple-negative breast cancer (TNBC) as more sensitive to ferroptosis, thereby providing the opportunity to explore new targets for cancer therapy. Ferroptosis is featured by uncontrolled lipid peroxidation and membrane injury caused by unbalanced redox homeostasis and iron-dependent oxidative stress[1]. However, recent evidence demonstrates attractive crosstalk between different programmed cell death pathways, e.g., ferroptosis and apoptosis[2]. In this context, our research team has showcased that Ru(III)-based complexes can serve as pro-apoptotic multitarget agents capable of effectively inhibiting proliferation, migration and invasion of breast cancer (BC) cells[3]. We here demonstrate that ferroptosis is implicated in the mechanism of action of a novel lipophilic Ru(III) complex named PalmiPyRu, which has proven to be effective against the TNBC[4]. Following treatments *in vitro*, we show significant effects on iron homeostasis via changes in cellular content of iron regulatory proteins, transferrin receptor, ferritin and ferroportin, leading to labile

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iron pool enlargement and consequent iron-dependent oxidative stress. Moreover, targeted experiments show reactive oxygen species (ROS) production with oxidative damage, coupled to a consistent decrease in cellular antioxidant potential. Glutathione (GSH) levels are significantly decreased after treatment with PalmiPyRu, as well as the expression of glutathione peroxidases (GPXs). Distinctive cytomorphological hallmarks highlight that TNBC phenotypes are directed towards a ferroptotic death pathway. These findings prove the potential of PalmiPyRu as a candidate anticancer drug in effectively inhibiting cancer cell proliferation through multimodal molecular mechanisms.
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P-32-015
Novel peptides targeting epidermal growth factor receptor for a selective cancer therapy

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The epidermal growth factor receptor (EGFR) is a well-studied membrane receptor, and its overexpression has been observed in a range of tumor types. Some therapeutic agents targeting EGFR are already in use; however, cancer cells can easily develop resistance to these treatments, and thus, there is significant research currently underway to develop new treatments that target EGFR and its family members. One promising approach is to design new blocking and/or targeting peptides. In this study, we present a computational approach for the design of four targeting peptides (1-4). The design was conducted using Rosetta software and a review of pertinent bibliographic data. Affinity assays demonstrated their effective binding to EGFR, particularly peptides 1, 2, and 4. Biological tests of these peptides in pancreatic adenocarcinoma BxPC3 cells, which overexpress EGFR, demonstrated that they are unable to activate the receptor pathway. However, they could interact with EGFR and compete with wild-type EGF. Moreover, western blotting studies revealed that only peptide 2 slightly stimulated EGFR activation and subsequent Erk/Akt phosphorylation. Proliferation experiments confirmed the inhibition of cell growth and subsequent death by these proteins in a two-dimensional culture model and in a tumoral spheroid model. In addition, western blotting experiments confirmed the activation of the EGFR degradation pathway, specially by peptide 1. Furthermore, proteins 1, 2, and 3 induced EGFR internalization from the cell surface. Of these, peptide 4 displayed the most promising blocking activity, while peptide 1 was the most internalized and recycled by the cell. The results presented here demonstrate the antitumor efficacy of these peptides and encourage further investigation of their efficacy in animal models. In the case of 1, this may demonstrate its potential for the targeted delivery of cytotoxic agents to cancer cells overexpressing EGFR.

P-32-016
Biological characterization of newly synthesized molybdenum nanoparticles for photothermal tumor therapy

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Pancreatic ductal adenocarcinoma (PDAC) is one of the leading causes of cancer-related mortality in Europe [1]. Photothermal therapy (PTT), which uses plasmonic nanoparticles (NPs) to absorb laser light in the near-infrared (NIR) biological windows, enables selective tumor cell targeting without harming healthy tissue [2]. Non-stoichiometric molybdenum oxide (MoO_x) is a cost-effective nanomaterial with strong photothermal properties, and its functionalization with antibodies against tumor-associated proteins, such as carbonic anhydrase IX (CAIX), enhances its specificity and efficiency [3]. PTT at high temperatures (> 50°C) induces necrosis and inflammation, whereas mild hyperthermia (< 48°C) shifts the cell death mechanism toward apoptosis, reducing adverse effects [4]. This work evaluated MoO_x-M75 NPs, functionalized with an anti-CAIX antibody, in PTT *in vitro* in 2D and 3D pancreatic cancer models. Upon NIR-I (808 nm) laser irradiation, MoO_x-M75 increased the temperature by 34°C in solution and nearly by 8°C in tumor cell cultures. NPs were internalized via clathrin- and caveolin-mediated endocytosis, localizing in early and late endosomes and lysosomes, as detected by indirect immunofluorescence and confocal microscopy. Flow cytometry and confocal imaging confirmed that PTT treatment led to a > 42% increase in apoptotic cells and a ~30% increase in necrotic cells, as indicated by caspase-3/7 activation, γH2AX detection, and Zombie Violet™ staining. These results demonstrate the efficacy of nanoparticle-mediated mild hyperthermic PTT using MoO_x-M75 and support its potential for pancreatic cancer therapy.
This work was supported by the Slovak Research and Development Agency (APVV-20-0485), and by SAS Grants for PhD Students (DoktoGrant APP0662).
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P-32-017
Modelling organoids from 3D spatial cartography expands the scope of precision medicine in breast cancer

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Molecular heterogeneity is a common phenomenon within tumours and often impacts on prognosis and response to treatment. In particular, recent studies emphasise the presence of metabolic heterogeneity within tumours that is partly intrinsic or can be imposed by changes in the tumour microenvironment (TME). Here, we use a multimodal MSI (Mass Spectrometry Imaging) approach comprising desorption electrospray ionization (DESI) and matrix-assisted laser desorption/ionization (MALDI, complemented with IMC (imaging mass cytometry) protein profiling to stratify breast cancer (BC) into metabolically distinct regions (metabotypes) that are present both within and between tumours. The high-throughput genetic characterisation of these metabotypes revealed the presence of clinically relevant genetic signatures that are highly predictive of responses to targeted therapies. These metabotypes were modelled in representative patient-derived organoids (PDOs) that helped to identify hidden vulnerabilities within highly heterogeneous tumours. This advanced metabolic profiling workflow has the potential to identify distinct regions within tumours with differential therapeutic vulnerabilities, hence providing an entirely novel approach to overcoming the challenge of tumour heterogeneity and expanding precision medicine in breast cancer.

P-32-018
Targeting PERK-lipid crosstalk as a strategy for cancer therapeutics

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Protein kinase R-like endoplasmic reticulum kinase (PERK) exhibits haploinsufficient tumor-suppressive activity in melanocytes. Effective targeting of PERK in cancer requires a detailed understanding of its dual functions. Our studies reveal how PERK regulates cell homeostasis under stress and provide critical insights for designing novel anti-cancer therapeutics targeting PERK and its lipid interactions. Lipid extractions and analyses were performed by Lipidomics Shared Resource, MUSC. Preparation of samples and analyses of endogenous bioactive sphingolipids was performed using a Thermo Scientific Vanquish Ultra-High Performance Liquid Chromatography (HPLC-MS/MS). Sphingosine binding to PERK and regulation of PERK activity were assessed using a radiometric kinase assay and a photoactivatable and clickable pacD-e-Sph system. Lipid-protein interactions were analyzed in real-time using surface plasmon resonance. WM239A melanoma cells and MEF cells were used in experiments. Our studies revealed that PERK possesses lipid kinase activity, directly phosphorylates diacylglycerol a precursor for phosphatidic acid and exhibits mitogenic properties that contribute to the activation of the RAS, MAPK, AKT and mTOR signaling pathways. We have determined the role of lipids, such as sphingolipids and ceramides, in modulating PERK activity. Sphingolipids are essential structural components of cellular membranes, but they also exert diverse regulatory effects on cell signaling pathways that mediate growth, proliferation, motility, adhesion, differentiation, senescence and apoptosis. We identified that D-e-Sph directly binds to PERK and acts as an endogenous PERK inhibitor. The specific domain of PERK required for D-e-Sph binding. Our studies will provide critical insights for designing novel anti-cancer therapeutics targeting PERK and its lipid interactions. This work was supported by Polish National Science Centre (NCN) Grant OPUS No. 2019/33/B/NZ5/03034; National Institutes of Health grants P01CA165997, P30CA138313, P30GM103339.

P-32-019
Investigating the effect of gilteritinib on FLT3 expression in acute myeloid leukemia

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Acute myeloid leukemia (AML) exhibits significant genetic heterogeneity, with FLT3 mutations representing a prevalent and adverse molecular aberration. While gilteritinib, a potent type I FLT3 tyrosine kinase inhibitor, has gained FDA approval for relapsed/refractory FLT3-mutated AML, elucidating its comprehensive mechanism of action is essential for therapeutic resistance management and biomarker identification. This investigation aimed to characterize modulatory effects of gilteritinib on FLT3 expression. The study demonstrated that gilteritinib administration induced dose-dependent suppression of both FLT3 phosphorylation and protein expression, with maximal inhibition observed following 48 hour exposure. Notably, the reduction in FLT3 protein levels occurred independently of alterations in FLT3 mRNA transcription, suggesting post-transcriptional regulatory mechanisms. Analysis across multiple AML cell lines revealed that gilteritinib-mediated FLT3 downregulation was observed in both FLT3 wild-type and

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mutant variants. Comparative evaluation of alternative FLT3 inhibitors, including midostaurin, crenolanib, and quizartinib, demonstrated analogous FLT3-suppressive capabilities, corroborating gilteritinib's effects. While these findings have significant therapeutic implications for optimizing gilteritinib-based interventions in AML, further investigation is warranted to elucidate the precise molecular mechanisms and clinical significance of FLT3 downregulation.

P-32-020
Dubermatinib synergistically enhances the anti-cancer activity of cisplatin in A549 cell line

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Lung cancer (LC) is the leading cause of cancer-related death. Non-small cell lung cancer (NSCLC) constitutes approximately 80% of all lung tumors. Platinum-based chemotherapeutics such as cisplatin (DDP) are an important treatment strategy for NSCLC. AXL is a member of receptor tyrosine kinases (RTKs) and is associated with poor prognosis in cancer. Mono- and combination therapies of many inhibitors targeting AXL have shown promising results in the search for cancer treatment. This study aimed to investigate the effects of the combination of Dubermatinib (TP-0903), an AXL inhibitor, and DDP in the A549 NSCLC cell line. Half-maximal drug inhibitory concentration (IC_{50}) values of both DDP and TP-0903 were calculated in A549 cells. Cell viability was determined by the SRB assay, and a statistically significant was considered as $p < 0.05$. Cells were incubated with TP/DDP monotherapies and TP + DDP combination for 72 hours to determine the anti-proliferative effect. Synergistic interactions of drugs were evaluated by calculating combination indexes with Sinergyfinder. The anti-cancer effects of effective drug combinations were evaluated using wound healing and colony formation assays. The combination of TP with DDP showed synergistic anti-cancer effects by reducing cell viability, colony formation ability, and cell migration in A549 cells. Combining dubermatinib with cisplatin may produce promising results in treating non-small cell lung cancer. Acknowledgements: This study was supported by Bursa Uludag University Scientific Research Projects Coordination Office with project number THIZ-2023-1647. We would like to thank them for their support to the Project.

P-32-021
The effect of dubermatinib (TP-0903) combined with gemcitabine on apoptosis and anti-cancer activity in BxPC-3 cell line

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Pancreatic ductal adenocarcinoma (PDAC) is one of the lethal solid tumors due to limited surgery and chemoresistance development. Receptor tyrosine kinases (RTKs) are a class of therapeutic targets that have provided successful treatments for various cancers, and AXL is also a member of RTKs. Dubermatinib (TP-0903) is an orally bioavailable small molecule AXL inhibitor that has passed evaluation in the first human study in patients with solid tumors. This study aimed to investigate the anti-cancer activity and effect on apoptotic markers of combined treatment with gemcitabine (GEM) and TP-0903 in the BxPC-3 cell line, a human pancreatic cancer cell line. BxPC-3 cells were treated with GEM, TP-0903, and a combination of both drugs. The cytotoxic effect on the cells was evaluated using the SRB assay. qRT-PCR determined Bcl-2 and Caspase-3 expression levels to evaluate apoptosis. The wound healing assay was performed to understand the effect of the treatment on cellular migration, and the colony formation assay was conducted to analyze clonogenic potential. Student's *t*-test was used for statistical analysis, and $p < 0.05$ was considered significant. Dubermatinib enhanced the antiproliferative effect of gemcitabine by inducing apoptosis in BxPC-3 cells. At the same time, combined treatment of GEM and TP showed synergistic anti-cancer effects by reducing cell migration and colony formation ability in BxPC-3 cells. The combination of dubermatinib and gemcitabine may be a promising drug potential for treating pancreatic cancer. Acknowledgements: This study was supported by TUBITAK within the scope of project number 124Z994 and Bursa Uludag University Scientific Research Projects Coordination Office with project numbers THIZ-2022-1044 and TGA-2024-1461. We would like to thank BUU SRP Coordination Office and TUBITAK for their support to the Project.

P-32-022
Optimization of glioblastoma viral therapy using the JAK/STAT inhibitor sunitinib

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The use of oncolytic viruses to preferentially infect and eliminate cancer cells while sparing normal cells represents a promising therapeutic approach for treating glioblastoma that is resistant to chemotherapy and radiotherapy. However, the efficacy of oncolytic virotherapy is often limited by innate immunity pathways, particularly by the induction of interferon type 1. We investigated the effect of interferon type 1 on protecting cells from viral oncolysis, as well as the impact of the JAK/STAT inhibitor sunitinib on enhancing lytic efficacy, using a panel of primary glioblastoma cultures and model glioblastoma cell lines (CT2A, GL261). We assessed the sensitivity of nine cell lines to twenty-five oncolytic viruses from various families. We discovered that non-pathogenic oncolytic strains can overcome the interferon-induced antiviral state. Our results indicate a decreased sensitivity of glioblastoma cells after interferon treatment, yet most of the tested enteroviruses were able to establish productive infections in tumor cells even after treatment with the highest concentrations of IFNα2β (< 500 U/ml). Conversely, the tested paramyxoviruses (Sendai and Newcastle disease virus) and vesicular stomatitis virus did not exhibit effective oncolytic properties following treatment with 30 U/ml. Treatment with sunitinib at non-toxic doses significantly enhanced the sensitivity of all tested cell cultures to viral infection. Given that sunitinib is an effective antitumoral drug for glioblastoma, its combination with oncolytic viruses may offer a safe and efficient therapy for recurrent glioblastoma. *In*

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vivo, sunitinib can dramatically amplify the oncolytic effects of these viruses. This research was supported by the Russian Science Foundation (grant no. 23-74-10102).

P-32-023
Senolytic potential of novel thioquercetin derivatives against cisplatin-induced senescent melanoma cells *in vitro*

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The senolytic activity (selective killing of senescent cells) of quercetin (3,3',4',5,7-pentahydroxyflavone), a naturally occurring flavonoid, has been reported. However, the potent and broad-spectrum senolytic activity of quercetin is only achieved upon co-treatment with the anticancer drug tyrosine kinase inhibitor, dasatinib. Thus, there is a need to design and obtain novel derivatives of quercetin with potentiated senolytic activity. This approach is especially important for the elimination of drug-resistant senescent cancer cells activated upon therapeutic treatment. In the present study, three novel thioquercetin derivatives (thioQ, thioQAc$_4$, and thioQAc$_5$) were synthesized and tested against several cellular models of melanoma with cisplatin-induced senescence program. As targeting the HSP90 activity also resulted in the induction of cell death signaling in different types of senescent cells, the senolytic action of thioQs was also evaluated upon co-treatment with 17-DMAG, a HSP90 inhibitor. Thioquercetin derivatives promoted apoptotic cell death in drug-induced senescent melanoma cells that was potentiated when 17-DMAG was added. These cell death results were associated with elevated production of reactive oxygen species (ROS). Several markers of cell death, proliferation, senescence, and inflammation were also assayed using imaging cytometry (BCL-2, CASP3, HSP70, HSP90, p21, p27, p57, AKT, pAKT, SA-beta-galactosidase activity, NF-κB, IL-6, and IL-8). In conclusion, thioquercetin derivatives, novel potential senolytic drugs, are proposed as evidenced using chemotherapy-induced senescence model and several melanoma cell lines *in vitro*. This work was supported by the National Science Centre (NCN, Poland) within M-ERA.NET 3 Call 2022 grant no. 2022/04/Y/ST5/00155 and PCI2023-143448 funded by MICIU/AEI/10.13039/501100011033 and co-funded by the European Union.

P-32-024
The impact of microfluidics-based bortezomib-loaded PLGA and PEG-PLGA nanoparticles on multiple myeloma cells

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Bortezomib is an antineoplastic agent which has been commonly used in multiple myeloma (MM) treatment. However, the resistance to bortezomib necessitates novel drug delivery approaches. In this regard, nanocarriers based on poly (lactic-co-glycolic acid) (PLGA), an FDA-approved biocompatible polymer, improve drug targeting when combined with polyethylene glycol (PEG). The preparation method of PLGA nanoparticles critically influences their size, cellular uptake, circulation, and tumor-targeting efficiency. In this study, PLGA and PEG-PLGA nanoparticles encapsulating bortezomib were prepared using a microfluidic device, ensuring monodispersity and reproducibility. To enhance nanoparticle synthesis, a herringbone microfluidic mixer was utilized, which increases surface area exchange by generating micro-vortices within a protruding micro-channel. Atomic force microscopy (AFM) analyses revealed a spherical and smooth morphology for both nanoparticle types. The size of nanoparticles ranged from 100 to 200 nm according to the performed Dynamic light scattering (DLS) measurements. The bortezomib encapsulation efficiency was greater for PEG-PLGA (20%) nanoparticles in comparison to PLGA (15%) nanoparticles. Drug release studies revealed that PLGA nanoparticles exhibited a higher drug release rate at basic pH, while the release was higher for PEG-PLGA nanoparticles under acidic conditions. The cytotoxicity was evaluated on RPMI 8226 MM cells over 48 hours, when comparing half-maximal drug inhibitory concentration (IC_{50}) values of free bortezomib (15 nM), PLGA (7 nM), and PEG-PLGA (5 nM) formulations. The results demonstrated that PEGylation enhanced the nanoparticle circulation and accumulation in MM cells, increasing the cytotoxicity. These findings underscore the reproducibility and biocompatibility of bortezomib-loaded PLGA and PEG-PLGA nanoparticles synthesized via microfluidics. This study offers valuable insights into nanoparticle-based drug delivery in MM and may contribute to future clinical research efforts.

P-32-025
The effect of combined drug therapy in prostate cancer cell lines

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Prostate cancer is one of the highly aggressive cancer types in elderly men. The therapy options may not be effective because of using alternative growth pathways. At that point, the solitary treatments would be inadequate. Nowadays, the combinatory drug approaches as novel treatments are better options for therapy. In our study, three essential pathways (c-MYC, WNT, and SHH) that have roles in cell proliferation, cell cycle, maintenance of stemness were targeted with small molecule inhibitors. KJ-Pyr-9 inhibits the c-MYC pathway by disrupting the MYC/Max complex. Pyrvinium pamoate inhibits the WNT pathway in an indirect way. Glasdegib is an inhibitor of the SHH pathway by blocking Smo protein. The half-maximal drug inhibitory concentration (IC_{50}) and IC_{25} doses of each inhibitor were determined on the PC3 cell line, then dose optimization studies were performed for combination. The optimized doses were applied on both 2D and 3D cultures. The combination of inhibitors showed a synergistic effect on 2D against prostate cancer cell lines PC3 and LNCaP. The migration rate of both cell lines decreased upon combined treatment. The combined treatment diminished both viability and size of 3D spheroids of

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PC3 and LNCaP cells. The proposed combined treatment in this study is novel and promising to overcome drug resistance and side effects of single agents. *The authors marked with an asterisk equally contributed to the work.

P-32-026
Evaluation of apigenin-chrysin-galangin combination on MCF-7 and MCF-10A cell lines: a potent anti-carcinogenic agent

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Flavonoids have been demonstrated to be useful in various cancer prevention mechanisms; use of flavonoids in combination to prevent cancer has been the subject of numerous studies. Breast cancer is still the most common cancer among women worldwide and chemotherapeutic drug side effects and resistance development continue to be major issues. To reduce negative effects of toxic chemotherapy and create new complementary therapy agents, researchers have therefore looked to natural products like flavonoids. For this reason, we aimed to investigate combined and synergistic effects of apigenin, chrysin and galangin on MCF-7 breast cancer cell line and MCF-10A fibrocystic breast epithelium, as control. We performed the cell proliferation experiments utilizing WST-1, apoptotic status by using Annexin V-PI assay. Also, we executed cell cycle assay and multicaspase assay by using Muse Cell Analyzer. According to WST-1 assay, starting from 15 µg/mL dose of Apigenin-Chrysin-Galangin combination caused inhibition in cell viability on MCF-7 cells at 48th hour; however, flavonoid combination did not show any similar effect on MCF-10A cells. 15 µg/mL doses at 48-hour exposure were determined as the most effective dose and this dosage were used in subsequent experiments. In Annexin V-PI assay, we have detected 70% apoptotic cells in MCF-7; in contrary 70% of cells remain live in MCF-10A cells and no necrotic cells were found in both cell lines. In multicaspase assay, we have detected that after treating with flavonoid combination, 65% of cells were found as live and 34% of cells were total caspase in MCF-7 cells and this finding may related to MCF-7 cells are caspase-3 mutant. According to cell cycle assay, flavonoid combination increased the percentage of MCF-7 cells in S phase (74%) at 48 h. In conclusion, our findings suggest that flavonoid combinations may be used as a complementary approach to current breast cancer therapy.

P-32-027 Abstract withdrawn

P-32-028
A distinct pattern of DNA damage from high-dose radioactive iodine therapy in patients with differentiated thyroid cancer with/without type 2 diabetes mellitus

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Recent studies reveal that the incidence of differentiated thyroid cancer (DTC) in women with type 2 diabetes mellitus (T2DM), a metabolic inflammatory condition, is continuously increasing [1,2]. We hypothesized that the extent of DNA damage resulting from high doses of I-131 therapy differs between the two groups (DTC/−T2DM vs DTC/ + T2DM). Peripheral blood samples were collected into EDTA tubes from 56 female patients with DTC/−T2DM and 25 with DTC/ + T2DM before and again 69 hours after the intake of a median I-131 activity of 90.54 mCi (3.35 GBq). DNA damage was quantified using spectrophotometry. The aggregate index of systemic inflammation (AISI) was calculated as (neutrophil count × monocyte count × platelet count)/lymphocyte count. The systemic inflammation response index (SIRI) was calculated as (neutrophil count × monocyte count)/lymphocyte count. In DTC/−T2DM patients, I-131 therapy resulted in DNA damage of about 18%. This suggests a reduced cell turnover [3] and efficiency in DNA repair post-irradiation. Conversely, in the presence of T2DM, the same therapy led to an increase in DNA synthesis of approximately 7%. This enhancement is likely due to altered radiation sensitivity and impaired apoptosis associated with insulin resistance and hyperglycemia. Furthermore, in DTC/ + T2DM patients, a median high BMI of 33.8 kg/m^2 was positively correlated with inflammatory markers AISI, SIRI, and DNA synthesis (r = 0.60). This indicates metabolic dysregulation can influence the DNA repair response to ionizing radiation. The interplay between metabolism, inflammation, and high-dose radiation response suggests that a "one-size-fits-all" approach may not be ideal in DTC patients undergoing I-131 therapy.
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P-32-029
Development and evaluation of the novel docetaxel-loaded double-reverse thermoreversible system for intramuscular treatment

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This study aimed to develop a novel solid lipid nanoparticle (SLN)-loaded dual-reverse thermoreversible system (DRTS) for intramuscular administration of docetaxel (DCT) to achieve sustained release and enhanced antitumor efficacy. DCT-loaded SLNs were prepared using homogenization and membrane emulsification techniques, optimizing the lipid mixture (tricaprin/tricaprylin = 9:1, w/w), drug, and surfactant at a 1.4/0.3/0.6 weight ratio. The resulting SLNs had a mean particle size of 157 nm and an entrapment efficiency of 93%. DRTS formulations were prepared by incorporating these SLNs into poloxamer-based solutions (P 188 and P 407), and their rheological properties were analyzed. The optimized SLN/P 188/P 407 (2.3/7.5/6.5%) formulation remained liquid at room temperature but gelled at physiological temperatures (30–36°C), allowing for easy intramuscular administration followed by rapid *in situ* gelation. *In vitro* release studies showed that DCT-DRTS exhibited a higher dissolution rate than DCT suspension but a delayed release compared to DCT hydrogel. Pharmacokinetic studies revealed that DCT-DRTS had a 3.2-fold higher area under curve (AUC) compared to DCT suspension, while reducing the initial burst effect and highest peak (C_{max}) relative to DCT hydrogel.

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Antitumor efficacy evaluation in tumor-bearing athymic nude mice demonstrated significant tumor volume reduction and body weight maintenance with DCT-DRTS. Immunohistochemical analysis revealed an increased number of caspase-3 and PARP-immunoreactive cells (indicating enhanced apoptosis) and fewer CD31- and Ki-67-positive cells (suggesting reduced angiogenesis and tumor proliferation). In conclusion, DCT-DRTS provides controlled drug release, reduced initial burst effect, and enhanced antitumor efficacy, making it a promising alternative for intramuscular DCT formulations.

P-32-030
Combination treatment with ixazomib and O-GlcNAcylation inhibitor induces synergistic apoptosis in pancreatic cancer cells

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Pancreatic cancer has a steadily increasing incidence and mortality rate year on year, and has the lowest survival rate among cancers. However, most anticancer drugs for pancreatic cancer have no clear effect or develop resistance, so new strategies to improve it are urgently needed. In this study, we confirmed that O-GlcNAcylation is closely related to cancer development and survival, and is significantly upregulated in PANC-1 pancreatic cancer. In PANC-1 cells, treatment with the proteasome inhibitor ixazomib induced cytoprotection through autophagy via aggresome formation. Ixazomib activated NF-κB to translocate to the nucleus, and upregulated histone deacetylase 6 (HDAC6). Interestingly, the O-GlcNAcylation inhibitor OSMI significantly inhibited ixazomib-induced aggresome formation, while significantly increasing p62/SQSTM1 (seqestosome-1) bodies containing ubiquitinated aggregates. In addition, combined treatment with ixazomib and OSMI upregulated the endoplasmic reticulum (ER) stress response, increasing C/EBP homologous protein (CHOP) expression and ultimately inducing apoptosis. Additional mechanistic studies demonstrated that the activity of HDOC6 and vimentin, which are necessary for aggresome formation, depend on the regulation of GlcNAcylation. These results in PANC-1 cells were also confirmed in a xenograft model, where combined treatment with relatively low doses of ixazomib and OSMI increased the sensitivity to apoptosis and enhanced the anticancer effect. Therefore, combination treatment with ixazomib and OSMI is expected to provide a potential therapeutic strategy for pancreatic cancer.

P-32-031
L-Carnitine and acetyl-L-carnitine induce metabolism alteration and cell death in colorectal cancer cells

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Colorectal cancer (CRC) remains one of the most prevalent and deadly malignancies worldwide, fueled by metabolic reprogramming and mitochondrial dysfunction that support tumor growth and progression. Increasing evidence indicated dietary bioactive compounds as contributing factors in the prevention and management of CRC. In this context, L-carnitine (Cnt) and short-chain acyl carnitines, including acetyl-L-carnitine (C_2Cnt), are amino acid-derived metabolites abundant in food of animal origin, which attracted attention for their antioxidant, anti-inflammatory and potential antitumor properties. On the other hand, to date, knowledge about the beneficial effects in preventing colon cancer is limited. In this study, we aimed at investigating the anticarcinogenic benefit of Cnt and C_2Cnt in CRC cells, HCT 116 and HT-29. Results showed that treatment with Cnt and C_2Cnt for 72 h induced oxidative stress with a concomitant reduction of cell viability, mitochondrial respiration, and glycolysis, ($p < 0.001$). These phenomena triggered autophagic flux and mitophagy via PINK1/Parkin modulation, promoting apoptotic cell death ($p < 0.01$). Of note, the combined exposure to Cnt and C_2Cnt showed a synergistic effect, enhancing tumor cell growth inhibition and metabolic dysfunction compared to single treatments ($p < 0.05$). These effects were associated with enhanced expression levels of SIRT4 ($p < 0.01$) and activation of AMPK signaling ($p < 0.01$), two key regulators of cellular energy homeostasis. Overall, present findings reinforce the role of nutritional factors in CRC prevention, suggesting Cnt and C_2Cnt as promising biomolecules to target CRC metabolic vulnerabilities.

P-32-032
CAVPENET-mediated modulation of PP1 activity inhibits prostate cancer growth in 2D and 3D cultures through metabolic reprogramming

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Prostate cancer (PCa) remains one of the most prevalent malignancies affecting men worldwide, with drug resistance posing a significant challenge to effective treatment. Consequently, there is an urgent need to develop novel therapeutic strategies. Protein phosphatase 1 (PP1) has recently emerged as a promising target for cancer therapy. Given the dynamic and context-dependent nature of PP1 interaction with its regulatory subunits, disrupting specific PP1 complexes represents a potential approach to impair tumor progression. Thus, the main goal of this work is to develop a bioactive peptide to disrupt a key PP1 complex for PCa progression. The peptide designed to interrupt the interaction between PP1 and

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caveolin-1 (CAV1) was synthetised using microwave-assisted solid phase synthesis and coupled to penetratin to allow an efficient cell delivery. The efficacy of the peptide, CAVPENET, was evaluated *in vitro*, using androgen-independent (PC-3) cell lines. Our results demonstrated that CAVPENET successfully internalized into PC-3 cells, leading to decreased viability and impaired migration ability. Mechanistically, these effects were attributed to PP1-dependent inhibition of AKT signalling, resulting in significant alterations in pathways related to glycolytic and lipid metabolism. CAVPENET peptide was also evaluated in a 3D PCa/prostate cancer-associated fibroblast (PCAF) spheroid model. Treatment with CAVPENET significantly reduced spheroids growth and viability, while no significant effects were observed in monotypic PCAF spheroids, suggesting selective anticancer activity. Collectively, these findings underscore the potential of PP1-disrupting peptides, particularly CAVPENET, as a promising therapeutic approach for PCa. Future studies should focus on further elucidating the molecular mechanisms and optimizing peptide stability to enhance drug-like properties. Matos, B., et al. Pharmaceutics 2024 Sep 12;16(9):1199. doi: 10.3390/pharmaceutics16091199.

P-32-033
Modified macrophages as a potential anticancer cell therapy

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Macrophages are a heterogeneous group of highly plastic cells that serve a multitude of functions and have two antagonistic polarization states: pro-inflammatory, anti-cancer M1 phenotype and anti-inflammatory, pro-tumorigenic M2 phenotype. In the tumor environment (TME) there is a specific population of tumor associated macrophages (TAMs) that play an important role in tumor progression. Reprogramming TAMs from M2 to M1 phenotype is considered a unique avenue for antitumor cell therapy development. The aim of the study was development of cell therapy using modified M1 macrophages (M1/IL-12/RFP) that destroy tumor cells by secretion of interleukin 12 (IL-12) into TME and transform existing TAM (M2) macrophages into M1 anti-tumor ones. Studies also determined the influence of M1/IL-12/RFP on the systemic immune response. The experiments were conducted on bone-marrow derived murine macrophages from BALB/c mice. Using adenovirus-driven engineering macrophages were modified to express IL-12 and red fluorescent protein. The amount of IL-12 secreted by modified macrophages was assessed by ELISA assay. Mice with well-developed 4T1 breast cancer tumors were treated with $1*10^6$ and $2*10^6$ M1/IL-12/RFP macrophages administered subcutaneously at 1-3 doses. Parameters of complete blood count in control and treated mice have been analyzed using the hematology analyzer. Additional post-therapeutic analyses were performed to observe changes in TME. Our data show that M1/IL-12/RFP macrophages release IL-12. *In vivo* studies showed that M1/IL/12 inhibited 4T1 tumors growth. There was noticed increased number of immune cells in mice blood. The results demonstrate that M1/IL-12/RFP macrophages have influence on tumor growth inhibition and cells number of the innate and adaptive immune system cells. However, further research is needed to investigate their precise effect in the tumor microenvironment. The work was funded by National Science Center Poland, UMO-2021/41/B/NZ5/03245.

P-32-034
VDAC1 antagonist molecules deeply rewire the channel electrophysiology in cancer cells

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Mitochondrial metabolism relies on the continuous and efficient exchange of ATP/ADP, NAD^+/NADH and other metabolites through the voltage-dependent anion-selective channel 1 (VDAC1), the most abundant b-barrel protein of the outer membrane. In many cancers, VDAC1 also drives the metabolic rewiring aimed at promoting the "Warburg effect", coupling glycolysis to oxidative phosphorylation. Based on the above, a set of novel VDAC-antagonists (VA) small molecules have been recently identified by AI-based screening. VA molecules selectively bind the NADH-anchoring pocket on VDAC1 by hampering mitochondrial respiration, disrupting cell metabolism in cancer-derived cell lines and organoids exclusively [1]. Though, whether and how these drugs directly affect VDAC ion channel properties and/or modulate its permeability have remained unexplored. To address this issue, we here provide a detailed biophysical characterization of VA-molecules/VDAC1 interaction using the planar lipid bilayer technique. Our results indicate that all five VA molecules hampered the voltage gating of the channel reconstituted into an artificial membrane and concurrently helped to stabilize the "open" state, by altering the flexibility of the N-terminal a-helix that forms the NADH-anchoring pocket by facing the b-barrel lumen. Furthermore, our drugs induced a thorough loss of cation preference in the closed states that turned VDAC1 into a non-selective channel. In conclusion, these results provide new insight into the action of VA molecules, leading to the further optimization and development of novel therapeutic avenues for the treatment of drug-resistant tumors and may increase awareness of the VDAC voltage-gating mechanism, which has not yet been fully elucidated. [1] Conti Nibali et al., iScience (2024), doi: 10.1016/j.isci.2024.109853. This work was supported by PNRR M4C2-Investment 1.4-CN000041 (Funded by the European Union—NextGenerationEU).

P-32-035
Tetrahedral DNA nanostructures for the targeted delivery of cisplatin to treat non-small-cell lung cancer

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Non-small-cell lung cancer (NSCLC), the most prevalent form of lung cancer, continues to present a poor prognosis despite conventional therapies. Cisplatin, a widely used chemotherapeutic agent, has shown effectiveness in treating NSCLC by inducing DNA crosslinking to inhibit cancer cell proliferation. However, its therapeutic utility is frequently limited by substantial side effects, cytotoxicity, and the development of multidrug resistance (MDR). This paper explores a targeted drug

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delivery strategy employing tetrahedral DNA nanostructures (TDNs) to enhance cisplatin's therapeutic efficacy while minimizing its adverse effects on healthy tissues. TDNs, with their high biocompatibility, stability, and functionalisable surface properties, present a promising platform for targeted drug delivery. The TDNs are engineered to carry cisplatin and are conjugated with an epidermal growth factor receptor (EGFR)-specific antibody, cetuximab, to target NSCLC cells specifically. This approach aims to utilise EGFR overexpression on NSCLC cells, directing TDNs precisely to cancer sites, where cisplatin is released via pH-sensitive linkers in the acidic tumour microenvironment. Preliminary simulations indicate that TDNs maintain structural integrity until reaching the target site, enabling localised cisplatin release. These insights suggest that TDN-mediated cisplatin delivery may offer a controlled approach to overcoming MDR and reducing systemic toxicity in NSCLC.

P-32-036
Non-small cell lung cancer targeted siRNA-lipid nanoparticles for regulation of ubiquitinase

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Many studies about the treatment of non-small cell lung cancer (NSCLC) have been conducted, however treatment efficiency and survival rates are still low and metastasis and recurrence of lung cancer are still major challenges. Tripartite motif-containing 28 (TRIM28), known as the ubiquitinase, is over-expressed in NSCLC. Over-expressed TRIM28 promotes the growth of NSCLC by inhibiting p53-MDM2 pathway. RNA interference (RNAi) is the biological process that regulates gene-expression by sequence-specific suppression. Small interfering RNA (siRNA) is one of the components of RNAi pathway. In this study, we investigated about NSCLC treatment using lipid nanoparticle (LNP), known as bioavailable and well-targeted vehicle, loaded TRIM28-targeted siRNA suppressed the P53-MDM2 targeted-ubiquitinase expression. LNP loaded TRIM28 siRNA (T28siLNP) successfully reduced the expression of TRIM28 in human NSCLC (A549) and mouse NSCLC (TC-1) and inhibited the growth of both NSCLCs following the p53-MDM2 pathway. For enhancing the tumor targeting, T28siLNP was coated by D-type PD-1 peptide (D-PPA) which bind with PD-L1 in high affinity (D-T28siLNP). This modification improved the tumor treatment efficiency with high suppression of TIRM28 expression. These results demonstrated that T28siLNP is a potential therapeutic material for non-small cell lung cancer.

P-32-037
Therapeutic cancer vaccine for treatment of metastatic cancer by immunogenic apoptotic bodies

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Many therapeutics have been developed for tumor therapy, but traditional chemo reagents are still used due to low responses against newly developed anti-cancer drugs. However, chemotherapy often does not completely prevent recurrence and metastasis. Thus, new therapeutic method is required for treatment of the patients with metastatic cancer. In this study, we generated an immunogenic apoptotic body (AB) from surgically extracted tumors and modified the surface membranes with monophosphoryl lipid A (MPLA). The surgically extracted tumor was prepared as single cell suspension and apoptosis induced by anti-cancer drug including doxorubicin, paclitaxel, etoposide or cisplatin. The surface of apoptotic body (AB) from tumor cell was then decorated with MPLA (iABs). The treatment of iAB effectively promoted activation of dendritic cells (DCs) as well as cytotoxic T lymphocyte (CTL) in the mouse. In the othortopic metastatic model of breast cancer mice, breast cancer-associated iAB effectively prevented its growth. In addition, CT-26 colon cancer-associated iAB also prevented growth of CT-26 tumor in the rectum of the mice. Furthermore, it has shown that the prevention of metastatic cancer was mediated by activation of CTLs. Therefore, tumor-associated iABs can be used as therapeutic vaccine for prevention and treatment of metastatic cancer.

P-32-038
Combination therapy of immunotherapy and tumor cuproptosis induction prevented metastatic cancer

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Although various new technologies for cancer treatment are being developed, they still have limitations in blocking metastasis and recurrence. Copper-based materials are gaining attention as new tumor therapeutics because it can induce cuproptosis of tumors and perform photothermal therapy (PTT), but they cannot completely block metastasis and recurrence due to low capacity of immune activation. To overcome these shortcomings, in this study, CuS-lipid nanoparticles (C-LNP) were synthesized by coating CuS nanoparticles with lipids containing immune stimulator, monophosphoryl lipid A (MPLA). CuLNPs responded to near-infrared laser with an increase in temperature, which induced cuproptosis in CT-26 colon cancer cells in the mice. At this time, C-LNP induced the maturation of dendritic

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cells in the tumor draining lymph node by combination of C-LNP contained MPLA and cuproptosis generated antigen. Furthermore, C-LNPs prevented the secondary challenged metastatic lung cancer growth, which mediated by CT-26 antigen specific activation of cytotoxic T lymphocytes. Therefore, C-LNP is expected to be used as a nanomedicine not only for treating primary tumors but also for blocking metastasis and recurrence of cancer.

P-32-039
Investigation of cytotoxic effects of *Datura innoxia* Miller ethanol extract on cancer cells

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Datura innoxia Miller, a poisonous plant species belonging to the Solanaceae family, is also commonly known as "thorny apple". This plant has both medicinal and poisonous properties due to the tropane alkaloids it contains and is therefore the focus of scientific research. This species, which contains tropane alkaloids such as scopolamine, atropine and hyoscyamine, has anticholinergic effects and has been used in various medical applications. However, taking high doses of these alkaloids can lead to poisoning. Therefore, the use of the plant should be carefully controlled. In traditional medicine, some parts of *Datura innoxia* are used as painkillers, anti-inflammatory and anesthetics. However, all parts of the plant can be poisonous, and consumption can lead to serious health problems. This plant species, which is rich in phytochemical content, has been examined in many studies. When the research topics in the literature were examined, it was determined that not enough studies were conducted on lung cancer and glioblastoma with this species. In this study, the extracts obtained by maceration of samples taken from fruits and leaves of *Datura innoxia* Miller with ethanol were applied to L929 (healthy fibroblast cell), A549 (lung carcinoma cell) and C6 (glioma cell) cell lines in cell culture medium at different doses to obtain half-maximal inhibitory concentration (IC_{50}) doses. The chemotherapeutic agent cisplatin which is used in current treatments was used as positive control. With the obtained results, the IC_{50} value was found to be 69.46 ± 9.04 μg/mL for the L929 cell line, 23.88 ± 3.45 μg/mL for the C6 cell line and 23.74 ± 2.09 μg/mL for the A549 cell line. When the results were compared with the positive control, it was determined that the plant extract gave lower IC_{50} value in the A549 cell line. The findings have proven that *Datura innoxia* Miller plant can be used against lung cancer with carrier or combined systems to be developed in the future.

P-32-040
Development of a new treatment strategy for gemcitabine/paclitaxel-resistant pancreatic cancer targeting autophagy

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Pancreatic cancer has a five-year survival rate of approximately 12%, mainly because most patients are diagnosed with advanced and inoperable disease. Combination chemotherapy regimens such as gemcitabine (GEM) with paclitaxel (PTX) and FOLFIRINOX are standard treatments; however, resistance to these therapies remains a significant challenge. Autophagy has been implicated in mediating this resistance. The Atg8 and Atg12 conjugation systems are both indispensable for autophagosome maturation, with the ubiquitin-like protein activating factor Atg7 playing a critical role in these pathways. In this study, we investigated the impact of Atg7 knockdown on GEM/PTX sensitivity in GEM/PTX-resistant pancreatic cancer MIAPaCa2 (GP-R) cells. Compared to the parental cells, GP-R cells exhibited reduced sensitivity to GEM/PTX, increased expression of autophagy-related proteins, and elevated basal autophagy levels. Notably, Atg7 knockdown in GP-R cells effectively inhibited both basal and GEM/PTX-induced autophagy, significantly elevated total and mitochondrial reactive oxygen species, and induced apoptotic cell death. These findings suggest that inhibiting autophagy via Atg7 knockdown enhances the sensitivity of GP-R cells to GEM/PTX treatment. In conclusion, targeting Atg7 to inhibit autophagy represents a promising strategy to improve the efficacy of GEM/PTX therapy in pancreatic cancer.

P-32-041
Investigation of the anti-angiogenic effects of antidepressant drugs imipramine on glioblastoma cell line

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Imipramine, a tricyclic antidepressant, inhibits norepinephrine and serotonin reuptake. Tricyclic antidepressants have shown potential effects on cancer both *in vitro* and *in vivo*, though mechanisms remain unclear. Although the exact pathways involved in these effects remain uncertain, multiple potential mechanisms have been identified. These mechanisms play crucial roles in cell proliferation and apoptosis. Repurposing existing drugs, such as imipramine, offers a promising approach to targeting angiogenesis in glioblastoma. Glioblastoma is an aggressive brain tumor characterized by high angiogenic activity, primarily driven by vascular endothelial growth factor A (VEGFA). In this study, the effects of imipramine on viability, VEGFA gene and protein expression, and VEGFA secretion in human glioblastoma cell line. The Alamar Blue assay showed a dose- and time-dependent reduction in viability with 100 μM imipramine for 48 hours. qRT-PCR revealed significant VEGFA downregulation, but western blot and ELISA showed no significant changes in VEGFA protein expression and secretion. These findings suggest that imipramine reduces VEGFA transcription but doesn't significantly affect translation or secretion, likely due to compensatory or post-transcriptional mechanisms. This study highlights imipramine's potential to decrease glioblastoma angiogenesis at the transcriptional level but emphasizes the complexity of targeting angiogenic pathways in tumor cells. Although imipramine shows potential as part of a multimodal therapeutic strategy, further research is required to validate these findings *in vivo*, investigate its impact on alternative angiogenic pathways, and assess its synergistic effects with other anti-cancer agents. This study contributes to the growing body of evidence supporting drug repurposing as a cost-effective strategy for glioblastoma treatment.

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P-32-042
Gnetin C enhances darolutamide sensitivity in darolutamide-resistant prostate cancer 22Rv1 cells via mitochondrial dysfunction

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Prostate cancer is the second most diagnosed cancer worldwide and the leading cause of cancer-related mortality in 48 countries. Although prostate cancer generally has a relatively good prognosis, many cases eventually develop resistance to treatment and progress to castration-resistant prostate cancer (CRPC). Androgen-deprivation therapy is the standard treatment for CRPC, utilizing androgen receptor antagonists such as enzalutamide, apalutamide and darolutamide. However, resistance to these drugs remains a major clinical challenge, necessitating the development of novel therapeutic strategies to overcome drug resistance in CRPC. Melinjo (*Gnetum gnemon L.*) a plant native to Southeast Asia, is known for its physiological activities, including antioxidant and anti-inflammatory effects. Among its bioactive compounds, gnetin C, a resveratrol dimer, exhibits potent antioxidant properties and has recently been reported to exert anticancer effects against prostate cancer. In this study, we investigated the potential of gnetin C to overcome drug resistance using our previously established darolutamide-resistant prostate cancer 22Rv1 (Dar-R) cells. Compared with parental 22Rv1 cell, Dar-R cells exhibited reduced expression of NAD^+ biosynthetic enzymes and reduction in the NAD^+/NADH ratio, leading to a mitochondrial dysfunction. Gnetin C treatment led to a decline in mitochondrial content, downregulation of mitochondrial fission factors, and a reduced mitochondrial oxygen consumption rate in Dar-R cells. These results showed that gnetin C induces further mitochondrial dysfunction in Dar-R cells. Furthermore, gnetin C synergistically enhanced the anticancer activity of darolutamide. These findings suggest that gnetin C is a promising natural compound that may overcome acquired anticancer drug resistance in CRPC by inducing mitochondrial dysfunction.

P-32-043
Combating chemotherapy resistance by silencing STAT3 in Her2- and Her2+ breast cancer cells by using silk nanocarriers

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Chemotherapy resistance is a significant challenge in oncology, and one of the research subjects aims to identify factors involved in chemoresistance and target them therapeutically. We compared normal and chemoresistant Her2- and Her2+ breast cancer cells to evaluate the role of an oncogene linked to cancer progression, STAT3, in chemoresistance. D2F2 breast cancer cells and their Her2-overexpressing variant D2F2E2 were cultured in increasing doxorubicin concentrations to obtain chemoresistant counterparts (D2F2/dox and D2F2E2/dox, respectively). The expression level of *Stat3* and genes involved in proliferation, apoptosis, metastasis, and chemoresistance was analyzed using the RT-PCR method. The level of total and phosphorylated STAT3 protein was also compared between these cell lines. Next, in the chemoresistant Her2- and Her2+ breast cancer cells, *Stat3* was silenced using si*Stat3*, and then the expression of previously analyzed genes was examined. Additionally, we examined the potential of silk nanoparticles for targeted delivery of si*Stat3* to Her2+ breast cancer cells. The initially developed H2.1MS1:MS2KN silk spheres were characterized by SEM, their cellular internalization by confocal scanning microscopy, and the effect of siSTAT3 delivered by silk spheres was determined by RT-PCR. We demonstrated that D2F2/dox and D2F2E2/dox cells exhibit elevated levels of STAT3 at both the mRNA and activated protein levels. Additionally, these cells exhibited altered expression of tumor process-related genes, and the further silencing of *Stat3* induced additional changes. Moreover, we indicated that si*Stat3* therapeutics could be efficiently delivered to Her2+ cancer cells using H2.1MS1:MS2KN silk spheres, and siRNA delivered in such modified cell characteristics. Such a drug delivery system (DDS) applied *in vivo* can protect oligonucleotherapeutics against endonucleases' activity and specifically deliver siRNA to increase its therapeutic efficiency.

P-32-044
Mitochondriotropic derivatives of fisetin induce cell death signaling in phenotypically different breast cancer cells by promoting mitochondrial dysfunction and targeting sirtuins

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Fisetin (3,3′,4′,7-tetrahydroxyflavone) is a promising natural compound with a broad-spectrum of biological activities. For example, fisetin may interfere with mitochondrial function to induce cancer cell death. However, due to low bioavailability of fisetin, its health-promoting effects may be limited. In the present study, to improve the accumulation of fisetin in the mitochondrial compartment and potentiate fisetin-related cytotoxicity against breast cancer cells, fisetin derivative $FCPh_2$ was transformed into mitochondriotropic derivatives with the n-butyltriphenylphosphonium cation attached at positions 3-O (mF3) or 7-O (mF7). Mitochondria-targeted fisetin derivatives caused depolarization of mitochondrial membrane potential and promoted mitochondrial stress as judged by real-time analysis of mitochondrial function (basal and maximal respiration, proton leak, ATP production, spare respiratory capacity, and coupling efficiency) that resulted in massive apoptotic cell death in estrogen receptor-positive (HCC1500, CAMA-1, HCC1428, and ZR-75-30) and triple negative (HCC1806, BT-549, MDA-MB-

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436, MDA-MB-453) breast cancer cells compared to unmodified fisetin. In contrast, mF3 and mF7 did not affect the viability of corresponding MCF-10F normal cells. Mito-fisetin also decreased the levels of mitochondrial sirtuins SIRT3, 4, and 5 modulating mitochondrial metabolism in breast cancer cells. Furthermore, mF3 and mF7 inhibited tumor size *in vivo* using the zebrafish model. In conclusion, we show that anti-breast cancer action of fisetin may be augmented by mitochondrial targeting to promote related cytotoxicity and elimination of breast cancer cells with different receptor status. This study was supported by the National Science Centre (NCN, Poland) grant OPUS 22 no. 2021/43/B/NZ7/02129.

P-32-045
Palladium-based photosensitizers for enhanced photodynamic therapy: efficacy in normoxic and hypoxic ovarian cancer cells

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Photodynamic therapy (PDT) is a promising cancer treatment that employs light-activated photosensitizers (PSs) to generate cytotoxic reactive oxygen species and/or free radicals, leading to cancer cell death. However, its efficacy is limited by several factors including the hypoxic tumor microenvironment. Therefore, developing PSs active under hypoxic conditions is crucial. This study evaluated two novel palladium-based photosensitizers (PS-Pd-1 and PS-Pd-2) for their potential in PDT under normoxic and hypoxic conditions. A UV-visible and fluorescent spectroscopy was used to determine the photophysical properties of the photosensitizers. Cytotoxic activity was assessed in two human ovarian cancer cell lines using the MTT assay. A2780 and OVCAR3 cells were treated with tested photosensitizers and irradiated at various light doses (2, 5, and 10 J/cm^2) with a wavelength of 425 nm. To evaluate dark toxicity, the additional control plates were kept non-irradiated. The luminescence- and fluorescence-based assay was used to analyze the induction of apoptotic and necrotic cell death pathways. Further studies, such as cell cycle distribution, uptake by cancer cells, and oxidative stress generation by tested compounds were performed using flow cytometry to better understand the anticancer mechanism of action. Moreover, confocal microscopy determined intracellular localization using specific trackers, while the real-time cytotoxic effects were monitored post-irradiation using IncuCyte Live-Cell Analysis System. In conclusion, our findings demonstrate that PS-Pd-1 and PS-Pd-2 effectively induce PDT-mediated cell death in ovarian cancer cells under normoxic and hypoxic conditions. Their subcellular localization, ability to generate oxidative stress, and capacity to induce cell death highlight their potential as novel photosensitizers for PDT in cancer treatment. This research was funded by the National Science Centre, Poland, grant number UMO-2021/43/B/NZ7/02476.

P-32-046
Establishment of humanized murine models for glioblastoma treatment

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Glioblastoma (GB) is the most common treatment-resistant brain tumor in adults, characterized by its invasive nature and high recurrence rate. Oncolytic viruses offer a promising therapeutic strategy, selectively targeting and destroying cancer cells. Non-pathogenic Coxsackievirus B5 (CVB5) and Coxsackievirus B3 vaccine strains effectively infect human GB cells but fail in murine models due to species-specific receptor differences, highlighting the need for suitable preclinical models to evaluate human oncolytic enteroviruses in immunocompetent mice. To address this limitation, we engineered CT2A and GL261 murine GB cell lines to express the human coxsackie and adenovirus receptor (CXADR), rendering them permissive to group B coxsackieviruses (CVBs) and adenoviruses. These humanized models demonstrated a more than 10^4-fold increase in susceptibility to CVBs and an enhanced replicative capacity, with a 10^5-fold higher total viral yield of CVB5 in CT2A-CXADR cells at MOI = 0.01 compared to parental CT2A cells. These models also exhibited greater sensitivity to adenovirus type 5, with infection at MOI = 0.1 leading to 60% cell death in CT2A-CXADR compared to 12% in parental CT2A, and 28% in GL261-CXADR compared to 1% in parental GL261. We further confirmed tumorigenicity and successful engraftment of obtained intracranial GB models in immunocompetent C57BL/6 mice. Moreover, therapeutic evaluation of CVB5 via four intratumoral injections at three-day intervals resulted in strong oncolytic activity, significantly improving survival compared to the untreated control group. This study establishes novel syngeneic murine GB models for evaluating human oncolytic enteroviruses in immunocompetent hosts, confirming the essential role of CXADR in the entry of CVBs and adenoviruses, and providing a representative platform for preclinical GB research. This work was supported by the Russian Science Foundation (grant no. 23-74-10102).

P-32-047
Overcoming FCGRT-dependent entry into host cells by bioselected oncolytic enterovirus

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Virotherapy is a promising approach for treating malignant tumors, leveraging the natural ability of certain viruses, including enteroviruses, to lyse tumor cells without damaging healthy tissues. One of the limiting factors in the application of enteroviruses is their receptor-dependent mechanism of entry. It primarily involves interaction with a co-receptor to destabilize the virion, thereby facilitating subsequent binding to receptor. For most enterovirus B members co-receptor is neonatal Fc receptor (FCGRT), while specific receptor varies depending on the viral strain. For Echovirus 1 (Farouk, E1F), which possesses oncolytic properties, main receptor is integrin alpha-2 (ITGA2).

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Although FCGRT is broadly expressed in healthy tissues, its expression in tumor cells is often significantly reduced or nearly absent. To overcome this barrier, we employed a directed evolution strategy using bioselection of E1F in HEK293T cells with damaged interferon response system and deletion of FCGRT. After 25 passages a novel strain (E1F OL) was obtained. It doesn't require FCGRT for efficient infection lysing knock-outed cells. The E1F OL strain was tested on a panel of primary and model human tumor cell lines of various histological origins. Unlike the parental strain, the bioselected one demonstrated cytopathic effects in FCGRT-negative tumor cell lines (multiplicity of infection, MOI ≤ 0.1). The expression levels of co-receptor FCGRT and receptor ITGA2 were assessed using qPCR and western blot analysis. Our findings revealed a strong correlation between FCGRT expression in tumor cell lines and the ability of E1F to lyse tumor cells, whereas no such correlation was observed for ITGA2. In contrast, for the E1F OL strain, neither FCGRT nor ITGA2 expression influenced cytopathic effects. This unique feature of E1F OL enables it to bypass the FCGRT-mediated entry pathway, making it a more versatile candidate for oncolytic virotherapy. This work was supported by the Russian Science Foundation (grant no. 23-74-10102).

P-32-048
New chalcone derivatives as promising therapeutic agents in ovarian cancer: cytotoxicity and mechanistic insights

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Ovarian cancer remains one of the most lethal gynecological malignancies, with high mortality rates primarily due to late-stage diagnosis, the absence of reliable biomarkers, and the development of treatment resistance. Epithelial ovarian cancer (EOC), accounting for approximately 90% of cases, is often diagnosed at an advanced stage, leaving patients with limited treatment options. While standard therapy—consisting of surgery and platinum-based chemotherapy—initially achieves high response rates, recurrence and chemoresistance within five years are nearly inevitable, underscoring the urgent need for novel therapeutic strategies. Chalcones, a subclass of flavonoids, have emerged as promising anticancer agents due to their diverse pharmacological properties. In this study, we evaluated the cytotoxic and molecular effects of newly synthesized chalcone derivatives in ovarian cancer cells. A primary screening using the NCI-SRB assay identified two highly potent derivatives, IMG003 and IMG008, with low IC_{50} values, indicating strong cytotoxic activity. Subsequent analyses, including propidium iodide (PI) staining, MUSE Annexin-V staining, and western blot, revealed the molecular mechanism of action these compounds on cell cycle and cell death of ovarian cancer cells. Our findings highlight the potential of chalcone derivatives as promising candidates for ovarian cancer therapy. These compounds not only demonstrate potent anticancer effects but also provide a foundation for further preclinical and clinical investigations, paving the way for innovative, targeted treatment strategies against ovarian cancer.

P-32-049
Anti-PD-L1 Fab-based immunophotothermal lipid nanoparticles enable treatment of breast cancer and its metastasis

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Over the past decade, various therapeutic trials have made great strides in cancer immunotherapy. Immune checkpoint blockade, in particular, has shown outstanding efficacy as a regulator of immune cell activity against cancer. Nevertheless, antibody-based immune checkpoint blockade have defects, including inadequate tumor penetration, and high-cost burden to patients and manufacturers. In addition, the diverse chemical action sites of antibodies present difficulties in precise drug attachment. Therefore, in this study, we synthesized the fragment of antigen-binding site (Fab) of the anti-PD-L1 antibody and conjugated it to the drug-contained lipid nanoparticle (LNP) in order to utilize both drug delivery efficiency and the antigen-binding ability of the antibody. Indocyanine green (ICG) was incorporated in the LNP for photothermal therapy (PTT) and the LNPs were decorated anti-PD-L1 Fab, which named anti-PD-L1 Fab and indocynine-green contained lipid nanoparticle (piLNP). For cancer therapy, 4T1(breast cancer) cells were injected subcutaneously to build the first transplanted tumor in BALB/c. For evaluation of metastatic tumor inhibitory effects of the piLNP after photothermal therapy (PTT), these mice received a second transplantation with the same cancer cells, 4T1 breast cancer, by intravenous inoculation. The accumulation of LNP in 4T1 tumor in the BALB/c was increased by anti-PD-L1 Fab decoration in the LNPs. In addition, near-infrared (NIR) laser irradiation elevated temperature in the tumor, which consequently eliminated 4T1 tumor growth. Furthermore, the mice cured from the first 4T1 challenge by piLNP with PTT prevented second lung metastatic 4T1 cancer infiltration challenge; the prevented effect was mediated by T cell-induced anti-cancer immunity. Thus, these data demonstrated that potential use of piLNP as immuno-photothermal therapeutic material against cancer and its metastasis.

P-32-050
HER2-dependent paraptosis and ferroptosis induction by cannabidiol in breast cancer

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HER2 (human epidermal growth factor receptor 2) plays a crucial role in breast cancer progression and is considered a key therapeutic target. Cannabidiol (CBD), a non-psychoactive phyto cannabinoid, has been reported to exhibit anticancer effects; however, its mechanism of action in HER2-positive breast cancer remains unclear. In this study, we evaluated the cytotoxic effects of CBD in HER2-positive (SK-BR-3, BT-474) and HER2-negative (MDA-MB-231, MCF-7) breast cancer cells and investigated its association with HER2 signaling. MTT assay,

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Live/Dead assay, and western blot analysis were performed to assess CBD-induced cell death. HER2 knockdown and overexpression experiments were conducted to determine whether CBD specifically targets HER2. The results showed that CBD significantly reduced cell viability in HER2-positive cells compared to HER2-negative cells. Moreover, CBD treatment led to a marked increase in reactive oxygen species (ROS) levels and downregulation of HER2 expression. Mechanistically, CBD induced both paraptosis and ferroptosis, as evidenced by the modulation of key molecular markers such as Alix, ATF4, CHOP (paraptosis), and GPX4, SLC7A11, TFRC (ferroptosis). HER2 knockdown abolished CBD-induced paraptosis and ferroptosis, whereas HER2 overexpression sensitized cells to CBD-induced cell death. Molecular docking and dynamics simulations further revealed that CBD interacts directly with HER2, stabilizing within the HER2 binding pocket through hydrogen bonding and hydrophobic interactions. These findings suggest that CBD targets HER2 to induce paraptosis and ferroptosis, providing a novel therapeutic strategy for HER2-positive breast cancer.

P-32-051
Synergistic anticancer effects of *Citrus aurantium* and auranofin through induction of apoptosis and modulation of the PI3K/Akt pathway in hepatocellular carcinoma

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Hepatocellular carcinoma (HCC) is the sixth most common cancer globally and the fourth leading cause of cancer-related deaths. Despite the need for more effective treatments, viable therapeutic options remain limited. This study investigates the anticancer effects of *Citrus aurantium* (CA) and auranofin in HCC. CA is known for its hepatoprotective effects, while auranofin is a gold compound that inhibits thioredoxin reductase, a key enzyme in cellular redox regulation. We aimed to determine if their combination could offer a novel strategy against HCC. To explore their synergistic effects, we integrated drug and disease target data, identifying 27 common targets between CA and auranofin. Enrichment analysis revealed associations with anticancer activity, apoptosis, and the PI3K/AKT pathway, critical processes in cancer progression and treatment resistance. *In vitro* validation in Hep3B cells showed that the combination of ethanol extract of CA (EECA) and auranofin significantly reduced cell viability. And combination treatment-induced apoptosis was confirmed using flow cytometry analysis, with an increased sub-G1 population and annexin V-positive cells. Moreover, mitochondrial dysfunction, assessed by JC-1 staining, showed notable disruption of mitochondrial membrane potential (MMP) after treatment with combination treatment. Additionally, ROS levels, measured by DCF-DA, were significantly elevated by combined treatment, and this increase was reversed by a ROS scavenger, confirming role of oxidative stress in EECA and auranofin-induced apoptosis. Furthermore, apoptosis induced by combination treatment was further enhanced by the PI3K/Akt inhibitor, which is consistent with our enrichment analysis results. In conclusion, our findings demonstrated that combined treatment of EECA and auranofin elicited synergistic anticancer effects in Hep3B cells through induction of apoptosis via ROS-mediated mitochondrial dysfunction and PI3K/Akt signaling pathway modulation.

P-32-052
The inhibition of endothelial DLL4-NOTCH1 signaling by 2′-hydroxyflavanone enhances anti-PD-1 therapy in melanoma

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Immune checkpoint inhibitors (ICIs) have revolutionized cancer therapeutics; however limited patient responses necessitate combination strategies to improve therapeutic efficacy. Among potential combination partners, drugs targeting DLL4-NOTCH1 signaling pathway—a critical regulator of vascular function—show promise as angiogenesis modulators, but their clinical development have been hindered by safety concerns. To address this challenge, we adopted a novel approach by screening natural compounds with a long history of human consumption. Building upon our earlier findings, we identified three inhibitors of DLL4-NOTCH1 signaling: steppogenin, sanggenon F, and dehydrovomifoliol. Steppogenin inhibited both DLL4 and NOTCH1 activities, while sanggenon F and dehydrovomifoliol selectively suppressed DLL4 and NOTCH1 activity, respectively. We assessed their impact on key angiogenic processes, including endothelial cell migration, sprouting, and proliferation, and elucidated the relative contributions of selective DLL4 or NOTCH1 inhibition to the anti-angiogenic effect. By comparing structurally similar compounds, we identified the 2'-hydroxyflavanone moiety as a key element for DLL4 inhibition. Notably, combining steppogenin with an ICI demonstrated that a nature-derived angiogenesis inhibitor can boost the anti-cancer effect of ICI in a mouse melanoma allograft model. This comprehensive analysis of structure-activity relationships and *in vivo* therapeutic evaluation provides valuable insights into the development of novel anti-angiogenic compounds for combination therapy with ICIs in cancer treatment.

P-32-053
Copper (II) increases anti-tumor activity of *Inula viscosa* extract in colon cancer cells

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Inula viscosa, commonly known as sticky inula, belongs to the Asteraceae family and has been used for medicinal purposes in the treatment of various diseases. Inula species contain numerous bioactive compounds, such as monoterpenoids, sesquiterpenoids, flavonoids, and glycosides, which contribute to their diverse biological activities, including anti-inflammatory, antipyretic, antiseptic, and antitumor effects. Studies have demonstrated the cytotoxic effects of *I. viscosa* on various cancer cells, which have been linked to the pro-oxidant activity of its polyphenolic compounds. It is well known that transition metals such as copper (II) and iron (III) enhance pro-oxidant activity of

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phenolic substances through the Fenton reaction. Therefore, in this study, we aimed to investigate the effects of *I. viscosa* extract combined with copper on cytotoxicity, apoptosis, and reactive oxygen species (ROS) production in colon cancer cells. The aerial parts of the plant were ground into small pieces, and a 70% methanol (MeOH-H O) extract was prepared. Different doses of the extract were applied alone and in combination with a non-toxic dose (150 μM) of copper(II) sulfate (CuSO) to colon cancer cells (HT-29) for 24 hours. After incubation, cytotoxic, apoptotic, genotoxic and ROS production activities were analyzed using MTT assay, acridine orange/ethidium bromide (AO/EB) dual staining, comet assay, and H2DCF-DA method. The combination of *I. viscosa* extract with copper (II) significantly increased cytotoxic, genotoxic, apoptotic and ROS production levels more than the non-combined groups. The IC_{50} value was determined as 340 μg/ml for *I. viscosa* alone and 276 μg/ml for the *I. viscosa* + copper (II) combination. In conclusion, the combination of *I. viscosa* and copper (II) enhanced anticancer activity in HT-29 cells through pro-oxidant activity.

P-32-054
Investigating Ad5-KUR04 as a novel therapeutic approach in cancer treatment

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Adenoviral therapy shows promise as a strategy to improve therapeutic outcomes in cancer treatment. Oncolytic viruses (OVs) not only directly kill tumor cells but also modify the tumor microenvironment and activate immune cells, such as lymphocytes, to enhance the host's immune response, potentially increasing the efficacy of cancer therapies. Additionally, OV treatment can be combined with other standard therapies, such as immune checkpoint inhibitors (ICIs). This study focused on the recombinant adenovirus Ad5-KUR04, which features an Ad5 backbone with modifications in the E1A and E3 regions. The experimental design utilized three ovarian cancer cell lines (OV-90, OVCAR3, OAW-42) and three mesothelioma cell lines (Mero-82, MSTO-211H, H226), with the HEK 293 and L929 fibroblast cell lines serving as controls. The aim was to determine the viral concentration required to induce cytotoxicity or reduce cancer cell viability. Over 10 days, cell viability was assessed using the MTT assay, with absorbance at 570 nm expressed as a percentage relative to the control. Significant cytotoxicity was observed when cell viability dropped below 70%. In the OVCAR3 cell line, cell viability decreased to 60% by day four at a viral concentration of 1000 VP, while a similar effect was observed in the OV-90 cell line, albeit delayed until day eight. The viability of healthy fibroblast cells remained above 75%, indicating minimal impact on non-cancerous cells. A similar response was noted in mesothelioma cell lines, with viability dropping below 70% by day eight. To further evaluate Ad5-KUR04 efficacy, cells were also incubated with the Ad5-KUR04 wild-type virus, revealing a slower cytopathic effect. While additional studies are needed to compare other cancer types, these results suggest that Ad5-KUR04 could represent a promising approach to enhancing cancer treatment. These preliminary findings support further investigation into alternative methods for treating cancer.

P-32-055
Targeted gene silencing using multi-microRNAs in breast cancer cells

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MicroRNAs are small, single-stranded, non-coding RNAs that play a key role in regulating gene expression at the post-transcriptional level. Given the structure of naturally occurring microRNAs in the body, it is possible to design artificial microRNAs with multiple regulatory sequence motifs in a single RNA strand, enabling them to target several disease-related genes simultaneously. This approach could be applied in advanced gene therapy where several genes are responsible for the severity of a disease. We explored the effectiveness of our technology based on artificial microRNAs designed to simultaneously silence three proteins involved in the branched-chain amino acid degradation pathway: dihydrolipoamide S acetyltransferase (DLAT), branched-chain amino acid transaminase 2 (BCAT2), and dihydrolipoamide branched-chain transacylase E2 subunit (DBT). Several vectors were designed incorporating self-amplification and exosome release-promoting motifs to enhance the delivery of multimicroRNAs. The resulting plasmids were named with the corresponding codes. Plasmids were extracted from bacteria and then transfected into breast cancer cells (BCC). Replicate DNA was produced from the RNA samples and real-time PCR was performed according to the TaqMan kit protocol. Protein expression inhibition was verified by western blot. The results showed a significant reduction in the expression of DLAT, BCAT2, and DBT genes following transfection with a plasmid containing the complete RNA system. Additionally, all three target proteins were effectively inhibited in cells transfected with the full-construct SF13 plasmid. These experiments demonstrate that our multimicroRNA-based system effectively reduces the expression of targeted genes. However, additional experiments are planned to precisely quantify the contribution of each added feature to the overall inhibition of the targeted genes. This study is funded by the Research Council of Lithuania, project number S-MIP-24-131. *The authors marked with an asterisk equally contributed to the work.

P-32-056
The mechanisms of acquired resistance of tumor cells to MDM2 inhibition

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In the process of carcinogenesis, the transcription factor p53 plays an important role in maintaining genome stability. TP53 is

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the most frequently mutated gene in human cancer (about 50% of tumors express mutant p53), which results in a loss of its oncosuppressive function. The main regulator of the p53 protein is the ubiquitin ligase MDM2, which is responsible for reduced p53 levels via several mechanisms. MDM2 suppression leads to p53 activation and could trigger apoptotic death of tumor cells by increasing the expression of p53-dependent proapoptotic factors (e.g., Bax, Puma, etc.). A serious problem in the use of various chemotherapeutics, including MDM2 antagonists, currently undergoing clinical trials, is the development of acquired drug resistance. Recently, to study the mechanisms of acquired resistance of tumor cells to MDM2 inhibition using multiple *in vitro*, *in vivo* and *in silico* approaches we have demonstrated that the development of resistance to MDM2 suppression (by RG7388) in neuroblastoma model is associated with the appearance of mutations in the TP53 gene: one mutation (His193Arg) was found to destabilize the interaction of p53 with DNA[1]. Moreover, it was observed that resistant neuroblastoma cells were less sensitive to cytostatic chemotherapeutic agents (doxorubicin, cisplatin, paclitaxel), which possessed enhanced proliferation and metabolism rates compared to parental cells. In summary, these features might contribute to tumor progression. Reference: 1. Pervushin N.V. et al. (2024) Apoptosis 29(11-12), 2197-2213. The study is supported by the RSF grant (project 23-74-30006).

P-32-057
Comparative effects of propolis and polyphenolic cocktail (PFK5120) on EGFR signaling in MCF-7 hormone positive breast cancer cell line

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Breast cancer (BC) has been identified as the leading cause of cancer-related mortality among women, with an observed increase in incidence of 0.5% per year since 2004. The antioxidant, anti-inflammatory, and antineoplastic properties of polyphenols, natural secondary metabolites of plants, are well-documented. However, the potential effect of polyphenols on the EGFR signaling pathway is poorly known. Therefore, we investigated the potential effect of the combination of natural propolis and a phenolic compound (PFK5120) on the EGFR signaling pathway and HER2 protein expression. MCF-7 cells were exposed to different specific doses of propolis (6.5-13-26 ug/mL) and PFK5120 (5-10-20 ug/mL) for 48 hours in a cell culture medium. The mRNA expression levels in the RAS/MAPK pathway were analyzed by real-time PCR, and HER2 protein expression was assessed by western blotting and immunohistochemical staining. The RT-PCR analysis showed that propolis caused a significant inhibition of the gene expression in the RAS/MAPK pathway of the EGFR panel at all the doses at 48 hours. Treatment with 20 μg/mL PFK5120 resulted in a similar general inhibition of this pathway, with the exception of increased MAPK3 (FC = 6.39) and RELA (FC = 3.04) gene expression at 48 hours. However, a significant increase in HER2 protein expression was observed 48 hours after administration of 20 μg/mL PFK5120 ($p = 0.0187$). The findings were confirmed by immunohistochemical staining results. Our data suggest that further studies using standardized polyphenol capsules may be more effective as new therapeutic agents in cancer treatment. These approaches may offer a natural alternative to conventional cancer drugs, providing treatment options with minimal to no adverse effects.

P-32-058
Green light-triggered multifunctional bilosomes as an effective tool for malignant melanoma treatment

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Melanoma, as the most aggressive of all skin cancers, still poses a formidable challenge to modern oncology, mainly due to the formation of numerous metastases, including to the surrounding lymph nodes, as well as distant metastases (most commonly to the lungs and brain). Consequently, we present a novel formulation combining anionic xanthene dye of photosensitizing activity (Rose Bengal, RB) with the natural pigment from the carotenoid family (astaxanthin, AST), co-encapsulated within polyethylene glycol (PEG)-coated positively charged bilosomes, which was developed and evaluated concerning particle size, ζ-potential, and morphology. The safety and efficacy of bilosomal formulations were assessed using *in vitro* models of malignant melanoma tumor (A375 and Me45) and normal human epidermal keratinocyte (HaCaT) cells. During the study, we demonstrated enhanced uptake of the nanocarriers containing hybrid cargo by pathological cells compared to non-loaded RB and AST molecules. Moreover, double-loaded bilosomes combined with green illumination (light dose 10 J/cm^2, λ_{max} 520-560 nm) significantly reduced cellular mitochondrial activity for both melanoma cell lines tested. After phyto-photoactivation, treatment of A375 and Me45 cells resulted in a more than 70% decrease in their viability, already at a low concentration of hydrophilic photosensitizer equal to 2 μM. In conclusion, our findings provide crucial information on an integrated approach to antitumor synergy by combining novel bilosomal-origin nanophotosensitizers with advanced phyto-photodynamic therapy. Acknowledgments: This work was supported by the National Science Centre (Poland) [UMO-2023/50/E/ST4/00603] and in part by Statutory Subsidy Funds of the Department of Molecular and Cellular Biology [SUBZ.D260.24.076] at Wroclaw Medical University. The authors would also like to express gratitude for the support of the Department of Physical and Quantum Chemistry at WUST.

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P-32-059
Development of a novel nano drug delivery system for cancer therapy: triggered drug release from cellulose nanoparticles with use of bacterial cellulase

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Targeted drug release technologies have emerged from advancements in cancer treatment strategies, aiming to minimize the damage caused by chemotherapy drugs. Doxorubicin (Dox) is a common chemotherapy drug that is effective on numerous cancer types but causes severe short-term and long-term side effects. In this study, a targeted drug release system was designed by combining cellulose nanoparticles (CNPs) and bacterial cellulase enzyme. The enzyme was partially isolated from two bacterial species (*Acinetobacter pittii* ROBY and *Ruminococcus flavefaciens* strain 17). Cellulase enzyme activities was further analyzed through a 3,5-dinitrosalicylic acid (DNS) enzyme activity assay, showing a higher enzyme activity in acidic environments, which is a desired condition due to the acidic nature of tumor microenvironment. On the other hand, Dox-loaded CNPs were generated with desirable features by dissolving microcrystalline cellulose in a NaOH-thiourea-urea complex system under high heat, which further includes addition and integration of Dox within the nanoparticulate structure. Before moving to cell culture studies, this approach was tested on bacteria in terms of CNP cultivation and the effect of released Dox on bacterial cell viability, showing that bacteria can successfully use non-drug loaded CNPs as a sole carbon source, while the release of Dox from Dox-loaded CNPs reduces bacterial cell viability in a considerable manner due to its cytotoxicity against bacterial cells. Currently, cell culture studies of the combination of bacterial cellulase enzyme and Dox-loaded CNPs (including bacterial cellulase enzyme lacking controls to determine the drug release is whether controlled or not; triggered only by the presence of cellulase enzyme) are ongoing to test the validity of this triggered release approach, and promising results have been achieved in early studies. *The authors marked with an asterisk equally contributed to the work.

P-32-060
Flecainide with ranolazine combination inhibits Nav 1.5 activity of triple-negative breast cancer cells (MDA-MB-231)

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Triple-negative breast cancer (TNBC) is an aggressive breast cancer characterized by high invasion and metastasis. Deaths are often due to metastasis and so reducing metastasis could improve cancer patients' survival and quality of life. Voltage-gated sodium channels (VGSCs) in cell membranes play a role in cancer, and the overexpression of Nav1.5 VGSC is linked to metastatic potential of breast cancer, making it a promising therapeutic target. Clinically used anti-epileptics and anti-arrhythmics have been shown to inhibit cancer cell migration and metastasis by blocking VGSCs. Ranolazine (RNL) is used in the treatment of angina pectoris. Flecainide (FLC) is an antiarrhythmic drug used in heart rhythm disorders and both block Na^+ ions. This study aims to investigate the effects administered alone and in combination of FLC and RNL on Nav1.5 expression in the highly invasive MDA-MB-231 cells. TNBC cells were treated with FLC (1 μM), RNL (5 μM), and FLC + RNL (1 μM + 5 μM) combination for 24 and 48 h. Cell proliferation was determined by MTT method, migration, by wound-healing, invasion by boyden chamber assay. Nav1.5 expression levels was analyzed via western blot and qRT-PCR. The VGSC blocker TTX reduced the motility by 26.0% in 48 h. FLC caused a 17.6% reduction in cell motility, while 5 μM RNL led to a 20.8% inhibition. TTX, inhibited the by 63.9%, RNL a similarly 65.6% and FLC by 42.6% in the invasion of MDA-MB-231 cells. TTX caused the strongest reduction in protein (11.0%) and gene expression (27.8%), while the combination treatment achieved in an 8.8% decrease in Nav 1.5 protein and an 18.6% reduction in gene expression. In our study, in which the combined effect of FLC and RNL was investigated for the first time, particularly FLC + RNL combination significantly inhibited Nav1.5 protein expression of MDA-MB-231 cells. Consequently, we conclude that the drugs show a synergistic effect when used in combination and offering a novel approach to limit metastatic activity of TNBC.

P-32-061
In vivo biocompatibility study of a silk-based drug delivery system for therapeutic oligonucleotides in cancer therapy

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Therapeutic nucleic acids often show limited efficacy in the clinical setting due to a lack of cellular specificity and fast degradation in the bloodstream. We developed a silk-based platform for delivering therapeutic oligonucleotides *in vivo* to address this issue. The carriers are produced from bioengineered MS2-type spider silk functionalized with poly-lysine peptide (KN) to enhance the binding of the nucleic acids. Despite silk's overall good biocompatibility and biodegradability, the potential toxicity of newly designed materials should be carefully studied. The study examines the potential *in vivo* toxicity and immunogenicity of MS2-type silk-based drug delivery carriers. To test the acute toxicity, three doses of spheres (5, 10, and 20 mg/kg b.w.) were administrated to mice, and then the mortality and any changes in mice behavior that may indicate any distress were analyzed. Based on the acute toxicity results (no lethal effect or any observable discomfort among all tested groups), we chose the dose of 20 mg/kg b.w. for the subacute toxicity study, in which spheres were applied to mice three times, and the blood samples for the analysis of biochemical markers were collected on specific days. In the immunogenic studies, the spheres were administered at 20 mg/kg b.w. three times and then analyzed: a) the presence of silk-specific antibodies in blood sera (14 days after initial administration of spheres), b) proliferation rates of cells isolated from the spleen after their restimulation, and c) the profile of the cytokines released by the restimulated splenocytes. The results showed no significant systemic toxicity and low

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immunogenicity after administration of MS2-type spheres to the mice. It suggests their potential application for nucleic acid delivery in cancer therapy. Next, the effectiveness of the silk MS2-based oligonucleotide delivery system in cancer treatment will be evaluated.

P-32-062
Fatty acid metabolism enhances metabolic adaptation and plays a key role in therapy resistance in glioblastoma cells

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Glioblastoma (GBM), the most aggressive primary brain tumor in adults, has a median survival of only 15-18 months despite advancements in surgery, radiotherapy, and chemotherapy. Resistance to standard treatments such as temozolomide (TMZ) and experimental therapies like tumor necrosis factor-related apoptosis-inducing ligand (TRAIL) remains a major challenge. While TRAIL selectively induces apoptosis in cancer cells, many GBM cells develop resistance through poorly understood mechanisms driven by tumor heterogeneity and non-genetic factors. To investigate metabolic adaptations linked to TRAIL resistance, we utilised TRAIL-sensitive (A172-S) and resistant (A172-R) GBM cell models established by Cingöz et al. Untargeted metabolomics profiling via ultra-high-performance liquid chromatography-high-resolution mass spectrometry (UHPLC-HRMS) identified fatty acid metabolism as the most prominently altered metabolic signature. Integrating metabolomics with transcriptomic data revealed significant correlations between metabolic changes and gene expression, highlighting key metabolic pathways involved in therapy resistance. Ongoing functional assays aim to determine the role of candidate genes within these pathways in TRAIL resistance. By elucidating these metabolic adaptations, our study seeks to identify novel therapeutic targets to overcome resistance in GBM, paving the way for improved TRAIL-based treatment strategies.

P-32-063
Nutraceuticals as potential therapeutic agents for cutaneous melanoma: evaluation of the anti-tumor effect of natural polyphenols encapsulated in novel delivery systems

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Cutaneous melanoma continues to be lethal, especially when diagnosed in advanced stages, with current therapeutic options being limited, either due to low efficiency or development of resistance to treatment. Thus, alternative methods are being investigated to act as adjuvants, including the use of natural compounds, capable of targeting tumor cells without being toxic to their normal counterparts. However, further research is needed to increase their bioavailability by developing efficient biocompatible delivery systems. In this regard, we tested the anti-tumor potential of curcumin (C) and/or quercetin (Q) encapsulated in multiparticulate drug delivery systems (MDDS) and embedded in a collagen-based scaffold (Coll) in terms of cell viability, proliferation, anti-inflammatory and anti-oxidant effect. After seeding human melanoma cells (MelJuSo) on the substrates, cell viability evaluation was performed using quantitative MTT [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide] and LDH (lactate dehydrogenase) assays, together with fluorescence microscopy (LiveDead assay). Specific markers of cell viability, proliferation, invasion and inflammation were analyzed at gene and protein level, while the amount of reactive oxygen species (ROS) was determined using a fluorimetric assay. Significant cytotoxicity was reported for the composites containing natural polyphenols, especially the one with both C and Q, compared to the simple Coll control. Moreover, reduced expression of specific markers, among them EGFR, MMP-2, BCL-2, IL-6, etc. was detected in the presence of C/Q, as well as decreased ROS over time. In conclusion, the combination of encapsulated nutraceuticals negatively affected melanoma cell growth, diminishing inflammation and oxidative stress, suggesting their potential as therapeutic agents for cutaneous melanoma. This research was supported by UEFISCDIPN-III-P2-2.1-PED-2021-2917/HEALSKIN.

P-32-064
Pleotropic effect of SMO protein inhibitors on TOP-resistant ovarian cancer cell lines

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Ovarian cancer is the most common cause of death among patients with gynecological cancers. The presence of cancer stem cells (CSC) results in the development of resistance to chemotherapeutic agents. The activity of the Sonic Hedgehog (Shh) pathway, a well-known CSC-specific pathway, may contribute to reduced treatment efficacy by increasing the expression of drug transporters from the ABC family. In this study, we used ovarian cancer cell lines resistant to topotecan (TOP) (A2780TR1 and A2780TR2), which are characterized by a high level of BCRP protein expression, as well as the parental A2780 cell line sensitive to TOP. The effect of the Smoothened (SMO) protein inhibitor Vismodegib (VIS) on ovarian cancer cells resistant to topotecan (TOP) (A2780TR1 and A2780TR2) was investigated. The cytotoxic effect of inhibitors and TOP was examined with the MTT [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide] assay. The effect of inhibitors on the ability of ovarian cancer cells to form colonies was also tested. The migration of cancer cells was examined with a wound healing assay. The activity of BCRP protein was analyzed using intravital imaging of transporter-specific dyes, as well as flow cytometry (Mitoxantron (MIT), Hoechst). IC_{50} value for VIS was similar for both resistant cell lines and the parental A2780 cell line. Treatment with inhibitors resulted in reduced colony formation and cell migration in investigated cell lines. We also

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observed increased sensitivity to TOP in the presence of VIS. This effect was not caused by changes in BCRP protein expression, as indicated by western blot. Flow cytometry and fluorescent microscopy analysis showed that VIS inhibits BCRP protein activity, causing higher accumulation of cytotoxic drugs (fluorescent dyes) in the cells. In conclusion, the Shh pathway inhibitor Vismodegib exerts a pleiotropic effect on drug resistant cells, resulting in the inhibition of cell growth, migration, and higher sensitivity to the cytotoxic drug topotecan.

P-32-065
Exploring the therapeutic potential of natural compounds as novel inhibitors of DYRK1B in pancreatic cancer

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Pancreatic cancer is an aggressive malignancy with a poor prognosis and a high mortality rate. Current treatments, including surgery, radiation therapy, and chemotherapy, have limited efficacy due to the cancer's high metastatic potential and intrinsic chemoresistance. Notably, 90% of pancreatic cancer cases involve metastasis and cancer cells often evade chemotherapy by entering a quiescent state. Previous studies indicate that quiescent pancreatic cancer cells overexpress dual specificity tyrosine-phosphorylation-regulated kinase 1B (DYRK1B), which promotes cell cycle arrest and reduces intracellular reactive oxygen species (ROS), thereby enhancing cell survival under chemotherapy-induced stress. This study aimed to develop DYRK1B inhibitors that drive quiescent cancer cells back into the cell cycle, sensitizing them to chemotherapy. Using iGEMDOCK computer-aided screening and enzyme-based assays, we identified Fv03, a potential natural DYRK1B inhibitor. The effects of Fv03 on cell viability, cell cycle progression, and ROS levels were evaluated in PANC-1 pancreatic cancer cells, which exhibit high DYRK1B expression. Our results demonstrated that Fv03 effectively reduced PANC-1 cell viability and inhibited DYRK1B activity, as evidenced by decreased phosphorylation of its downstream substrate, $p27^{Kip1}$ at Ser10. Additionally, Fv03-induced DYRK1B inhibition increased ROS levels, leading to cancer cell death. In conclusion, this study provides fundamental insights into DYRK1B as a promising therapeutic target and underscores the need for further optimization of Fv03 to enhance its clinical potential. These findings pave the way for the development of novel therapeutic strategies for chemoresistant pancreatic cancer.

P-32-066
Anti-cancer effects of ATRA and curcumin combination therapy in a glioblastoma cell line

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Glioblastoma (GBM) is an aggressive brain tumor characterized by high recurrence rates and poor prognosis due to therapy resistance. Conventional treatments, including chemotherapy, radiotherapy, and surgery, remain insufficient due to GBM's complex molecular profile and the acquisition of drug resistance through various epigenetic and protein regulatory mechanisms. Epithelial-mesenchymal transition (EMT) plays a critical role in GBM progression by enhancing cellular migration and invasiveness, leading to metastasis and lower survival rates. Recent studies suggest that targeting EMT and inflammatory pathways may provide new therapeutic avenues. All-trans retinoic acid (ATRA), a vitamin A metabolite, has been reported to suppress EMT and induce apoptosis in various cancer types, including breast, lung, and pancreatic cancers. Additionally, curcumin, a polyphenolic compound derived from *Curcuma longa*, is known for its anti-inflammatory and anti-cancer properties, enhancing apoptosis while protecting normal tissues. In this study, we aimed to investigate the anti-cancer effects of ATRA and curcumin combination therapy on GBM cell viability, migration, and apoptosis. U87 glioblastoma cells were treated with ATRA and curcumin alone and in combination at several doses. Cell viability and migration were assessed using the xCELLigence real-time system, while apoptosis was analyzed via flow cytometry. Our results demonstrate a clear synergistic effect of ATRA and curcumin, dose and time dependent. Interestingly, cells treated with free doses showed initial proliferation within 24 hours, emphasizing the importance of dose optimization. These findings suggest that ATRA and curcumin combination therapy holds promise for overcoming GBM treatment limitations by enhancing therapeutic efficiency and reducing drug resistance. Further studies, including *in vivo* and clinical evaluations, are necessary to confirm these effects and facilitate clinical research.

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P-32-067
Effects of gallic acid on the endoplasmic reticulum stress pathway and intestinal epithelial stem cells in experimentally induced colon cancer in rats

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Colon cancer results from the transformation of normally functioning colon epithelium to adenomatous polyps. Reports suggest that food components or diet may gradually deteriorate the gut microbiota composition, leading to colorectal cancer. Investigation of new parameters is important in terms of early diagnosis and development of new treatment methods, as well as revealing the pathophysiology of the disease. In this study, we investigated the possible prophylactic and therapeutic effects of gallic acid (GA) in rats with experimental colon cancer. Seventy-two Wistar male rats were randomly divided into 6 groups. The duration of the experiment was 20 weeks. The first group was the control group. Rats in the second and third groups were given 50 mg/kg GA in the first and last 10 weeks of the experiment, respectively. Rats in the fourth group were injected with 30 mg/kg dimethylhydrazine (DMH) once a week for the first 10 weeks. Rats in the fifth group were given GA + DMH during the first 10 weeks. Rats in the sixth group were given DMH for the first 10 weeks and GA for the next 10 weeks. Rat samples were used to measure oxidative stress, calcium, total protein, HIF-1α, and midkine. The expression of LGR5 protein and proteins in the UPR signaling pathway in rat colon tissue was measured by western blotting. Midkine levels were found to be significantly higher in the fourth group than in the control group ($p < 0.05$). It was observed that expression of GRP-78, ATF-4, and LGR5 was significantly increased in the fourth group compared to the control group. As a result, pathways related to oxidative stress, stem cell and endoplasmic reticulum stress may be a guide in the prophylactic or therapeutic effects of gallic acid in the colon cancer since they show significant differences between groups. Further research is necessary to clarify these findings.

P-32-068
The design of immunoconjugates for targeted photosensitizer delivery

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Photodynamic therapy (PDT) is an established approach to the treatment of oncologic diseases. It is based on cytotoxic activity of photosensitizers—small molecules generating reactive oxygen species (ROS) under visible light—that is manifested upon irradiation of the tumor. Despite its efficacy, classic PDT lacks selectivity, as photosensitizers accumulate in healthy tissues, causing off-target phototoxicity. Photoimmunoconjugates (antibody-photosensitizer conjugates) merge the benefits of photodynamic therapy and immunotherapy. Their tumor specificity stems from antibody-mediated targeting of tumor-associated antigens. In addition, ROS production induces immunogenic cell death, potentially triggering the abscopal effect—immune-mediated elimination of distant metastases. This phenomenon, rarely seen in conventional PDT or radiotherapy, has been observed with photoimmunoconjugates *in vivo*. Currently, there are several unresolved issues related to the development of an optimal design for photoimmunoconjugates. Most of the known PDT drugs lose some of their efficacy when conjugated to an antibody. In addition, even though photosensitizers are delivered by a target-specific vehicle, they can still cause serious damage to blood and organs when accidentally exposed to light upon delivery in the bloodstream, i.e. before they reach the targeted tumor (Thankarajan et al. (2022) J. Controlled Release 343, 506-517). These issues can be addressed by our proposed design of next-generation photoimmunoconjugates, in which the photosensitizer, based on halogenated hemicyanine, remains inactive during systemic circulation and is selectively activated only upon internalization by tumor cells, leveraging the unique enzymatic profile of cancer cells. This approach minimizes off-target phototoxicity while maximizing ROS generation at the tumor site, thereby significantly enhancing both therapeutic efficacy and safety.
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P-32-069
Combined targeting of AKR1C3 and AR in the treatment of aggressive castration-resistant prostate cancer

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Castration-resistant prostate cancer (CRPC) represents an aggressive evolution of prostate cancer characterized by resistance to the Androgen-deprivation therapy (ADT), that mainly involves the use of abiraterone (ABI, CYP17A1 inhibitor) and enzalutamide (ENZA, androgen receptor (AR) inhibitor) as standard treatment options. A variety of factors contributes to the emergence of CRPC, one of the most important being the androgen axis: while other pathways can contribute to castration-resistance, AR remains a critical driver in the continuum of CRPC. AR is a cytoplasmic receptor which, after androgen binding, homo-dimerizes and translocates to the nucleus, where it acts as transcription factor. One of the targets of transcriptional activity of AR is the gene encoding for AKR1C3, an enzyme involved in oxidoreductive reactions of a variety of substrates including endogenous steroids, prostaglandins and exogenous compounds. Its principal function

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consists in regulating the availability of androgens, estrogens and progestins for their respective receptors. Its overexpression has been strictly correlated with emergence of resistance to ADT in CRPC. Given the close correlation between AR and AKR1C3 in CRPC, these proteins are here studied as targets for small molecules that could be co-administered with standard-of-care drugs. Using a selected small library of potent and selective AKR1C3 inhibitors we previously developed[1], we report on transcriptome analysis on LnCAP and 22RV1 cell lines after treatment with these compounds, demonstrating that they have a down-regulation effect on a series of AR-related genes involved mainly in cell proliferation and DNA duplication and repair. Furthermore, we tested their efficacy in co-administration with ENZA. The results obtained highlight the potential usefulness of targeting AKR1C3 and AR to treat CRPC. [1]Previously published in: Pippione A.C. et al. (2018) Eur J Med Chem, 150, 930-945; Pippione A.C. et al. (2022) Eur J Med Chem, 237, 114366.

P-32-070
Breaking barriers in glioblastoma therapy: a novel near-infrared-absorbing photodynamic therapy (PDT) agent with a powerful type I mechanism

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Glioblastoma multiforme (GBM) remains one of the most aggressive and treatment-resistant brain tumors, underscoring the need for innovative therapies. Photodynamic therapy (PDT) has emerged as a promising approach, utilizing photosensitizers to generate reactive oxygen species (ROS) upon light activation, selectively inducing cancer cell apoptosis. This study introduces a novel near-infrared (NIR)-absorbing PDT agent, designed to enhance tissue penetration and target GBM effectively. The agent, synthesized in free and caged forms exhibits strong absorption at 750 nm and emission at 780 nm, enabling deeper tissue penetration critical for GBM treatment. The compound demonstrates minimal dark toxicity, low molecular weight, and high photostability, making it an ideal candidate for clinical applications. Photophysical characterization revealed high singlet oxygen (Φ) and fluorescence (Φ_F) quantum yields, further highlighting its potential for PDT. Cytotoxicity studies on U87 and U118 glioblastoma cell lines demonstrated IC_{50} values of 3.24 μM and 6.86 μM, respectively, while the L929 healthy fibroblast cell line showed an IC_{50} of 6.41 μM, indicating selective toxicity toward GBM cells. Mechanistic studies using scavenger assays confirmed a Type I photodynamic mechanism, with cell viability significantly increasing in the presence of ROS scavengers like N-acetylcysteine and mannitol. Confocal imaging further validated intracellular localization and activation of the photosensitizer. This NIR-activated PDT agent offers superior tissue penetration and selective GBM targeting through a Type I mechanism, presenting a significant advancement over conventional PDT. Ongoing research on the caged version aims to explore its potential for spatiotemporal control in GBM therapy. ERC-supported project NIR-PDT (Grant Agreement ID: 852614).

P-32-071
The role of self-assembled nanocarrier complexed with model drug (doxorubicin, Dox) in anticancer therapy

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Intratumoral clonal heterogeneity (ICH) is a common phenomenon occurring in cancers, underlying the ineffectiveness of anti-cancer therapies. Our study focused on: 1) analyzing the selective delivery of a model drug (oxorubicin, Dox), via a ribbon-like self-assembled carrier composed of self-associating molecules (SN): Congo red (CR) or Evans blue (EB), delivered through immunotargeting; 2) applying a model system of ICH to verify the possibility of immunotargeting SN and drug release at the target site of a specific cancer cell clone. The aim was to develop selective, targeted (through immunotargeting and SN carriers) drug delivery to a specific population of cells within a tumor characterized by clonal heterogeneity. The following were determined: 1) a dose of SN that is non-toxic to cells and at which SN is capable of self-association, 2) the IC_{50} dose for free and carrier-bound Dox, 3) the binding of SN with antibodies bound to cancer cells, 3) Dox release, 4) increased Dox anti-cancer effect on the cell in the presence of SN. We evaluated the effects of this therapeutic strategy on the proliferation and apoptosis of human breast cancer (BC) cells (MDA-MB-231) using MST (microscale thermophoresis) and Annexin assay. Analyses were conducted to assess the impact of added anti-CD146 antibodies, drug, drug with SN (CR/EB-Dox), and SN alone. The model of ICH was developed, indicating the involvement of CD146 in acquiring a mesenchymal profile by cancer cells, correlating high CD146 expression with poor prognosis in BC patients. ICH and dynamic changes in CD146 protein expression have been demonstrated in BC, possibly because high CD146 levels correlate with acquiring a mesenchymal profile. Low levels of this protein facilitate the transendothelial migration process. Funding sources: the international project (no. U1C/P04/NO/02.24) in the strategic program Initiative of Excellence at the Jagiellonian University.

P-32-072
Losartan protects against short and long-term radiotherapy-induced testicular damage via testosterone restoration and metabolic remodeling

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Testicular dysfunction is a common side effect of radiotherapy due to off-target damage. Germ cells are highly vulnerable, and

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while Sertoli and Leydig cells are more resistant, they are still affected, impairing spermatogenesis and steroidogenesis. With rising youth cancer rates, strategies to preserve fertility are crucial. Losartan (LOS) has shown potential to mitigate this damage. This work aimed to determine if radiotherapy induces short- and long-term alterations in testicular metabolism and if LOS can restore this function. Male Wistar rats (n = 47, 12 weeks old) received 2.5 Gy of ionizing radiation to the scrotum (1.05 Gy/min; Varian Clinic 6EX). LOS-treated rats received 34 mg/kg twice daily by gavage for 7 days before, during and after irradiation. Animals were euthanized at 2 and 60 days post-exposure, to represent short and long-term effects, respectively. Reproductive organs were weighed, serum hormones assessed (ELISA), sperm parameters analyzed following World Health Organization guidelines, testicular mRNA expression quantified (qPCR) and oxidative stress markers, such as lipid peroxidation, protein carbonylation, and protein nitration (slot-blot) measured. Metabolomic profiles were obtained via 1H-NMR. Short-term irradiation reduced seminal vesicle weight, increased FSH, and decreased sperm concentration. Long-term effects included reduced testicular and epididymal weight, impaired sperm quality, increased protein carbonylation, and altered metabolic profiles. LOS mitigated short-term weight loss but not sperm decline. Long-term, LOS improved sperm quality, reduced oxidative stress, and promoted adaptive metabolic responses. Irradiation-based cancer therapy causes structural and functional testicular damage and changes the testicular metabolome of rats, while LOS has the potential to be used as a radioprotector to mitigate the adverse short- and long-term effects of radiation on male fertility.

P-32-073
Anti-tumor efficacy of stem cells armed with targeted therapeutics in lung cancers

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Targeted cytotoxins, such as bacterial toxin-based therapies, have shown promise in cancer treatment; however, their clinical translation has been obstructed due to their off-target effects and systemic toxicity. To combat this, stem cells have emerged as promising therapeutic delivery vehicles owing to their migration capacities and tumor-homing properties. Formerly, human neural stem cells secreting interleukin-13 fused *Pseudomonas* exotoxin (ILPE) were tested and shown to have therapeutic benefits when combatting glioblastomas. In this study, human mesenchymal stem cells (hMSCs) are used to deliver IL13PE against lung cancer due to their expression of the IL13Ra2 receptor, a target also found in glioblastoma. To establish targeted-toxin-released mesenchymal stem cells (hMSC-IL13PE), first, human mesenchymal stem cells (hMSCs) were engineered to be toxin-resistant by transfecting them with mutant elongation factor 2-encoding single-stranded oligonucleotides (ssODN1). These ssODN1 integrated-hMSCs were then gradually exposed to diphtheria toxin (DT) to allow for the subsequent selection of resistant clones. Then, recombinant IL13PE pDNA was introduced into resistant stem cells to enable targeted toxin release, with their expression verified through molecular analysis. After engineering the stem cells, their therapeutic effects on firefly luciferase (FLUC) labeled cancer cell lines were evaluated by assessing cancer cell viability in conditioned media and co-culture experiments. Cell viability assays were performed using luciferase-based detection for transduced cells, demonstrating a significant cytotoxic effect on targeted cancer cells while preserving the survival of resistant hMSCs. These findings emphasize the potential of hMSCs as effective delivery vehicles for IL13PE, providing a targeted approach to lung cancer treatment. This study is funded by TÜBITAK with project number 117S42.

P-32-074
High fat diet changes critical metabolic reprograming genes in meningioma

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Poor dietary habits, particularly high sugar and fat intakes, are linked to cancer progression. Meningiomas, the most common primary adult brain tumors, exhibit aberrant fatty acid (FA) metabolism, which correlates with poor prognosis and tumor grade [1]; however, the underlying molecular mechanisms and the link to cancer's progression remain unclear. Our study investigates how palmitate, a prevalent dietary lipid, affects meningioma metabolic reprogramming. Using meningioma cell lines representing each grade of the disease, we modeled the disease and assessed palmitate tolerance via MTT [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide] viability assays hence found comparable ranges across all grades. We analyzed our cell lines' RNA sequencing data with Ingenuity Pathway Analysis (IPA) and identified key metabolic adaptation genes. By maintaining extracellular palmitate at a constant level, we simulated a fat-rich tumor microenvironment and examined gene expression changes in critical targets using qPCR. Notably, we observed downregulation of aldehyde oxidase 1 (AOX1), a key FA metabolism enzyme. Reduced AOX1 expression has been shown to increase NADP levels, enhancing pentose phosphate pathway (PPP) flux, nucleotide synthesis, and cell invasion in prostate cancer [2]; this metabolic shift, previously implicated in prostate cancer, appears to contribute to meningioma malignancy. Furthermore, AOX1 downregulation correlated with higher tumor grade for our cell lines, suggesting an adaptive mechanism for tumor progression. Our findings indicate that targeting FA metabolism could be a promising therapeutic approach for malignant meningiomas. Future research will employ genetic manipulation to elucidate AOX1's role in tumor survival and assess its potential as a therapeutic target.
References: [1] Jiang J et al. (2013) APMIS 121, 997–1003. [2] Wu J et al. 2023, Urol Int 107, 517–525

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P-32-075
Drug screening in tumor organoids as a promising decision support system in precision oncology

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Precision oncology aims to tailor treatments based on patient-specific tumor characteristics, and tumor organoids have proven to be powerful preclinical models for personalised drug screening. These three-dimensional (3D) structures, derived from patient tumor samples, closely mimic the histological and genetic characteristics of the original tumour and provide a reliable platform for drug response testing. In this study, we developed organoid models of liver and ovarian tumours to investigate their potential for treatment decisions. Patient-derived tumor tissue was cultured in a Matrigel matrix to form self-assembling organoids. Characterisations were confirmed by immunohistochemistry and whole exome/transcriptome sequencing for genotypic/phenotypic analyses to confirm their tumourigenic properties. High-throughput drug screening was performed using the CellTiterGlo assay to assess sensitivity to standard chemotherapies and targeted therapies. Our results show a heterogeneous response of tumor organoids to drugs, reflecting the variability observed in clinical practice. Liver tumor organoids showed a common response to bevacizumab, which is applicable according to the guidelines. Ovarian tumor organoids responded differently to platinum-based chemotherapy. Importantly, the results correlated with patient treatment outcomes, highlighting the clinical relevance of organoid-based screening. These findings highlight the potential of tumor organoids as a decision support system in precision oncology, allowing personalized treatment selection. Further validation in clinical trials could establish organoid-based drug testing as a standard approach for optimizing cancer therapies.

P-32-076
Specificity analysis of Elk-1 suppression in neuroblastoma cells using antisense oligonucleotides

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Elk-1 is a member of the ternary complex factor (TCF) subfamily within the E-twenty-six transcription factor superfamily, along with Elk-3 and Elk-4. It is activated through the mitogen-activated protein kinase signalling pathway and playing a crucial role in various biological processes such as cell viability, proliferation, and cancer. Studies have shown that Elk-1 is overexpressed in brain cancer-initiating cells and is involved in the transcriptional regulation of stemness genes. Antisense oligonucleotides (ASOs), a type of RNA-based therapeutic approach, are promising molecules for the treatment of various diseases. ASOs are single-stranded synthetic oligonucleotides that bind to specific regions of target RNA, altering RNA and protein levels through various mechanisms. While ASOs are designed based on target RNA sequences, ensuring specificity among highly homologous proteins is a critical challenge. In this study, we aimed to explore that potential use of Elk-1 targeted ASO molecules for suppression of Elk-1 in cancer cells, particularly in neuroblastoma. For this, neuroblastoma cell lines were transfected with different anti-Elk-1 ASO molecules, and changes in Elk-1 and Elk-3 protein levels were analysed using western blot. As a result, ASOs targeting Elk-1 mRNA were shown to significantly reduce Elk-1 protein levels while not affecting the expression of Elk-3, which shares high sequence similarity with Elk-1. Future studies aim to further evaluate the specificity of the ASO targeting Elk-1 by analysing the expression of Elk-4. The determination of the *in vitro* efficacy of these Elk-1-specific ASOs will make significant contributions to their potential use as an effective treatment method in cancers such as neuroblastoma. *The authors marked with an asterisk equally contributed to the work.

P-32-077
Silencing of Elk-1 in glioblastoma using antisense oligonucleotides

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Glioblastoma multiforme (GBM), a highly malignant and deadly cancer, remains resistant to conventional treatments such as chemotherapy and radiotherapy. This highlights the need for the development of novel therapeutic options. In recent years, a number of RNA-based therapeutics, including small interfering RNA (siRNA) and antisense oligonucleotides (ASOs), have received approval from the US Food and Drug Administration. Small synthetic single-stranded oligonucleotides, ASOs, that target specific RNA sequences can alter RNA and protein levels or enable splice correction by influencing various molecular mechanisms without modifying the gene sequence. Elk-1, a member of the E-Twenty-Six transcription factor family, takes roles in proliferation of brain tumors. It was also shown that Elk-1 is overexpressed in brain tumor-initiating cells and regulates the expression of stemness genes. Considering the role of Elk-1 in brain tumors, it is hypothesized that silencing Elk-1 could offer a promising strategy for developing effective treatments for GBM, and the aim of this study is to demonstrate the efficacy of Elk-1-targeted ASOs with distinct sequences in silencing Elk-1 gene expression. For this purpose, glioblastoma cells cultured as monolayer and spheres were transfected with different ASOs, and western blot analyses were performed. The results have shown that Elk-1 targeting ASOs cause a notable alteration in Elk-1 protein levels in glioblastoma cells. These findings indicate that Elk-1-targeted antisense oligonucleotides have considerable potential as drug candidates for the development of effective treatments for GBM and other brain cancers. *The authors marked with an asterisk equally contributed to the work.

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P-32-078
Attenuated oncolytic vaccinia virus for treatment of glioblastoma

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MicroRNAs play a crucial role in gene regulation, controlling key processes of tumor growth and metastasis. They are widely used as prognostic markers of cancerous tumors, and their selective expression in tumor cells makes them a useful tool for targeted viral therapy. miR-21 is one of the most studied oncogenic microRNAs and is overexpressed in glioblastomas (GB). GBs often exhibit defective interferon signaling. The LIVP strain of vaccinia virus (VV) demonstrates differential oncolytic activity in these tumors, suggesting a potential mechanism for targeted therapeutic intervention. To investigate this further, we engineered a recombinant VV strain incorporating a miR-21-5p binding site upstream of an open reading frame encoding human interferon-α (IFNα) and the fluorescent protein tagRFP. Experimental results indicate that IFNα expression does not significantly compromise the viral ability to replicate in tumor cells or prevent the formation of infectious foci. In tumor cells with high miR-21 expression, the transcript with binding site is cleaved, preventing IFNα and tagRFP expression. Conversely, in normal cells with low miR-21 levels, IFNα is expressed at high levels, triggering an antiviral response that inhibits viral replication and protects the surrounding tissue. The expression level of miR-21 was assessed in a panel of primary and model GB cell lines, and viral kinetics were analysed. Our results confirmed that microRNA interference efficiently regulates IFNα/RFP expression in the oncolytic virus, supporting the concept of a microRNA-attenuated viral design. This work was supported by the Russian Science Foundation (grant no. 23-74-10102).

P-32-079
Anticancer activity of pleurocidin family NRC-03 peptide against cervical cancer cells

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NRC-03 is a 26-residue cationic antimicrobial peptide belonging to the pleurocidin family, derived from skin mucous secretions of winter flounder (*Pleuronectes americanus*). Although pleurocidin-like peptides were originally demonstrated as broad-spectrum antimicrobials, many studies reveal their potent cytotoxic properties against cancer cells, including breast cancer or leukemia cells. The cationic structure of NRC-03 allows it to specifically target cancer cells that have negative charges leading to the rupture of their membranes and ultimately causing cell death. Importantly, NRC-03 exhibits lower toxicity towards normal cells. To date, the ability of NRC-03 to inhibit the growth of cervical cancer cells has not yet been studied. Moreover, the molecular mechanisms governing the NRC-03-induced death of cervical cancer cells remain unknown. This study focuses on elucidating the possibility of employing the NRC-03 peptide as a therapeutic agent against cervical cancer cells and decoding the mechanism of cytotoxicity of this peptide. To achieve that, the viability of cervical cancer HeLa cells, their morphology as well as their proliferation capability was explored using colorimetric and microscopic methods. The induction of apoptosis and other cell death mechanisms was investigated using flow cytometry, fluorescence microscopy and gene profiling. Biocompatibility of the peptide was assessed using a vaginal epithelial cell line (VK2/E6E7) and human erythrocytes. We demonstrated that the NRC-03 peptide decreases significantly the viability of cervical cancer cells while offering the appropriate biocompatibility. Our results strongly encourage further studies on the utility of NRC-03 peptide as an anticancer molecule in the treatment of cervical cancer. Funding: Financial support was received from the Medical University of Bialystok (B.SUB.25.407 to EP).

P-32-080
Cytotoxic effects of polyphenolic cocktail (PFK5120), salicylic acid and indole-3-carbinol combinations on gastric adenocarcinoma cell line

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Cancer is the leading cause of morbidity and mortality worldwide. Among all cancer types, gastric cancer is one of the most lethal cancer due to its high incidence, poor prognosis and low survival rates. Studies have revealed the anticancer properties of plant secondary metabolites via apoptosis inducing effects. Changes in the expression levels of BAX, BCL2 and BAD genes, which have key roles in mitochondrial apoptosis, have been shown in previous studies. In this research, AGS cell line, and HUVEC cell line were treated with PFK5120, indole-3-carbinol (I3C), salicylic acid (SA) individually and combined forms at certain doses and times. Cytotoxicity analysis was performed by WST-8 method, and real-time PCR method was used to indicate the changes in the expression levels of BAX, BCL2, and BAD genes. According to WST-8 analysis, at 48 h, PFK5120 exhibited a strong cytotoxic effect on AGS cells across all doses, I3C showed a strong cytotoxic effect at 15 μg/mL dose, and SA had cytotoxic effects at doses of 60 μg/mL and 125 μg/mL ($p < 0.0001$ for each). On the other hand, WST-8 analysis revealed that the treatment with combinations showed more cytotoxic effect on cancer cells, and the combined treatment caused a lower cytotoxic effect on HUVEC control cells. Individual treatments caused an upregulation in BAX and BAD expressions and a downregulation in BCL2 expression. Also, while the combination of PFK5120/I3C shows the similar results as individual applications, PFK5120/I3C/SA application demonstrates an opposite result. In this context, the effect of the combined use of I3C and SA on apoptosis mechanisms should be investigated in more detail. In particular, future studies should clarify whether these compounds exert their antiproliferative effects through different signaling pathways. The findings obtained provide significant contributions to the evaluation of plant-derived secondary metabolites as potential adjuvant agents in cancer treatment.

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P-32-081
Olive leaf extract enhances the cytotoxic activity of NK-92 cells against HT-29 colon cancer cells

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Olive leaf extract (OLE) is rich in bioactive compounds with potential anticancer properties. This study aimed to investigate the effects of OLE on HT-29 colon cancer cells and its influence on NK-92 cell cytotoxic activity under co-culture conditions. The phenolic, flavonoid, and antioxidant capacities of OLE were analyzed. HT-29 and NK-92 cells were treated with varying concentrations of OLE, and cell viability was assessed using MTT [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide] and ATP assays. Apoptosis rates were determined through AO/EtBr staining, while cell migration was evaluated using migration assays. A co-culture system was established in 96-well plates with a seeding ratio of 1:5 (HT-29 to NK-92 cells). The cells were treated with different concentrations of OLE and incubated for 48 hours. The viability of HT-29 cells was then assessed using the MTT assay. Granzyme and perforin levels were quantified using ELISA according to the manufacturer's protocol. OLE exhibited cytotoxic effects on HT-29 cells, with an IC_{50} value of 540 μg/mL at 24 hours. It also induced apoptosis in HT-29 cells and significantly reduced cell migration in a dose-dependent manner at 24 hours. Co-culture experiments revealed that the IC_{50} value of OLE decreased to 200 μg/mL, indicating that OLE enhances the cytotoxic activity of NK-92 cells against HT-29 cells. Furthermore, granzyme and perforin levels significantly increased following OLE treatment, suggesting an augmentation of NK-92-mediated cytotoxicity. In conclusion, OLE exerts significant cytotoxic effects on HT-29 cells and enhances NK-92-mediated cytotoxicity against HT-29 cells. Flavonoids found in plants exhibit anticancer activity at high doses; however, achieving these concentrations *in vivo* is often challenging. Nevertheless, flavonoids can exert cytotoxic effects even at lower doses by enhancing the activity of immune system cells, such as NK cells.

P-32-082
Double-functionalized silk nanospheres for delivery of oligotherapeutics to VEGFR-overexpressing tumor microenvironment cells

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siRNA enables efficient silencing of target gene expression, but its delivery to the cells is limited and requires a delivery system. Targeting VEGF receptors, which are overexpressed in both cancer and endothelial cells of the tumor microenvironment, to deliver therapeutic siRNA is a promising strategy for cancer treatment. Bioengineered silk shows high promise for drug delivery. It can be functionalized with a targeting peptide or nucleic acid-binding sequence to facilitate the targeted delivery to specific cells. In this study, bioengineered silk proteins VE1MS1 and VE2bMS1, designed to target VEGFR1 and VEGFR2, respectively, were blended with MS2KN silk, containing a nucleic acid-binding domain. Agarose gel electrophoresis confirmed the oligonucleotides' binding affinity to the VEGFR-functionalized silks and their MS2KN blends. Then, silk proteins were formulated into nanospheres, and their physicochemical properties, including size, morphology, zeta potential, cytotoxicity, and siRNA loading to the spheres, were established. The incorporation of therapeutic oligonucleotides into spheres was achieved with 60% efficiency. Flow cytometry analysis revealed that VEGFR-targeting spheres loaded with siRNA efficiently bound to VEGFR-overexpressing H1975 (90%), HCC4006 (70%) non-small-cell lung cancer cells, and HUVEC endothelial cells (65%). Moreover, specific cellular internalization of VE1MS1 and VE2bMS1 spheres and intracellular delivery of siRNA were confirmed by confocal microscopy. qRT-PCR and western blot analysis showed that HIF-1α-siRNA delivered via silk nanospheres lowered target gene expression in H1975 and HUVEC cells compared to scrambled-siRNA control. HIF-1α silencing modulated the expression of factors related to tumor angiogenesis and progression at mRNA and protein levels. Our results show that VEGFR-targeting silk spheres are a promising tool for efficient delivery of oligotherapeutics to VEGFR-overexpressing cells and silencing effect induction.

P-32-083
Impact of thiazolyl hydrazone derivatives on primary and metastatic breast cancer cell lines: relation to apoptosis and necroptosis

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This study describes the synthesis of six novel thiazolyl hydrazone derivatives (4B, 4C, 4D, 4G, 4H, 4L) and assesses their cytotoxic effects on primary (MCF-7) and metastatic (M4A4) breast cancer cell lines. Furthermore, the impact of these derivatives on apoptotic and necroptotic pathways in breast cancer cell lines was evaluated. Thiazolyl hydrazone structure were synthesized in three steps. Hydrazide formation, thiosemicarbazide conversion and thiazolyl hydrazone synthesis under suitable conditions. The structures were confirmed by IR, ^{1}H-NMR, ^{13}C-NMR, 2D-NMR (HSQC) and elemental analysis. MCF-7 and M4A4 cells were cultured and treated with various concentrations (5 μM, 10 μM, 25 μM, 50 μM and 100 μM) of the all compounds for 24 and 48 hours. The cytotoxicity was evaluated with 3-(4, 5-dimethylthiazolyl-2)-2, 5-diphenyltetrazolium bromide (MTT) assay. The distribution of caspase 3, Bax, Bcl-2, cytochrome C, Ki-67, RIPK1, RIP3 and MLKL were analyzed using indirect immunoperoxidase staining. MTT assay results, analyzed using Graphpad Prism 8.0.1, determined optimal doses for each compound and cell line, followed by 48-hour treatments. Immunohistochemical analysis

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revealed that apoptosis activation in M4A4 cells via caspase 3 expression (4C, 4D, 4G, 4H and 4L) and in MCF-7 cells through Bcl-2 reduction (4C, 4D, 4L) and an increased Bax/Bcl2 ratio (4D and 4L). Proliferation marker, Ki-67, immunoreactivity was also decreased in MCF-7 and M4A4 cells after applying of 4B, 4C, 4D, 4H ve 4L or 4B, 4C compounds, respectively. RIP 3 immunoreactivity was decreased in M4A4 for 4C and 4L and MCF-7 for 4B, 4D, 4H and 4L compounds, while other compounds showed similar immunoreactivity to the control group. Different thiosemicarbazide compounds exhibited varying effects on primary and metastatic breast cancer cell lines. Notably, compounds 4D and 4L effectively induced apoptosis rather than necroptosis in both cell lines, highlighting their potential as promising anticancer agents.

P-32-084
Investigating the *in vitro* effects of natural plant extracts on human cancer-related gene expression

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Plant extracts had been applied since ancient times in wound healing and the treatment of different kinds of diseases including human cancer diseases. Many chemical compounds used as medical agents originated from plants. Olives (*Olea europaea*) are ancients plants that were cultivated and domesticated around and near the Mediterranean Sea. Oil extracted from these plants, besides being of nutritional value, were used also in the human wound healing. Its potential in the regulation of important disease related genes were not studied extensively on the molecular level yet. Sun dried black olives were extracted after dry grinding with either pure ethanol or menthol different concentrations to either glioblastoma (GBM) cell lines U87-MG and U251 or to the hepatocellular carcinoma (HCC) cell line HepG2. Hypoxia induced HIF-1α and N-myc down-regulated gene 1 (NDRG1) reached an expression level at both the mRNA and the protein level making 50% of the maximum expression level after 24 h of hypoxia when ethanol-dissolved extracts were added, while using methanol-dissolved extracts exerted a down-regulation to nearly 35% of the maximum hypoxic expression level. All the cell lines examined showed this expression pattern, while for HIF-1α the highest down-regulation was in the treated U251 cell lines followed by HepG2 cell lines Caspase 3 expression was not affected by the application of olive extracts while Sp1 and CEBPα were also down-regulated in a differential manner. Further, treatment of these cells with the extracts resulted in a significantly lowering of the cell migration. These observations open the potential for the treatment optimization of GBM and HCC by the application of olive extracts as (anti-angiogenesis/anti-cancer) therapeutic target gene(s) regulators, thereby enhancing the applied treatment modalities and potentially preventing tumour initiation and tumourigenesis.

P-32-085
Hypericum alpestre inhibits PI3K/Akt/mTOR and HIF-1α signaling while activating caspase-3 in MDA-MB-231 cells: a comparative study with dactolisib and doxorubicin

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Targeting hypoxia-associated pathways like HIF-1α and PI3K/Akt/mTOR, alongside activating apoptotic executioners such as Caspase-3, is crucial in managing aggressive cancers like triple-negative breast cancer (TNBC). Natural compounds are increasingly recognized for their multi-targeted therapeutic potential, and we investigated the bioactivity of *Hypericum alpestre* in modulating these key oncogenic and apoptotic pathways. MDA-MB-231 cells were treated with 0.125 and 0.25 mg/mL of *Hypericum alpestre* extract. Immunocytochemistry (ICC) was used to assess HIF-1α, PI3K, Akt, mTOR, and caspase-3 expression, with results compared to dactolisib (PI3K/mTOR inhibitor, 1 μM) and doxorubicin (pro-apoptotic chemotherapeutic, 2 μM). Hypoxic and insulin-stimulated conditions were applied to activate these pathways. *Hypericum alpestre* significantly reduced HIF-1α expression and suppressed PI3K, Akt, and mTOR activation in a dose-dependent manner. Both tested concentrations exhibited inhibitory effects comparable to dactolisib and doxorubicin. Additionally, *Hypericum alpestre* markedly enhanced caspase-3 activation, promoting a pro-apoptotic shift in treated cells. These findings highlight the extract's dual role in suppressing pro-survival signaling (HIF-1α/PI3K/Akt/mTOR) and promoting apoptosis (caspase-3), making it a strong candidate for anticancer therapy. Its comparable efficacy to dactolisib and doxorubicin suggests its bioactivity may stem from active phytochemicals such as flavonoids, hypericin analogues, or other polyphenols. *Hypericum alpestre* effectively inhibits the HIF-1α/PI3K/Akt/mTOR axis while enhancing caspase-3-mediated apoptosis in MDA-MB-231 cells. Its activity at both tested concentrations mirrors that of dactolisib and doxorubicin, supporting its potential as a promising plant-derived anticancer agent for further preclinical development.

P-32-086
Targeting arginine metabolism with plant-derived compounds in Hela cell lines: a novel strategy for cancer therapy

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As cancer incidence rises globally, improving treatment strategies remains a major biomedical challenge. While chemotherapy and immunotherapy have advanced cancer management, their efficacy is often limited by adverse effects and resistance. Recent studies highlight the potential of combining herbal extracts with chemotherapeutic agents to enhance outcomes. This study evaluated the antitumor effects of *Inula helenium* and *Alchemilla smirnovii* Juz (AS) extracts on HeLa cells. Their combination with arginase inhibitors L-NAME and nor-NOHA was assessed

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for synergistic effects on nitric oxide synthase (NOS) and arginase pathways. Arginase activity was measured using the diacetyl monoxime colorimetric method, and cytotoxic effects via MTT [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide] assay. Malondialdehyde (MDA) levels in cell culture medium were determined using the Ohkawa thiobarbituric acid-malondialdehyde method, and NO levels via Griess assay. NOS activity in cell lysates was measured by L-arginine to L-citrulline conversion. Treatment with *A. smirnovii* significantly reduced arginase activity, while *I. helenium* (IH) also showed a decrease. The combination of plant-derived compounds with arginase inhibitors further enhanced cytotoxicity in HeLa cells. Increased MDA levels in all treated groups suggest enhanced lipid peroxidation, potentially contributing to anticancer effects via oxidative stress. NOS activity was dose-dependent: at 0.25 mg/mL, AS increased NOS activity 1.3-fold ($p < 0.05$), while at 0.125 mg/mL AS decreased it by 1.18-fold ($p > 0.05$). IH at 0.25 mg/mL increased NOS activity 1.72-fold ($p < 0.05$), while 0.125 mg/mL showed no significant change. These results confirm NOS and arginase as potential therapeutic targets in oncology. Targeting arginine metabolism with arginase inhibitors and plant-derived compounds holds promise for novel anticancer strategies.

P-32-087
Synergistic inhibition of HIF-1α and PI3K/Akt signaling and activation of caspase-3 by emodin and metformin combination in MDA-MB-231 cells

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Emodin and metformin, two pharmacologically active compounds with distinct origins, exhibit overlapping antitumor activities. Emodin, a natural anthraquinone, has anti-proliferative and anti-angiogenic effects, while metformin, an antidiabetic drug, suppresses tumor growth via metabolic reprogramming and AMPK activation. Both interfere with the PI3K/Akt/mTOR pathway and hypoxia-inducible factor-1 alpha (HIF-1α), key regulators of tumor progression and therapy resistance. This study examined their combined effects on HIF-1α expression, PI3K/Akt/mTOR pathway activity, and Caspase-3-mediated apoptosis in triple-negative breast cancer cells. MDA-MB-231 cells were cultured under hypoxia-mimicking conditions induced by cobalt chloride (CoCl, 100 μM) and insulin stimulation. Cells were treated with emodin (40 μM), metformin (10mM), or their combination, and expression levels of HIF-1α, PI3K, phosphorylated Akt, mTOR, and caspase-3 were assessed using immunocytochemistry (ICC) and western blotting. Both emodin and metformin individually reduced HIF-1α expression and inhibited PI3K, p-Akt, and mTOR signaling under hypoxic and insulin-stimulated conditions, while their combination led to significantly greater suppression. Co-treatment also strongly enhanced caspase-3 activation, indicating a synergistic pro-apoptotic effect. ICC and western blot analyses confirmed that the emodin + metformin combination was more effective than either compound alone in inhibiting oncogenic signaling and inducing apoptosis. The combined administration of emodin and metformin synergistically disrupted the HIF-1α/PI3K/Akt/mTOR axis and enhanced caspase-3 activation. While emodin likely interferes directly with oncogenic signaling, metformin amplifies metabolic stress via AMPK activation, collectively driving hypoxia-targeted antitumor activity. This dual-action approach highlights their potential for overcoming hypoxia-driven resistance in TNBC. *The authors marked with an asterisk equally contributed to the work.

P-32-088
Interconnection between hyperglycemia, cancer, and arginine metabolism: a unified therapeutic approach

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Hyperglycemia and diabetes are associated with profound metabolic dysregulation, particularly in nitric oxide (NO) bioavailability and arginine metabolism. Emerging evidence suggests that these metabolic disturbances contribute not only to insulin resistance and vascular complications but also to tumor progression and therapy resistance in cancer. Our studies have explored how modulating the L-arginine/NOS/arginase axis can serve as a therapeutic strategy for both metabolic disorders and oncological conditions. In our diabetes model, L-norvaline and L-arginine supplementation significantly improved glucose homeostasis by enhancing NO production, reducing oxidative stress, and restoring endothelial function. These findings parallel our observations in cancer models, where alterations in arginine metabolism influence the PI3K/Akt signaling pathway, affecting tumor growth, metastasis, and drug resistance. Notably, hyperglycemia-induced oxidative stress shares mechanistic similarities with the inflammatory and angiogenic responses observed in cancer, further linking the two diseases. Our combined therapy approach using *Rumex obtusifolius* extract and 5-fluorouracil (5-FU) demonstrated a 29.5% increase in apoptosis, along with a significant reduction in TNF-α, VEGF-α, MMP-2, and COX-2 levels—key regulators of inflammation, vascular remodeling, and cellular proliferation. Given that chronic hyperglycemia is known to promote these same pathways, our findings suggest that metabolic interventions targeting arginine metabolism could not only mitigate diabetic complications but also reduce cancer susceptibility and improve treatment efficacy. This study underscores the need for an integrated therapeutic approach that bridges metabolic disorders and oncology. By regulating arginine metabolism and NO bioavailability, we propose a novel strategy that could optimize diabetes management while simultaneously offering protective effects against cancer progression.

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P-32-089
Investigation of the anti-tumor activity of a novel triazole derivative, CTAPM1, alone and in combination with docetaxel in breast cancer cells

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Multidrug resistance (MDR) remains a significant challenge in all breast cancer types, despite the application of various treatment modalities. Glutathione-S-transferases (GSTs) are a family of enzymes associated with MDR in tumor cells. This study investigates the anti-tumor activity of CTAPM1 (Turkish Patent and Trademark Office, Patent No: 2018/19936), a novel, isozyme-specific inhibitor of GSTP1, in breast cancer cell lines. MDA-MB-231, MCF-7, 4T1 breast cancer cell lines, and non-tumorigenic mammary epithelial MCF12A cells were treated with varying concentrations of CTAPM1 and ethacrynic acid, a benchmark GST inhibitor, alone or in combination with docetaxel for 24, 48, and 72 hours. The cell viability was assessed using MTT [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide] assay. The cell migration was performed using scratch assay. To identify the synergistic effects of the compounds in combination with docetaxel in cancer cells, we employed CompuSyn, Synergy Finder Plus, and Combenefit software. Intracellular GSTP1 activity was measured based on its ability to conjugate 1-chloro-2,4-dinitrobenzene (CDNB) with glutathione (GSH). Results indicated significant cytotoxic effects for CTAPM1 on the cancer cells, while it was only cytotoxic at very high concentrations on non-tumorigenic epithelial cells. It was also found that CTAPM1 was more effective than ethacrynic acid in reducing the migration ability of cancer cells. Additionally, CTAPM1 reduced intracellular GSTP1 activity in MDA-MB-231 and 4T1 cells. This study is the first to explore the GSTP1 inhibitory activity of CTAPM1 in breast cancer cells and its potential anti-tumor effects. In conclusion, CTAPM1 emerges as a promising drug candidate that may enhance treatment efficacy, alone or in combination with docetaxel. This study is supported by The Scientific and Technological Research Council of Turkiye (TUBITAK, Project number: 123S096) and Hacettepe University Scientific Research Projects Coordination Unit (Project number: THD-2023-20995).

P-32-090
In silico identification and evaluation of antibiofilm agents to overcome drug resistance in hepatocellular carcinoma

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Hepatocellular carcinoma (HCC) is one of the most aggressive malignancies, with high mortality rates due to limited treatment options and the frequent emergence of resistance to sorafenib and regorafenib as the first- and second-line therapies. Recent studies suggest that bacterial biofilms in the tumor microenvironment contribute to drug resistance by protecting cancer cells from therapeutic agents. This study employs an *in-silico* approach to identify antibiofilm agents with potential anticancer properties to overcome drug resistance in HCC. A computational approach was implemented, beginning with molecular docking studies targeting key biofilm-associated proteins, including quorum sensing regulators, extracellular polymeric substance synthesis enzymes, and efflux pump inhibitors. Target proteins were selected from bacterial strains associated with biofilm formation, particularly ESKAPE pathogens and additional biofilm-forming strains. These strains were analyzed in UniProt, and we selected only the non-redundant ones, which formed a refined dataset with 23 target proteins. FDA-approved and investigational small molecules were chosen from the ZINC20 database as the ligands to be docked against these target proteins. Docking studies were used to detect the high-affinity binders, which will then be assessed for their interactions with drug resistance-related proteins in HCC. We have reached out to more than 20 small molecules with an antibiofilm activity potential. Considering the key proteins of the signaling pathways shown to be involved in developing drug resistance in HCC as their secondary targets, these selected ligands are planned to be analyzed further for their anticancer potential. Including molecular dynamics (MD) simulations in our research, we aimed to confirm the stability of these interactions. This study introduces a novel therapeutic strategy targeting tumor-associated biofilms to enhance the efficacy of existing HCC treatments. *The authors marked with an asterisk equally contributed to the work.

P-32-091
Emodin inhibits HIF-1α expression in MDA-MB-231 cells via suppression of the PI3K/Akt/mTOR pathway

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Hypoxia-inducible factor-1 alpha (HIF-1α) plays a key role in cellular adaptation to hypoxia and is frequently upregulated in triple-negative breast cancer (TNBC), promoting tumor progression, angiogenesis, and therapy resistance. Targeting HIF-1α and its regulatory pathways is a promising therapeutic strategy. Emodin, a natural anthraquinone, has demonstrated

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antitumor properties, but its effects on HIF-1α expression remain insufficiently explored. We investigated emodin's impact on HIF-1α in MDA-MB-231 cells using immunocytochemistry (ICC) and western blot analysis. Hypoxia was mimicked by CoCl (100 μM), while insulin activated the PI3K/Akt/mTOR pathway. Cells were treated with emodin alone or in combination with these agents, and expression levels of HIF-1α and key signaling proteins were assessed. ICC revealed that emodin (40 μM) reduced HIF-1α nuclear accumulation in CoCl- and insulin-treated cells, and western blot confirmed a significant decrease in HIF-1α protein levels. Emodin also suppressed PI3K, phosphorylated Akt, and mTOR, suggesting inhibition of the PI3K/Akt/mTOR pathway. Previously, *in silico* docking and HPLC-MS/MS profiling showed that *Rumex obtusifolius* extract, rich in emodin, binds key PI3K/Akt components. These findings support that emodin directly inhibits this cascade, reducing HIF-1α expression under hypoxic and insulin-induced conditions. This experimental confirmation strengthens its proposed mechanism and highlights the therapeutic potential of emodin-rich extracts in combating hypoxia-driven tumor progression and resistance in TNBC.

P-32-092
Laser-induced high-energy electron FLASH irradiation of cancer cells for development of new radiotherapeutic strategies

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Radiotherapy serves as a key treatment tool in oncology. However, due to significant toxicity incurred to normal tissues, conventional X-ray therapy has proved its limitations. In the last decade, an increased interest emerged in the use of alternative radiation sources/strategies with improved tissue penetration profile and tumor targeting control. Among these, ultra-high dose rate radiation (also known as FLASH) has shown important potential in treating solid tumors, while sparing normal tissue from radiation-induced damage. Here, we present recent data on cancer cells response to new high-energy electron FLASH irradiation generated by the interaction of a high-intensity laser beam with gas jets. In order to compare the effects produced by electron FLASH radiation on cancer and normal cells, we used a co-culture model consisting of A375 melanoma cells and normal human epidermal melanocytes. Both cell types were simultaneously exposed to FLASH, followed by image cytometry quantification of phosho-γ-H2AX foci. The obtained results provided evidence of a differential DNA damage response of cancer and normal cells to the applied treatment [previously published in: Orobeti S et al. (2024) Sci Rep 14:14866]. This validated the new FLASH irradiation source as a potential new radiotherapeutic strategy to counteract tumors. Next, we started adapting *in vitro* cell assays for characterization of radiation induced cell response. Dose-dependent cytotoxic effects of X-ray exposure were successfully observed in melanoma and ovarian cancer model cell lines for doses up to 6 Gy. Flow cytometry showed an increase in early apoptosis in cancer cells at 72 h post-irradiation. However, all cells expanded 3 weeks post-irradiation (resistant) showed 98% viability. These optimized set-ups will allow future investigations on the molecular mechanisms of response to the newly developed electron FLASH irradiation source. Further work is in progress for improving its stability and uniformity.

P-32-093
Dupα7, a non-functional variant of α7-nAChR, mediates resistance of metastatic melanoma cells to the antitumor protein SLURP-1

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Melanoma is an aggressive tumor, the progression of which is accompanied by a decreased expression of the three-finger protein SLURP-1 of the Ly6/uPAR family [1]. This protein regulates epithelial homeostasis by allosteric inhibition of nicotinic acetylcholine receptors of the alpha 7 type (α7-nAChR). SLURP-1 and peptide mimicking its first loop (Oncotag) inhibit carcinoma progression *in vitro* and *in vivo* [2] (In this work, we studied the possibility to control melanoma cell homeostasis by a recombinant analogue (rSLURP-1). We showed that a content of endogenous SLURP-1 in the plasma of melanoma patients is reduced in comparison with healthy donors. In contrast to carcinomas, rSLURP-1 inhibited migration only of some patient-derived metastatic melanoma cells. rSLURP-1-resistant metastatic melanoma cells demonstrated higher levels of expression of a chimeric non-functional variant of α7-nAChR called as dupα7 [3]. Dupα7, which together with α7 subunit can form hybrid α7-dupα7 receptors, contains a truncated ligand-binding domain. Knockdown of the CHRFAM7A gene coding dupα7 returned melanoma sensitivity to rSLURP-1. In electrophysiological experiments in *Xenopus laevis* oocytes, reduced rSLURP-1 inhibitory activity at α7-dupα7 receptors in comparison with α7-nAChR was revealed. We suggest that the increased dupα7 level in some melanomas results in inability of rSLURP-1 to control their homeostasis due to its low activity at these hybrid receptors. Thus, our results indicate that targeting of α7-nAChR with rSLURP-1 may be a promising strategy for melanoma therapy, but the dupα7 influence on the regulation of the cholinergic system in cancer cells should be taken into account. The work was supported by the Russian Science Foundation (project No. 23-74-00040). References [1] (Bergqvist 2018 Int J Dermatol 57(2):162-170). [2] Shlepova 2023 Front Cell Dev Biol 11:1256716. [3] Di Lascio 2022 Int J Mol Sci 23, 3463.

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P-32-094
Evaluation of prostate-specific antigen (PSA) as prognostic factor related to therapy for prostate cancer patients

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The aim of this study is to assess the dynamic changes of prostate-specific antigen (PSA) according to different therapy methods for prostate cancer (PCa) patients and the relation with severity of disease. This was a prospective observational analysis of diagnostic data from 54 men, between the ages of 53 and 80 years, during January 2023 to December 2024, at a tertiary care oncology hospital. Patients were treated with radical prostatectomy (PR), androgen deprivation therapy (ADT), and radiotherapy-volumetric modulated arc therapy (VMAT). The following data were recorded: age, PSA levels in ng/mL (initial diagnosis, before and 2-4 months after radiotherapy), Gleason Score (GS). Study participants had a mean age 70.4 ± 5.8 years with an initial median interquartile range (IQR) of PSA levels 19 (11.3-45.2). Median interquartile range (IQR) for GS was 2(1-3). Bone metastases were found in 14.8% patients. All patients were treated by VMAT with a mean dose of 6700 ± 814 cGY. 13 (24%) of patients were treated previously with PR and needed radiotherapy because of PCa recurrence after a median time of 5 (3-9) months. 17 (31.4%) patients were treated previously with ADT. Median levels of PSA after radiotherapy follow up were 0.68(0.21-2.54) with 2 (3.7%) patients remaining with high levels of PSA. We didn't find statistically significant relation between initial PSA levels, or after radiotherapy follow up and presence of bone metastasis. Biochemical recurrence of PCa according to PSA was found in all treatment methods even though, less frequently in patients treated with radiotherapy. PSA level wasn't found as a significant predictive factor for severe prognosis of PCa patients. *The authors marked with an asterisk equally contributed to the work.

P-32-095
The effects of telomerase mediated telomere-targeting novel drug candidate compounds on oxidative DNA damage and DNA repair on A549 cells

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Cancer cells extend their telomeres through the activity of the telomerase enzyme, allowing them to evade cell cycle control checkpoints and achieve immortality. Telomerase expression is present in approximately 85-95% of cancer cells, whereas its activity is absent in almost all somatic cells. Hence, telomerase-mediated telomere-targeted therapies have gained significant attention in recent years. 6-thio-2'-deoxyguanosine (6-thio-dG, aka THIO) and ribo-thio are telomerase substrate precursor nucleoside analogs, they induce telomeric DNA damage and cell death within 72-96 hours in telomerase-positive cancer cell lines when incorporated at telomeric ends by telomerase. The aim of this study is to investigate the effects of novel telomerase mediated telomere-targeting drug candidates on oxidative DNA damage and DNA repair on the A549 non-small cell lung cancer (NSCLC) cell line. The candidate drug molecules, 6-thio-dG and ribo-thio (MAIA Biotechnology, Chicago, USA), were tested on A549 cells at two different concentrations (0.3 μM and 1 μM) for 72 hours. DNA extraction midiprep kit (Qiagen) was used to isolate DNA from untreated and treated A549 cells. Orbitrap LC-MS and LC-MS/MS techniques were used to evaluate their effects on base excision repair (BER) proteins hAPE1 and hPARP1, as well as on the DNA damage marker 8-hydroxy-2'-deoxyguanosine (8-OH-dG). Based on our findings, 6-thio-dG and ribo-thio exhibited a statistically significant increase in 8-OH-dG when compared to the untreated control group, especially at 1 μM concentrations, suggesting the potential for DNA mutations in cancer cells. Furthermore, a notable elevation in hPARP1 and hAPE1 expression was observed in cells incubated with both 6-thio-dG and ribo-thio at 0.3 μM and 1 μM concentrations compared to the untreated control group. Our data suggest that both 6-thio-dG and ribo-thio induce DNA repair pathways in response to the increased DNA damage in cancer cells.

P-32-096
Potential of laser-irradiated chlorpromazine for triple-negative breast cancer therapy

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Chlorpromazine (CPZ), a well-known antipsychotic, has demonstrated anticancer properties through multiple mechanisms. This study represents a novel approach in triple-negative breast cancer treatment using laser-irradiated CPZ. We aimed to investigate the antitumor activity of CPZ photoproducts generated by laser irradiation on a breast cancer cell model. CPZ hydrochloride solution was irradiated for 5, 10, 20, 40, and 60 min using a 266 nm pulsed Nd:YAG laser. Both laser-irradiated and non-irradiated CPZ solutions were applied to triple-negative breast cancer (MDA-MB-231) and normal breast (MCF-12A) cells for 24 h, at concentrations of 5, 10, 15, 20, and 30 μM. The cytotoxic potential of CPZ photoproducts was assessed using MTT [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide], LDH (lactate dehydrogenase), Live/Dead, and clonogenic assays, while their antitumor mechanisms were further investigated through ROS production analysis and the expression of PI3K, Akt, and β-catenin. The results revealed a dose-dependent cytotoxic effect of CPZ photoproducts on MDA-MB-231 cells, with significantly higher toxicity compared to non-irradiated CPZ. Less toxicity was observed in normal MCF-12A cells. The cytotoxicity correlated with irradiation time and ROS production, with maximum effects observed at 40 min of laser exposure. At concentrations above 15 μM, irradiated CPZ induced membrane damage and

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inhibited the ability of cancer cells to form colonies. Additionally, PI3K, Akt, and β-catenin expression slightly increased at low doses, suggesting a survival response, but decreased at high doses, indicating a shift toward apoptotic pathways. The absence of similar effects in non-irradiated CPZ confirms that photoproducts are responsible for the observed cytotoxicity. These findings highlight the therapeutic potential of CPZ photoproducts as a novel approach for triple-negative breast cancer treatment, supporting further investigation into their molecular mechanisms and clinical applicability. Acknowledgements to project ELI-RO/RDI/2024_022.

P-32-097
Repurposing existing drugs to discover allosteric inhibitors of ERK5

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The prominent role of ERK5 in cancer development has led to the discovery of small molecule inhibitors of ERK5, aiming to prevent tumour growth and metastasis. However, challenges such as drug resistance, insufficient selectivity, and opposing effects hinder the effectiveness of these inhibitors. Certain inhibitors were even discovered to activate ERK5 signalling paradoxically, complicating their clinical application. In this regard, allosteric inhibitors may offer improved ERK5 selectivity with potentially lower levels of paradoxical activation. Our methodology focuses on discovering new allosteric ERK5 inhibitors through drug repurposing. Computational screening of potential allosteric binding pockets and molecular docking and dynamics simulations revealed 22 potential allosteric inhibitors among FDA-approved drugs, targeting two distinct ligand-binding pockets on ERK5 kinase surface. The cellular thermal shift assay (CETSA) showed that five of the 22 drugs bound to ERK5. Follow-up measurements from the ERK5 kinase assays indicated that three of these drugs are ERK5 kinase inhibitors. We are currently leading additional experiments to explore their allosteric activity. *The authors marked with an asterisk equally contributed to the work.

P-32-098
Efficiency of porphyrin-based photodynamic therapy using scintillating nanoparticles as energy mediators for ovarian cancer therapy

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Ovarian cancer is a common gynecologic cancer with treatment challenges. An option for this is photodynamic therapy (PDT) based on the generation of reactive oxygen species (ROS) by a photosensitizer (PS) upon light activation. X or γ-rays could be an excitation source for activating PS with scintillating nanoparticles (NPs) as energy mediators to convert the X or γ-rays to UV/visible light. This study proposes a comparative analysis of anti-cancer efficiency of two porphyrin-based PS (tetratosylate porphyrin – TMPyP4 and tetrasulfonate porphyrin – TPP_SO_3Na) conjugated with $CeTiO_2$ scintillating NPs under irradiated versus non-irradiated conditions. $CeTiO_2$-PS conjugates and free components were applied to ovary cancer cells (SK-OV-3) at various concentrations of porphyrins (0.01–0.1 μg/mL TMPyP4 and 0.01–0.5 μg/mL TPP_SO_3Na) for 24 h to allow cell internalization. The cells were exposed to the secondary radiation field generated by a high-power and short-pulse laser system (0.2 Gy) the next day. Untreated and non-irradiated cells served as control. After 24 h, the PDT efficiency was estimated by evaluating cell viability, reactive oxygen species (ROS) production, and cell death. Cell survival and induction of DNA damage were explored by clonogenic and Comet assays. The results showed the presence of dead cells after the treatment with the highest dose of $CeTiO_2$_TPP_SO_3Na in non-irradiated conditions. A slight decrease in cell viability was observed in irradiated compared to non-irradiated conditions after the exposure to conjugates. An inhibition of colony-forming capacity and production of intracellular ROS were also noticed. Under irradiation, $CeTiO_2$_TPP_SO_3Na induced more pronounced DNA lesions compared to non-irradiation and $CeTiO_2$_TMPyP4. We conclude that TPP_SO_3Na conjugated to scintillating NPs presented enhanced anti-tumoral activity than TMPyP4, highlighting the potential of PDT for improving the ovarian cancer therapy. Acknowledgements to ELI-RO/RDI/2024_022 project. *The authors marked with an asterisk equally contributed to the work.

P-32-099
Expression levels of lncRNA H19 in MCF-7 breast cancer cells following Paclitaxel and Doxorubicin treatment

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Breast cancer remains a formidable global health challenge, requiring ongoing research efforts to improve therapeutic strategies. Paclitaxel, a microtubule-stabilizing agent, has shown efficacy in the treatment of breast cancer, particularly by disrupting mitotic spindle dynamics. Doxorubicin, a well-established anthracycline chemotherapeutic agent, is widely used in breast cancer treatment. The long non-coding RNA (lncRNA) H19 plays a key role in regulating cellular processes such as proliferation and metastasis. In this study, we investigated the differential expression levels of lncRNA H19 in MCF-7 breast cancer cells treated with paclitaxel and doxorubicin, two commonly used chemotherapeutic agents with different mechanisms of action. Quantitative real-time polymerase chain reaction (qRT-PCR) was used to evaluate the expression of lncRNA H19 in these cells. As a result, MCF-7 cells treated with paclitaxel showed a 2.06-fold increase, whereas those treated with doxorubicin exhibited a 2.08-fold decrease in lncRNA H19 expression compared to untreated MCF-7 cells, as determined by the $2^{-\Delta\Delta CT}$ method. In conclusion, the high expression levels of lncRNA H19 in paclitaxel-treated breast cancer cells may be associated with the cellular response to chemotherapy.

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P-32-100
Molecular perspective on gemcitabine resistance in PANC-1 and PANC-1GemR pancreatic cancer cells: the potential role of polyphenolic cocktail (PFK5120) in overcoming chemoresistance

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Pancreatic cancer is an aggressive malignancy with a high mortality rate primarily due to late diagnosis and limited treatment options. The efficacy of gemcitabine (GEM) used in standard chemotherapy is decreasing due to the development of resistance. To overcome this problem, natural polyphenols are being investigated as potential adjuvant treatment options. PFK5120, a polyphenol mixture of anticarcinogenic compounds developed by our team and currently in the patent process. This study examined the molecular underpinnings of GEM resistance in pancreatic cancer cells and how PFK5120 may affect it. mRNA expression levels were analyzed using the RT-PCR method. Gene expression analyses in four experimental groups (control, GEM, PFK5120, and GEM + PFK5120) revealed significant molecular alterations associated with drug resistance. PFK5120 alters KRAS and AKT/mTOR signaling pathways, which are crucial to chemotherapy response. These alterations are achieved by modulating different resistance mechanisms in both cell lines. Specifically, PFK5120 suppressed KRAS expression in PANC-1 cells, whereas the combination treatment activated SRC and AKT signaling pathways. Furthermore, the combination therapy suppressed PTEN and NFKB, supporting its potential role in enhancing chemotherapy sensitivity. In PANC-1GemR cells, PFK5120 changed AKT, PIK3CA, and mTOR pathways. This finding, which points to alternative signaling mechanisms in resistant cells, suggests that PFK5120 may affect treatment response. The observation of suppressed NFKB in resistant cells further supports the potential for this alteration to influence inflammatory responses and resistance mechanisms. The results of this study provide a better understanding of the molecular signaling mechanisms underlying gemcitabine resistance, reveal the potential of PFK5120 to enhance chemosensitivity, and suggest that phenolic compound combination therapies may be a novel and effective pancreatic cancer treatment.

P-32-101
Bush medicine in Trinidad and Tobago: a quest for novel plant-based anti-cancer treatments

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"Bush medicine" as it is colloquially termed in Trinidad and Tobago, refers to traditional plant remedies used for the treatment of various ailments. This study was undertaken to identify ethnomedicinal plants used locally by bush medicine practitioners (BMPs) in their healing practices for cancer, and to investigate the phytochemical composition, antioxidant activity (AOA) and cytotoxic activity (CA) of their extracts. Ethical approval was granted, and a structured questionnaire was used to interview thirty (30) consenting BMPs. Five (5) understudied plants were collected at different locations in Trinidad, identified and vouchered at The National Herbarium. The different plant parts were repeatedly macerated in 95% ethanol resulting in eight (8) crude extracts of varying yields, designated as: BB, CH, MaR, MS, JBB, JBF, JBL and JBS. Total phenolic content (TPC) and total tannin content (TTC) were determined using the Folin-Ciocalteu colorimetric method and gallic acid as a standard, while the total flavonoid content (TFC) was quantified via the aluminium chloride colorimetric method with quercetin as a standard. AOA was measured using the DPPH free radical scavenging assay with ascorbic acid as a standard. Screening of the extracts for anticancer effect *in vitro* was conducted on human-derived cell lines, A549 lung cancer, MDA-MB-468 triple negative breast cancer, and PC-3 prostate cancer using the MTT assay. The highest TPC (402.40 ± 0.09 mgGAE/g) and the best AOA (IC_{50} = 0.08 mg/mL) were recorded for the MS extract. CH and JBL extracts showed the highest TTC (249.36 ± 0.01 mgGAE/g) and TFC (80.79 ± 0.05 mgQE/g), respectively. MaR extract displayed preferential CA against MDA-MB-468 (IC_{50} = 6.86 μg/mL) while showing reduced CA for A549 and PC-3 (IC_{50} = 39.45 μg/mL and > 100 μg/mL, respectively). Further studies are underway to explore the potent effect of MaR against breast cancer and to identify the bioactive component(s) responsible for its cytotoxicity. *The authors marked with an asterisk equally contributed to the work.

P-32-102
Development of a novel small circular DNA decoy inhibitor targeting STAT3 for cancer therapy

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Aberrant activation of Signal Transducer and Activator of Transcription 3 (STAT3) is a hallmark of several cancers, including ovarian and breast cancer. Active STAT3 translocates to the nucleus, where it regulates the expression of several genes involved in cell cycle progression, survival and proliferation, through recognition of specific DNA sequences, called gamma-activated sites (GAS), by the DNA-binding domain (DBD) of STAT3. STAT3's involvement in these key processes makes it a promising therapeutic target. Current STAT3 inhibitors, including chemical compounds and linear DNA-based decoys, face significant challenges, such as limitations in specificity and rapid degradation inside the cell. This study introduces a novel approach using a small circular DNA decoy inhibitor containing three GAS motifs highly specific for the DBD of STAT3. The circular structure enhances the stability of the molecule, potentially improving its half-life inside the cell and the multiple binding sites increase its ability to capture STAT3 more effectively. To construct the circular DNA decoy, single-stranded oligonucleotides with GAS sequences were ligated into a circular form using complementary splint oligonucleotides, followed by annealing to form double-stranded constructs. Circularity was confirmed through enzymatic restriction analysis. A control

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circular molecule with mutated GAS sites was also synthesized in parallel. A multistep precipitation protocol was utilized to purify and desalt the final compounds. Next, the ability of the circular DNA decoy to impact STAT3 signaling in cancer cells was evaluated. Preliminary results indicate that the obtained construct can be successfully transfected into tumor cell lines. Further experiments are ongoing to assess its effects on STAT3 activity and cell viability. This circular DNA decoy holds promise as a more stable and specific inhibitor for the therapeutic targeting of hyperactive STAT3 signaling in cancer.

P-32-103
A novel cytotoxic fusion protein targeting B7-H3 for acute myeloid leukemia therapy

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B7-H3 (CD276) is a transmembrane ligand that is highly expressed in a diverse range of malignant diseases including acute myeloid leukemia (AML), while being almost inexistent in normal adult tissues. This pronounced differential expression makes it a highly promising potential biomarker for diagnostics and personalized therapies in cancer. However, current approaches to targeting B7-H3 suffer from limitations such as suboptimal specificity, efficacy, or manufacturing challenges. To address these barriers, we developed a novel targeted cytotoxic protein by fusing a B7-H3-specific affibody with magainin 2, an antimicrobial peptide with known cytotoxic effects on cancer cells. A rational design approach was used, which integrated structural compatibility, binding affinity and cytotoxic function to ensure specificity and stability. The affibody was selected based on its well-characterized high affinity and specificity for B7-H3, while magainin 2 was chosen for its potent cytolytic properties. The resulting construct was expressed in *Escherichia coli*, followed by a double purification through immobilized metal affinity chromatography (IMAC), tag cleavage and separation. Its sequence was then validated through ESI-tandem mass spectrometry. The solubility and the stability at 37°C of the resulting fusion protein were high and it showed no antibacterial effects, suggesting that the rational design employed was successful in avoiding unintended toxicity, thus addressing a caveat of many cytotoxic peptides. The cytotoxic potential and specificity of the obtained compound were tested on AML cells that overexpress B7-H3 and cells with naturally absent B7-H3, respectively. Further cell experiments involving the compound are underway to evaluate a range of effects. This study demonstrates a rationally designed, efficiently produced cytotoxic protein that holds potential as a targeted therapeutic for AML.

P-32-104
Abstract moved to P-30-134

P-32-105
Biological responses of normal and breast cancer cells to X-ray activated photodynamic therapy

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Combining ionizing radiation with photodynamic therapy (PDT) represents a promising strategy for enhancing cancer treatment, by enabling the activation of photosensitizers (PS) within deep-seated tumors using high-penetration X-ray radiation. In this study, we investigated the potential of scintillating CeTiO nanoparticles (NPs) to convert ionizing radiation into visible light, thereby activating tetratosylate porphyrin (TMPyP4) for the targeted suppression of breast cancer cells. The anti-tumor activity of TMPyP4-loaded CeTiO NPs was evaluated in triple-negative breast cancer (MDA-MB-231) and normal breast epithelial (MCF-12A) cell lines under both irradiated and non-irradiated conditions. Cells were treated with the conjugate at varying TMPyP4 concentrations (0.01–0.1 μg/mL) for 24 h followed by exposure to a secondary radiation field (0.2 Gy) generated by a high-power, short-pulse 10-petawatt laser system. Cell viability and proliferation were assessed using MTT [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide], Live/Dead, and clonogenic assays, while oxidative stress and genotoxicity were evaluated through reactive oxygen species (ROS) production and comet assay, respectively. The results demonstrated a dose-dependent and cell-type-specific response, with significant inhibition of cancer cell proliferation upon irradiation in the presence of TMPyP4-loaded CeTiO NPs, correlating with increased ROS production. Cancer cell colony formation was also markedly suppressed, and moderate DNA damage was observed following irradiation. Comparative analysis with CeTiO NPs and porphyrin alone confirmed the enhanced therapeutic efficacy of the conjugate. In conclusion, TMPyP4-loaded CeTiO NPs, activated by ionizing radiation, exhibit potent anticancer effects, highlighting their potential as a novel approach for improving breast cancer therapy.
Acknowledgements to project ELI-RO/RDI/2024_022. *The authors marked with an asterisk equally contributed to the work.

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P-32-106
Gold nanoparticles and tyrosine kinase inhibitors: a new approach to targeted therapy in pancreatic cancer

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Pancreatic cancer is a malignant tumor with one of the worst prognoses among solid tumors. This cancer is highly aggressive, invasive, and resistant to treatment. Despite numerous efforts to introduce new therapies, treatment results remain unsatisfactory, necessitating the search for groundbreaking methods that can improve the prognosis of patients. The implementation of targeted therapy in pancreatic cancer is crucial, as it offers greater efficacy with minimal side effects. Biomedical nanomaterials, especially gold nanoparticles (AuNPs), can play a significant role due to their unique properties, such as small size, high surface reactivity, biocompatibility, and ease of functionalization. Previous studies suggest that excessive activation of tyrosine kinase receptors and the PI3K/AKT/mTOR pathway may play a key role in the development of pancreatic cancer. The aim of our study was to investigate the interaction between AuNPs and the tyrosine kinase inhibitor OSI-774 and evaluate their effect on human pancreatic cancer cell lines of different malignancy grades (PANC-1, BxPC3). Our results showed a stable interaction between AuNPs and OSI-774. The optimal molar ratio of AuNPs to OSI-774 was 1:500. The combination of these compounds inhibited cancer cell proliferation in a dose- and time-dependent manner. FACS analysis showed that the AuNP:OSI-774 complex induced apoptosis in pancreatic cancer cells, caused changes in the cell cycle, and inhibited cell migration and invasion. Further studies of complexes formed by gold nanoparticles and tyrosine kinase inhibitors may open new perspectives in the treatment of pancreatic cancer. Our research was funded by the Polish Ministry of Science and Higher Education from state budget resources as part of the "Student scientific groups create innovations" program (grant number: SKN/SP/601857/2024).

P-32-107
Molecular modelling and biophysical analysis of Congo Red and PKC-412 in the treatment of pancreatic cancer

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Pancreatic cancer is a malignant tumor with one of the worst prognoses among solid tumors, characterized by resistance to treatment. Therefore, there is an urgent need to introduce new methods of targeted therapy. Previous studies have shown that overexpression of receptor tyrosine kinases, such as c-KIT or PDGFR, can increase the proliferation, migration, and invasion of cancer cells. The aim of our study was to analyze the aggregates formed between a supramolecular carrier (Congo red, CR) and a tyrosine kinase inhibitor (PKC-412), as well as to investigate the effect of the free inhibitor and its aggregate with Congo red (CR-PKC412) on selected properties of pancreatic cells with varying malignancies. To better understand the interactions between Congo red and PKC-412, we used molecular modeling in addition to biophysical methods. These approaches allowed us to determine the optimal molar ratio, which was then used in *in vitro* studies on pancreatic cancer cell lines. A significantly greater decrease in the viability of the tested 3D cultures was observed after 48 hours of incubation with CR-PKC412, resulting in a lower IC_{50} value for the tested coaggregate compared to PKC-412 alone. Moreover, a higher resistance of PANC-1 and BxPC3 spheroid cells to the tested compounds was observed compared to the 2D culture model. The results of our studies also showed that both PKC 412 (alone) and its coaggregate with Congo red exhibited strong antitumor activity, inhibiting the growth of pancreatic cancer cells and reducing their viability, survival, and migration. The obtained results shed new light on the potential use of midostaurin and CR-PKC412 in the anticancer therapy, emphasizing the importance of developing novel and more effective therapeutic strategies. Our research was funded by the Polish Ministry of Science and Higher Education from state budget resources as part of the "Student scientific groups create innovations" program (grant number: SKN/SP/601857/2024).

P-32-108
Supramolecular drug delivery systems: exploring the complex of CP-673451 and Congo Red in targeted therapy for bladder cancer

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Targeted therapy is increasingly recognized as a fundamental strategy in cancer pharmacotherapy. Therefore, the method of drug administration, particularly its targeted delivery, plays a crucial role in therapeutic efficacy. Among the most promising and innovative drug delivery carriers are supramolecular structures formed by self-assembly, such as Congo red. The aim of our study was to analyze the aggregate formed between the selected tyrosine kinase inhibitor, CP-673451, and the supramolecular carrier, Congo Red, and to evaluate *in vitro* the effects of both the drug alone and the drug-carrier coaggregate on bladder cancer cells. Using a range of biophysical techniques, the optimal molar ratio of the carrier to the drug was determined, and a preliminary evaluation of the coaggregate formation was conducted. The experimental results showed that

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the tested compounds inhibited both the proliferation and migration of bladder cancer cells in a dose- and time-dependent manner. Furthermore, FACS analysis independently confirmed that the compounds induced apoptosis. Additionally, alterations in cytoskeletal organization were observed after exposing the cells to the compounds. Further research into the role of receptor tyrosine kinases in bladder cancer may enhance our understanding of the disease's biology, potentially leading to the development of more targeted and effective cancer therapies in the future. We acknowledge the financial support from the National Science Centre, Poland (grant no. K/MNT/000232).

P-32-109
The enhancement of novel PEG-functionalized porphyrin photosensitizers using antioxidant enzyme inhibitors

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Photodynamic therapy (PDT) is a clinically approved cancer treatment with minimal systemic adverse effects. To enhance the physicochemical properties and cellular localization of photosensitizers (PSs), polyethylene glycol (PEG)-porphyrin conjugates have been developed [1]. PDT-induced phototoxicity relies on ROS, while alterations in antioxidant enzymes—catalase, glutathione synthetase (GS), and superoxide dismutase (SOD)—can contribute to cancer cell resistance [2]. To evaluate the effects of antioxidant enzyme inhibitors—catalase (3-AT), GS (BSO), ZnCuSOD (DDC), and MnSOD (2-ME)—on the activity of newly synthesized PSs. SKOV-3 ovarian carcinoma cells were seeded for 24 h, and treated with PS and an antioxidant enzyme inhibitor for h. Then the cells were irradiated at 425 nm (5 J/cm^2) and, after 24 h, analyzed using MTT, GSH/GSSG-Glo™, and DCFH-DA assays. Only co-treatment with PS and BSO significantly enhanced phototoxicity. A reduced GSH/GSSG ratio confirmed BSO inhibition. Additionally, the co-treatment significantly elevated intracellular reactive oxygen species (ROS) levels. The antioxidant enzyme, especially GS, plays a crucial role in the PDT resistance pathway of cancer cells. BSO could reduce the GSH/GSSG ratio, leading to the phototoxicity enhancement of PS. Moreover, co-treatment of PS and BSO dramatically generated higher intracellular ROS levels compared with the PS single treatment. Nevertheless, not all antioxidant enzyme inhibitors exhibited efficacy. The characteristics of PS determine the primary mediator and mechanism in which the antioxidant enzyme interacts differently. Consequently, selecting an appropriate antioxidant enzyme inhibitor tailored to the specific PS is crucial for optimizing PDT outcomes.
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P-32-110
Targeting pyruvate dehydrogenase kinase 3 with p-cresol: integrative experimental, computational, and cellular approaches reveal a potent inhibitor for cancer metabolism modulation

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Pyruvate dehydrogenase kinase 3 (PDK3) plays a critical role in cellular metabolism by inhibiting the pyruvate dehydrogenase complex, driving metabolic reprogramming in cancer. Targeting PDK3 offers a promising strategy for modulating aberrant metabolic pathways in malignancies. This study aimed to identify and characterize p-cresol as a novel and potent inhibitor of PDK3 using an integrative approach. A multi-faceted strategy combining computational docking and molecular dynamics simulations, *in vitro* enzyme inhibition assays, and cell-based studies was employed to assess the binding affinity, inhibitory potency, and cellular impact of p-cresol on PDK3 activity. Computational studies revealed strong binding interactions of p-cresol within the ATP-binding pocket of PDK3, with key stabilizing hydrogen bonds and hydrophobic contacts. Enzymatic assays confirmed its inhibitory effect with a micromolar IC_{50} value, demonstrating high specificity for PDK3 over related kinases. Cellular experiments further established that p-cresol effectively reduces cancer cell proliferation and inducing apoptosis in a dose-dependent manner. p-Cresol emerges as a potent and selective PDK3 inhibitor, with significant potential for therapeutic intervention in cancers driven by metabolic dysregulation. This study highlights its dual computational and experimental validation, paving the way for further preclinical development.

LB-R-32-01
Analytic hierarchy process (AHP)-based evaluation of the metaverse's impact on biology and biotechnology education for BSc students: unlocking new dimensions in learning

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This study evaluates the impact of metaverse-based learning on biology and biotechnology education for BSc students using the analytic hierarchy process (AHP), a structured decision-making approach that enables the systematic evaluation of multiple factors that influence educational effectiveness. As immersive technologies continue to transform education, the metaverse provides interactive, experiential learning environments that overcome the limitations of traditional teaching methods, particularly in science subjects where visualization and hands-on engagement are critical. To evaluate the role and effectiveness of metaverse-based instruction, this study used AHP to analyze key criteria including student engagement, interactivity, knowledge retention, accessibility, cost-effectiveness, collaboration, simulation accuracy, and instructor readiness. Expert judgments from professionals in the fields of biotechnology, education, and immersive technology were compiled through pairwise comparisons to determine the relative importance of these factors. The results show that interactivity, engagement and

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knowledge retention were the most influential factors for improving learning outcomes, while cost-effectiveness, accessibility and simulation accuracy were critical for the feasibility and wider adoption of metaverse-based training. In addition, collaborative learning and instructor readiness were found to be essential elements for successful integration into curricula. These findings provide a data-driven foundation for integrating the metaverse into biology and biotechnology education and offer actionable recommendations for educators, institutions, and policy makers. By highlighting the most impactful elements, this study underscores the potential of the metaverse to enhance student learning experiences, optimize digital education strategies, and inform future developments in immersive educational technologies. *The authors marked with an asterisk equally contributed to the work.

LB-R-32-02
Regulation of ER stress and unfolded protein response by lipids in cancer

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The rapid proliferation of tumor cells creates a microenvironment characterized by metabolic stress, including limited availability of glucose, oxygen, and essential growth factors. Cancer cells adapt to these conditions by engaging the unfolded protein response (UPR), a cellular signaling network that mitigates endoplasmic reticulum (ER) stress and promotes survival. Mammalian cells contain three ER transmembrane protein that function as proximal effectors of the UPR such as Ire1 PERK and ATF6. Recent studies show the intersection between lipids metabolism and ER stress signaling. We investigated the impact of inhibiting acid ceramidase activity using LCL-521 on PERK activation. Based on our model, acid ceramidase inhibition is expected to prevent the generation of specific lipids, leading to increased PERK activity. Consistent with this hypothesis, we observed a significant increase in PERK activation. Moreover, this effect was mitigated by the addition of exogenous lipids, confirming a regulatory relationship between lipid metabolism and PERK-dependent control of cell survival. Additionally, our data suggest that specific PERK mutations can drive oncogenic activity. Several PERK mutants promoted significant colony growth, whereas wild-type PERK did not, indicating a potential oncogenic function. To further explore this, we developed a mouse model harboring specific PERK mutations. Lipid profiling was conducted using mass spectrometry to analyze control and PERK knockdown U20S cells (various sphingolipids, including ceramides, dihydroceramides, and sphingomyelin). PERK mutant mice were generated at the International Institute of Molecular and Cell Biology, Warsaw, Poland. We hope that our research will help find innovative therapies targeting PERK and its role in lipid regulation. This research was supported by grants: Polish National Science Centre (NCN) OPUS No. 2019/33/B/NZ5/03034; National Institutes of Health P01CA165997, P30CA138313, P30GM103339. *The authors marked with an asterisk equally contributed to the work.

LB-R-32-03
Enhancement of the anti-cancer activity of multi-kinase inhibitors by inhibition of Atg4B via dairy-derived fatty acids in hepatocellular carcinoma cells

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Hepatocellular carcinoma (HCC) is a leading cause of cancer-related death worldwide. Multi-kinase inhibitors such as Cabozantinib (Cab) and Lenvatinib (Len) are standard therapies; however, their clinical efficacy is often limited by adverse effects and the development of resistance. Autophagy, a cellular degradation process exploited by cancer cells for survival and drug resistance, has emerged as a promising therapeutic target in HCC. Previously, we identified 21f as a novel inhibitor of Atg4B, a key enzyme involved in autophagosome formation. Based on the structure of 21f, we further revealed that long-chain fatty acids, including docosahexaenoic acid, possess Atg4B-inhibitory activity and enhance chemosensitivity in cancer cells. In the present study, we focused on dairy-derived fatty acids and investigated their Atg4B inhibitory activity, autophagy modulation, and effects on chemosensitivity in HCC cells. *In vitro* cleavage assays revealed that three dairy-derived fatty acids (trans-vaccenic acid, rumenic acid (Rum), and elaidic acid) inhibited Atg4B, with Rum exhibiting the most potent effect. Docking simulations suggested that a salt bridge between a carboxyl group of Rum and Arg229 of Atg4B contributes to its potent inhibitory activity. In HepG2 cells, Rum suppressed Cab- and Len-induced autophagy and significantly enhanced their anticancer effects. Combined treatment led to increased accumulation of lipid droplets, lipid peroxides, and HNE-modified proteins, along with a marked increase in reactive oxygen species. These results suggest that ferroptosis may contribute to the enhanced anticancer activity of Cab or Len by Rum. Collectively, these findings suggest that Rum, a naturally occurring dairy-derived fatty acid, may serve as a novel adjuvant to enhance the therapeutic efficacy of multi-kinase inhibitors in HCC treatment.

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LB-R-32-04
Functional consequences of targeting thyroid hormone signaling in prostate cancer

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Prostate cancer (PCa) is the second most frequently diagnosed malignancy in men and is largely driven by androgen receptor (AR) signaling. However, approximately 20% of patients develop resistance to AR-targeted therapies, underscoring the urgent need for alternative therapeutic approaches. Emerging evidence suggests a link between hyperthyroidism and an increased risk of PCa, with thyroid hormone receptors (THRs) implicated in cancer progression. Notably, sequencing analyses of PCa samples from an in-house cohort revealed an enrichment of mutations in genes associated with thyroid hormone (TH) signaling. In this project we aim to investigate the role of TH signaling in PCa pathogenesis. We observed, that pharmacological inhibition of TRβ using the selective antagonist NH-3 significantly reduced tumor volume and mass in 22Rv1 and LNCaP xenograft models. *In vitro*, NH-3 effectively suppressed PCa cell proliferation. RNA sequencing revealed transcriptomic changes in TH-related pathways, as well as AR signaling, cell cycle regulation, and lipid metabolism. These effects were confirmed by decreased expression of AR and AR-regulated genes, as well as reduced fatty acid synthase (FASN) protein levels. Increased uptake of the radiolabeled fatty acid analog [^{18}F]FTHA suggested a compensatory response to impaired *de novo* lipid synthesis. Our findings support the therapeutic potential of targeting TH signaling in PCa by disrupting AR signaling, altering lipid metabolism, and inducing cell cycle arrest. Given the observed alterations of genes affected by TH signaling, further investigation is needed to clarify its role in PCa progression and to evaluate its therapeutic potential.

LB-R-32-05
Cytotoxic screening and mechanistic insights into vincarubine, a potent anticancer alkaloid candidate from *Vinca minor* L.

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Approximately 60% of chemotherapeutic agents are natural products, emphasizing the need to explore plant sources for novel bioactive compounds, since the cytotoxic effects of many plant-derived molecules remain under-investigated. Given the established cytotoxic potential of the *Vinca* genus, we conducted a single-dose (10 μM) cytotoxicity screening of alkaloids isolated from *Vinca minor* L. Among tested alkaloids, vincarubine demonstrated the strongest cytotoxic activity against a panel of human tumor cell lines. Further evaluation confirmed its growth-inhibitory effects across multiple cancer histotypes. We also investigated its impact on cell viability and proliferation through kinetic and end-point assays. Finally, we explored the molecular mechanisms underlying the antiproliferative activity of vincarubine, comparing its effects to those of vincristine, an established *Vinca* alkaloid and known conventional chemotherapeutic drug. Experimental results demonstrated that vincarubine significantly inhibited the growth of A549 lung adenocarcinoma cells in a dose-dependent manner, with effects observable starting at a concentration of 5 μM. Additionally, treatment with 10 μM vincarubine led to a marked reduction in cell viability. Flow cytometry analysis revealed that exposure to 10 μM dose of vincarubine for 24 h resulted in a decrease in the proportion of cells in the G2 phase, accompanied by an increase in the G1-phase cell population. Western blot analysis of cell cycle regulatory proteins showed elevated levels of phosphorylated Rb at Ser807/811, alongside a reduction in phosphorylated Akt at Thr308. Furthermore, vincarubine treatment disrupted the organization of cytoskeletal proteins, including tubulin and actin, and altered the spatial distribution of focal adhesion components such as phosphorylated FAK at Tyr397, paxillin, and vinculin. Acknowledgements: The work was supported from EU-Project "OncoPharm" No. CZ.02.01.01/00/23_021/0008442. *The authors marked with an asterisk equally contributed to the work.

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LB-R-32-06
Gamma irradiation-induced and stress degradation under ICH recommended stress conditions of erlotinib

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Erlotinib (OSI-774), an FDA-approved epidermal growth factor receptor (EGFR) inhibitor, is extensively used in treating non-small cell lung cancer (NSCLC) and other malignancies. This study explores erlotinib's chemical instability under various stress conditions, including hydrolytic, oxidation, thermal, and photolytic stress, as well as its environmental impact due to drug residues in effluents. A stability-indicating reverse-phase liquid chromatography (RP-LC) method was developed to quantify erlotinib and its degradation products in pharmaceutical formulations. Gamma irradiation, an advanced oxidation process, was evaluated for its efficacy in degrading erlotinib in aqueous solutions. The study revealed a significant increase in degradation efficiency with higher absorbed doses and acidic pH conditions. These findings underscore the potential of gamma irradiation as an effective environmental remediation strategy for removing pharmaceutical contaminants and provide the first investigation of erlotinib's degradation via gamma irradiation.

LB-R-32-07
Immunotherapy of non-muscle invasive bladder cancer with novel mycobacterial agents as an alternative to BCG

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Non-muscle invasive bladder cancer (NMIBC) has a high recurrence rate, making it a common cancer type that significantly burdens healthcare systems. The primary treatment is transurethral resection of the bladder tumor (TURBT), often followed by adjuvant therapies like Bacillus Calmette-Guérin (BCG), the first FDA-approved immunotherapy. However, despite being a standard therapy, BCG can disseminate systemically after intravesical administration and may lead to life-threatening infections. Therefore, the search for alternative treatment strategies remains ongoing. This study aims to identify novel mycobacterial agents and potential combination therapies as alternatives to BCG for treating NMIBC. For this purpose, *Mycobacterium chubuense* (National Patent Registration Number: TR 2011 01873) and *Mycobacterium brumae* (National Patent Registration Number: TR 2011 01874) extracts, which were previously patented by our team, were used. After culturing the mycobacteria in Middlebrook 7H9 broth, extracts of bacterial cells were prepared. These extracts were applied to bladder cancer cells, and their dose- and time-dependent anti-tumoral effects were examined using the MTT assay. Comparison of each mycobacterial extract with control cells revealed a direct cytotoxic effect on the cancer cells. Our studies confirming the anti-tumor effects of these extracts are ongoing, both *in vitro* and *in vivo*. This study uniquely investigates the effectiveness of non-living mycobacterial extracts for treatment, eliminating the risk of bacteremia and sepsis. These results may contribute to the discovery of new and more effective agents for the treatment of bladder cancer. This study is supported by the Scientific and Technological Research Council of Türkiye (TUBITAK) under Grant Number 223S730. The authors thank TUBITAK for their support.

LB-R-32-08
High-throughput analysis of FDA-approved drugs in combination with increased fatty acid oxidation reveals new possibilities for hepatocellular carcinoma treatment

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Hepatocellular carcinoma (HCC) is the primary liver malignancy and amongst the deadliest cancers worldwide. Being an extremely heterogeneous tumor, and also because of the different etiologies concurring with its development, HCC still urgently needs new knowledge in the pathogenetic and therapeutic fields. HCC cells predominantly demonstrate the "Warburg effect", shifting from oxidative phosphorylation to glycolysis even in the presence of oxygen, ensuring a rapid energy supply to sustain increased proliferation [previously published in: Liberti MV, Locasale JW. (2016) Trends Biochem Sci. doi: 10.1016/j.tibs.2015.12.001]. Therefore, we suggested that stimulating oxidative metabolism could be a promising strategy to counteract the Warburg effect and limit cancer cell energy supply [previously published in: Di Leo et al. (2019) Oncogene. doi: 10.1038/s41388-018-0545-0]. We demonstrated that treatment with hexanoic acid (HA), a medium-chain fatty acid that crosses the mitochondrial membrane independently of transporters, reduces HCC cell proliferation and sensitizes cells to chemotherapeutic agents [previously published in: Castelli et al. (2023) Cancer Lett. doi: 10.1016/j.canlet.2022.216010]. These results prompted us to screen the efficacy of FDA-approved drugs on HCC cells proliferation, identifying those that have a cytostatic/cytotoxic effect, which is potentiated in combination with HA. We highlighted that HA increased the susceptibility of HCC cells to drugs targeting tyrosine kinase receptors, RAS/RAF/MEK/ERK, and PI3K/PTEN/AKT/mTORC pathways, which regulate the transcription of genes involved in cell growth, proliferation, and survival. Our results outlined a possible repositioning of FDA-approved drugs in the treatment of HCC, which can be exploited therapeutically depending on the levels of HCC lipid catabolism.

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LB-R-32-09
Development of thiazole-based anticancer agents for targeted therapy of lung cancer by utilizing AI-driven, *in silico* tools and nanotechnological approaches

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Non-small cell lung cancer (NSCLC), which accounts for approximately 85% of lung cancer cases, poses a major threat to public health. Despite significant advances in diagnostic and therapeutic approaches, the overall survival rate for NSCLC patients still remains low. Epidermal growth factor receptor (EGFR) is reported to be overexpressed in NSCLC. EGFR plays a key role in cancer-related signaling pathways associated with cell proliferation, apoptosis, angiogenesis and metastasis. Due to the importance of the thiazole scaffold for targeted anticancer drug discovery, new thiazole derivatives were designed by means of the mol2drug application, which is integrated with cutting-edge artificial intelligence (AI) algorithms for highly accurate predictions about the potential efficacy and the safety profile of a molecule based on its image. MTT assay was employed to examine their cytotoxic effects on A549 human lung adenocarcinoma and L929 mouse embryonic fibroblast cells. The nanoparticle (NP) formulations of the most promising anticancer agents (1, 4, 5, 8, 10, 11) were prepared using nanoprecipitation method. *In vitro* mechanistic studies were carried out for compounds 1, 4, 5, 8, 10, 11 and their NPs. According to flow cytometry-based apoptosis and ELISA-based EGFR inhibition assays, the NP formulations of compounds 1, 4 and 11 were found to exert potent cytotoxic and apoptotic effects on A549 cells through EGFR inhibition. The NP formulations of compounds 1, 4 and 11 induced apoptosis and inhibited EGFR more than erlotinib, the positive control. Furthermore, molecular docking studies suggested that compounds 1, 4 and 11 showed high affinity to the active site of the EGFR TK enzyme (PDB ID: 1M17). Based on *in silico* pharmacokinetic (QikProp and SwissADME) studies, these compounds are also predicted to possess favorable oral bioavailability and drug-likeness. Taken together, compounds 1, 4 and 11 stand out as potential anticancer agents for targeted therapy of NSCLC.

LB-R-32-10
Overcoming chemoresistance in triple negative breast cancer with antimicrobials

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Drug resistance in cancer treatment interferes with effective therapeutic outcomes and often leads to metastasis. We have recently shown that drug-resistant cancer cells exhibit increased mitochondrial oxidative phosphorylation (OXPHOS) compared to sensitive counterparts, a phenomenon often observed in patients after chemotherapy as well as in cancer stem cell models. For that reason, resensitization of drug-resistant cancer cells to chemo- and radiotherapy may improve treatment response. Recently, our laboratory and others have proposed the use of antimicrobials against Gram-negative bacteria as compounds capable of targeting mitochondria of OXPHOS-dependent chemotherapy-resistant malignant tumors. To this end, we tested dozens of antimicrobial agents from the chemical library on cell models of chemoresistant triple-negative breast cancer and selected several leads capable of discriminating between chemosensitive and chemoresistant cancer cells and not affecting poorly dividing somatic cells. We applied these results *in vivo* and confirmed the inhibition of tumors derived from drug-resistant but not drug-sensitive cells. The combination of selected antimicrobial and chemotherapeutic agents can enhance the anticancer effect. Next, we performed proteomic analysis and characterized the relevant molecular pathways of antimicrobial activity in cancer resistant cells. As a result, we identified an increase in cellular and mitochondrial oxidative stress, enhanced mitophagy, and decreased matrix metalloproteinase activity. In addition, we applied these drugs to perform chemopreventive studies in mice to show how OXPHOS suppression reduces the likelihood of resistant tumors in the long term. Overall, these promising results suggest that the combination of selected OXPHOS-inhibiting drugs with conventional chemotherapy may provide an alternative approach to treating chemoresistant tumors and preventing metastasis in patients. *The authors marked with an asterisk equally contributed to the work.

LB-R-32-11
Aurora B inhibition amplifies cisplatin-induced apoptosis in DLD-1 cells

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Aurora kinases (AURKs), a family of serine/threonine kinases, serve as mitotic regulators with three isoforms in humans: AURKA, AURKB, and AURKC. Overexpression of AURKs occurred in various human cancers, including colorectal adenocarcinoma (CRC). Among family members, AURKB overexpression correlates with clinicopathological parameters and survival rates in CRC. AURKB inhibition leads mitotic defects and apoptosis as AURKB is required for chromosome condensation and bi-orientation. This study assessed the effects

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of BI-831266, a selective AURKB inhibitor, alone and with cisplatin, on cell viability, colony formation, migration, and apoptosis in DLD-1 colorectal cancer cells. Crystal violet cell viability, 2D-clonogenic survival, wound healing, and Annexin V/PI apoptosis assays were performed. The combination indexes (CIs) were calculated by CompuSyn software. Results showed BI-831266 suppressed DLD-1 cell viability and proliferation in dose-dependent manner at 48 h and revealed greater cytotoxic effects with cisplatin. Wound healing assay showed 42.2 ± 4.9% and 33.3 ± 4.0% wound closure at IC_{50} values of BI-831266 and cisplatin, respectively, and 30.7 ± 3.5% wound closure in combination treatment. BI-831266 impaired migration and invasion of DLD-1 cells, alone and with cisplatin. Single treatments induced moderate apoptosis levels, which increased substantially in combination. Total apoptosis rate reached 32% after 48 h of combination treatment, with late apoptosis rate increasing from 2.4% to 9.8%. The combination treatment showed significant effect on late-stage apoptotic cell death. Our initial findings, first time, show that combining selective AURKB inhibitor BI-831266 with cisplatin reveals synergistic interaction in DLD-1 cells at specific doses, suggesting potential as a therapeutic strategy for cancer treatment and warranting further investigation. This study was funded by Scientific Research Projects Coordination Unit of AGU (Project No:FAB-2023-187).

LB-R-32-12
Telomere-targeting therapeutics RiboTHIO and THIO synergize with radiotherapy and immune checkpoint blockade to suppress lung tumor growth

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Telomere dysfunction represents a compelling therapeutic vulnerability in telomerase-positive cancers. RiboTHIO and THIO are novel telomere-targeting agents that induce telomeric DNA damage and activate anti-tumor immunity. This study aimed to evaluate their cytotoxic, immunomodulatory, and radiosensitizing properties in lung cancer. *In vitro* cytotoxicity of RiboTHIO and THIO were assessed on A549 lung adenocarcinoma cells to determine IC_{50} values. A heterotopic LLC-1 lung tumor model was established in immunocompetent C57BL/6 mice. Mice were treated intraperitoneally with single agents or in combination with anti-PD-L1. Clonogenic assays were performed to evaluate radiosensitization at 2, 4, and 8 Gy. Cell death modalities were investigated by Annexin V/PI staining. For A549 cells, IC_{50} values of both agents was 2.2 μM. THIO and RiboTHIO treatment significantly attenuated tumor progression *in vivo* (mean tumor size 0.7–0.8 ± 0.1 cm vs. 1.0 ± 0.2 cm in controls). Tumor volume increased by 100% in the control group but only 40% in THIO and RiboTHIO-treated mice. Median survival was extended from 27 to 36 days, with survival rates of 25% (THIO) and 40% (anti-PD-L1). Immune profiling revealed increased infiltration of CD4 T helper cells in RiboTHIO group (38.1 ± 4%) and marked elevation of CD8 cytotoxic T cells in all treated groups, peaking in the THIO + anti-PD-L1 arm (52.1 ± 2.5%). Colony formation assays demonstrated moderate inhibition with monotherapy but substantial synergy with radiotherapy. Combination of 8 Gy with 3 μM RiboTHIO or THIO nearly eradicated colony formation. Annexin V/PI analysis confirmed apoptosis as the dominant mechanism of cell death. RiboTHIO and THIO display potent anti-tumor activity by impairing clonogenic survival, enhancing radiation-induced cytotoxicity, and remodeling the immune landscape. These findings support their potential as promising immuno-radiosensitizing therapeutics for lung cancer treatment.

Advanced laboratory techniques (in medical biochemistry)

P-33-001
Interference of HbD variant in the measurement of HbA1c by Lifotronic H8 ion exchange high-performance liquid chromatography (HPLC)

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Hemoglobin A1c (HbA1c) is used to monitor long-term glycemic control. In the presence of hemoglobin variants, erroneous results may be observed in HbA1c measurements. In this case, we describe a patient whose HbA1c result was measured by ion exchange high performance liquid chromatography (HPLC) device and found to be 3.1%. Hemoglobin D (HbD) variant was detected in electrophoresis measurements performed for further investigation, and the patient's true HbA1c value was found to be 5.1%. In the case of very low HbA1c results or measurements that are inconsistent with the patient's clinic, analysis by methods such as electrophoresis, which is resistant to interference from haemoglobin variants, is recommended instead of ion exchange HPLC.

P-33-002
Evaluation of different purification strategies for honeybee vitellogenin

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Honeybee vitellogenin (Vg) is a monomeric 180 kDa storage phospholipoglycoprotein that can be cleaved into two smaller fragments (150 kDa and 40 kDa) [previously published in: Havukainen H. et al. (2011) J. Exp. Biol. 214(4), 582-592]. Vg is crucial in honeybee social organization, antioxidant defense, immunity, and lifespan [previously published in: Havukainen H. et al. (2013) JBC 288(39), 28369-28318; Corona M. et al. (2007) PNAS 104(17), 7128-7133]. Despite recent advances, the mechanisms underlying its biological functions in bees remain not fully understood. Due to its complex structure, variability, and ability to interact with numerous molecules, studying Vg presents significant challenges in selecting optimal experimental conditions. To investigate Vg's functions in greater detail, isolation of a purified protein standard from bee hemolymph is

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essential. In this study, several purification techniques were optimized and compared, mainly low-pressure liquid chromatography methods including ion-exchange chromatography (with both weak and strong anion exchangers), affinity chromatography, gel permeation chromatography, and magnetic bead-based purification method. Different conditions were tested for each method to identify the most effective approach. The purification success was always verified by SDS-PAGE, where a 180 kDa protein was detected. Ion exchange chromatography seems to be the most promising approach from all methods tested with the best effectivity using strong anion exchangers. The purified product will be subsequently subjected to further analysis and used to study the properties of Vg in bees.

P-33-003
Partial method validation of trimethylamine N-oxide (TMAO) by liquid chromatography-tandem mass spectrometry (LC-MS/MS)

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Trimethylamine N-oxide (TMAO) is formed from dietary precursors such as choline and carnitine by the oxidation of trimethylamine by the gut microbiota. Accurate measurement of TMAO levels in biological samples is critical to understanding the role of this metabolite in health and disease processes. In this study, a liquid chromatography-tandem mass spectrometry (LC-MS/MS) method was developed for the quantitative analysis of TMAO, and partial method validation was performed by modifying the study of Heaney et al. [1]. The analyses were performed using the ABSCIEX API3200 LC-MS/MS. The Q1/Q3 atomic mass values were determined as m/z 59→76 for TMAO and m/z 68→85 for the internal standard D9-TMAO. Chromatographic separation was performed using a Phenomenex Luna (50 mm × 4.6 mm, 5 μm) C18 column. HPLC water with 0.1% formic acid in phase A and methanol with 0.1% formic acid in phase B were used as the mobile phase. The Turbo Ion Spray method in positive mode was used as the ionisation source. The calibration curve was constructed with eight points using an artificial matrix instead of a biological matrix to avoid endogenous TMAO interferences. The range of 0.61–10.000 ng/mL was evaluated and an acceptable linearity with $R^2 > 0.999$ was achieved in each validation batch. When evaluating precision and accuracy, the intra-day %CV was 6.2 and the inter-day % CV was 7.3, indicating that the method has a high reproducibility. The limit of detection was 0.6 ng/mL and the limit of quantification was 2.0 ng/mL. The method developed in this direction proved to be suitable for clinical and pharmacokinetic studies as it showed acceptable linear response, precision and accuracy. The validated method enables precise monitoring of TMAO levels in human plasma and will contribute to a better understanding of disease progression and evaluation of therapeutic interventions. Reference: 1. Heaney, LM et al (2016) Anal Bioanal Chem. 408(3), 797–804.

P-33-004
Macromolecule delivery into human cells by osmotic shock is applicable for high-throughput experiments

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From identifying molecular drug targets and analyzing protein functions to studying drug-cell interactions, successful breakthroughs depend on the effective delivery of macromolecules that are not taken up by cells. We explored an osmotic pressure-driven intracellular delivery, proposing a detailed mechanism of this process [previously published in Karpińska A. et al. (2022) *J. Colloid Interface Sci.*, 627, 270-282]. Recently, we investigated the effectiveness of introducing various selected molecules into different human cells using a high-throughput multi-well plate format. After optimizing the osmotic shock delivery procedure, we successfully introduced fluorescent, neutral 20 kDa dextran (size of about 4 nm in radius), EGFP protein, and human albumin into lung cancer (A549), cervical cancer (HeLa), and triple-negative breast cancer (MDA-MB-231) cells. The study was conducted by measuring (on a plate reader) the fluorescence intensity of cells after one hour from cellular delivery compared to control – cells subjected to osmotic shock but without a fluorescent probe. For example, for 100 μM of dextran delivered into HeLa cells, we obtained a 60% higher intensity than the control. For the same molecule introduced into A549 cells, the percentage of fluorescence was equal to 36%, which shows the differences in intracellular delivery between tested cancer cells. We foresee that the presented research will facilitate the progress of a wide range of biological and medical studies by presenting a proposal for rapid intracellular introduction of various molecules into human cells, applicable to high-throughput experiments. Research financed from the Project No. FENG.02.07-IP.05-0127/23 being implemented under the action 1/2023 of the Foundation for Polish Science co-financed by the European Union from the funds of Priority 2 of the European Funds for a Modern Economy Program 2021–2027 (FENG) Action 2.7 Proof of Concept.

P-33-005
Enhancing transfection efficiency for nucleic acid delivery into human cells via osmotic pressure changes

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Delivering nucleic acids into human cells, called transfection, has been widely used due to its broad application for studying cellular processes and molecular mechanisms of diseases. There are various transfection methods and reagents with specific advantages and disadvantages. However, there is still no transfection strategy that suits all cell types and gives homogeneous efficiency in cell population. We investigated the delivery of macromolecules, including nucleic acids, to human cells by osmotic pressure changes [previously published in Karpińska A. et al. (2022) J. Colloid Interface Sci., 627, 270-282], showing that the nanostructure of the solution with high osmotic pressure determines the success of delivering process. Using fluorescence correlation spectroscopy (FCS)

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as a primary research method, we developed an optimized technology for quick, simple and universal intracellular delivery that also take the type of an introduced molecule into consideration. Here we present variations in the original formulation aiming in enhancing nucleic acid delivery by modifying charge ratios of the formulation. In our experiments, we are testing transfection efficiency using different technology modifications in the perspective of the usability for the most popular techniques in cell biology (multi-well plate reader and flow cytometry). Our results show that we can obtain similar effectiveness to commercially available transfection methods with less cytotoxicity, especially after 48. h. We believe that by adapting technology to the equipment available in most research laboratories, we will contribute to the development of targeted therapies. Research financed from the Project No. FENG.02.07-IP.05-0127/23 being implemented under the action 1/2023 of the Foundation for Polish Science co-financed by the European Union from the funds of Priority 2 of the European Funds for a Modern Economy Program 2021–2027 (FENG) Action 2.7 Proof of Concept.

P-33-006
Engineering intracellular vesicle membrane permeability by manipulating osmotic pressure at different timescales

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Efficient intracellular delivery of macromolecular cargo is a critical challenge in cell biology, impacting research and therapeutic applications. While natural cellular uptake pathways are effective and well-defined, artificial methods suffer from low efficiency and cell-type specificity, with the example of transfection techniques yielding endosomal escape rates below 2%. We investigate osmotic pressure-driven intracellular delivery as a promising alternative. Historically, this method faced limitations due to poorly understood mechanisms and associated cytotoxicity. We recently found the crucial role of the polymeric entangled regime in pinocytic vesicle disruption, shifting the focus from a mere concentration of osmotically active compounds to specific intramolecular orientations [previously published in Karpińska *et al.* (2022) *J. Coll. Interf. Sci.*, 627, 270-282]. As a consequence, we started to explore milder osmotic conditions. Here, we present data demonstrating successful intracellular delivery of nanoparticles, proteins and nucleic acids into human cells (HeLa, A549, MDA-MB-231), achieved through controlled, reduced osmotic pressure gradients and relaxed incubation times (from seconds to minutes). We achieved a 4-fold cellular osmotic shock reduction (312 to 78 kPa) followed by only a 20% reduction in pinocytic vesicle disruption force. This approach provided effective intracellular delivery with higher cell viability than traditional, high-osmolarity shock methods. Furthermore, the relaxed time regimes benefit high-throughput platforms, such as multi-well plates, where precise timing of pipetting is challenging. This work establishes osmotic pressure-driven vesicle disruption as a versatile, cargo- and cell-type-independent delivery method. Research financed under the action 1/2023 of the Foundation for Polish Science co-financed by the European Union from the FENG Program Action 2.7: Proof of Concept (project No. FENG.02.07-IP.05-0127/23).

P-33-007
Development of a method for determining organophosphate and pyrethroid metabolites in urine: preliminary results

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It has been indicated that organophosphate and pyrethroid insecticides (OPI), although important for pest control in agriculture, could negatively affect human health, even at low exposure levels. This highlights the need for effective methods for OPI biomonitoring, especially in vulnerable populations, like children. The development of accurate and reliable analytical methods is crucial for effective biomonitoring and risk assessment. For that purpose, a method for determining six OPI metabolites in human urine was developed using gas chromatography–mass spectrometry: dimethylphosphate (DMP), dimethylthiophosphate (DMTP), diethylphosphate (DEP), diethylthiophosphate (DETP), 3-phenoxybenzoic acid (3-PBA) and 2-methyl-3-phenylbenzoic acid (MPA). Liquid–liquid extraction was used for sample preparation, along with acid hydrolysis to deconjugate pyrethroid metabolites. Various solvents, its combinations and temperature-time parameters were optimised for extraction and hydrolysis efficiency. Precision, expressed as the relative standard deviation (%RSD) of five replicate measurements, ranged from 3.1% for 3-PBA to 13.5% for DEP, while measurement accuracy ranged from 98.4% for MPA to 101.8% for DEP. The limits of detection (LOD) ranged from 0.12 ng/mL for DMTP to 0.21 ng/mL for 3-PBA. The validated method was applied to analyse 423 urine samples from primary school boys (mean age 11.5 ± 0.4) from Zagreb County, Croatia, to assess pesticide exposure. The percentage of positive samples was 80.6%, with DEP being the most commonly detected metabolite at 50.8%, while MPA was detected in only 2.84% samples. DEP was detected over the broadest range, from 0.5 to 453.8 ng/mL. These findings show that the implemented method provides a robust and efficient tool for large-scale studies, contributing significantly to our understanding of contemporary exposure to pesticides in the young population. This research is funded by the Croatian Science Foundation (HrZZ IP-2019-04-7193).

P-33-008
Correlation between blood gas and hematology analyzer results for hemoglobin levels in emergency laboratory settings

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We aimed to determine the correlation between hemoglobin (Hb) levels measured by blood gas (BG) and complete blood count (CBC) analyzers in the emergency laboratory. We retrospectively evaluated 52 820 BG and CBC samples analyzed simultaneously at Dr. Lütfi Kırdar City Hospital between January and September 2024. BG tests were performed using the Radiometer ABL800 Flex (Kobenhavn, Denmark), while CBC tests were conducted with the MINDRAY BC-6800 (Shenzhen, China). Hb results were categorized based on reference intervals and gender. Data analysis included Spearman correlation, Bland-Altman and Passing-Bablok regression analyses. Bias and limits of agreement for each Hb level

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were compared with allowable total error (TEa) as defined by the Clinical Laboratory Improvement Amendments (CLIA). A strong correlation was observed between Hb levels, with a Spearman rank correlation coefficient ($r > 0.80$) for Hb levels < 15 g/dL in females and < 17 g/dL in males. A moderate correlation ($r = 0.680$) was noted for Hb levels > 15 g/dL and > 17 g/dL. The bias for females was 3.3%, 3.0% and 2.8% for Hb levels < 11 g/dL, 12–15 g/dL and > 15 g/dL, respectively. For males, the bias was 3.1%, 3.1% and 2.9% for Hb levels < 12 g/dL, 12–17 g/dL and > 17 g/dL respectively. The limits of agreement for bias exceeded the TEa (5.9%) and significant proportional and constant errors were detected in the Passing-Bablok analysis across all Hb levels. Clinicians should be aware of the difference between BG and CBC Hb measurements and different methods should not be used interchangeably for patient follow-up.

P-33-009
Development of a dispersive liquid-liquid microextraction (DLLME) method for cortisol determination in serum and validation with LC-MS/MS

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Cortisol, a steroid hormone produced by the adrenal cortex, regulates metabolism, immune response, and blood pressure. Accurate cortisol measurement is crucial for diagnosing adrenal dysfunctions like Cushing's syndrome and Addison's disease, as well as monitoring stress responses. This study developed a rapid, sensitive, and eco-friendly dispersive liquid-liquid microextraction (DLLME) method for cortisol determination in serum samples. The method outperforms traditional techniques like liquid-liquid extraction (LLE) and solid-phase extraction (SPE) by reducing solvent use, increasing extraction efficiency, and shortening analysis time. DLLME was optimized using dichloromethane, chloroform, and carbon tetrachloride as extraction solvents, with ethanol, methanol, and acetone as dispersive solvents. Post-extraction analysis used Waters Acquity UPLC coupled with a Waters Xevo TQD LC-MS/MS system. Chromatographic separation was achieved on an Acquity UPLC C18 column (50 mm × 2.1 mm, 1.7 μm) with gradient elution using water and methanol containing 10 mM ammonium formate as mobile phases. The flow rate was 0.4 mL/min, and total analysis time was 5 minutes. The method was validated for accuracy, precision, linearity, limit of detection (LOD), and limit of quantification (LOQ). Chloroform and acetone (100/500 μL) provided the highest extraction efficiency. The method showed a wide linear range (5–500 ng/mL), low LOD (1.33 ng/mL), low LOQ (5 ng/mL), high precision (%RSD < 10%), and excellent accuracy (recovery rates: 92–108%). A comparative study with ELISA confirmed a high correlation between the DLLME-LC-MS/MS method and ELISA, validating the reliability of the new approach. This study demonstrates that DLLME is a cost-effective, rapid, and eco-friendly alternative for cortisol determination in clinical and biochemical analyses. Future research could extend this method to other biological matrices and steroid hormones. *The authors marked with an asterisk equally contributed to the work.

P-33-010
Development of a practical LC-MS/MS method for the measurement of trimethylamine-N-oxide levels in plasma and its application in underwater rugby athletes

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Trimethylamine N-oxide (TMAO), a gut microbiota-derived metabolite linked to cardiovascular and chronic kidney diseases, has gained attention for its role in disease progression and response to environmental factors like hypoxia. This study developed a rapid, sensitive, and reliable LC-MS/MS method for TMAO quantification, optimized under UPLC conditions. A 12-point calibration curve using an artificial serum matrix ensured accuracy, with a linear range of 24 nM–50 mM, a quantification limit of 25.98 nM, and a detection limit of 8.6 nM. The method demonstrated excellent precision and reproducibility. The method was applied to plasma samples from underwater rugby athletes ($n = 20$) and sedentary individuals ($n = 20$). Athletes exhibited significantly higher TMAO levels (2.065 μM [IQR 1.313-3.935]) compared to sedentary controls (1.455 μM [IQR 1.093-1.986]; $p = 0.096$), with similar trends observed in both male ($p = 0.0197$) and female ($p = 0.0479$) subgroups. Despite the athletes' exposure to hypoxic conditions during training, no correlation was found between TMAO levels and hypoxia-inducible factor 1-alpha (HIF-1α), a key hypoxia marker. This suggests that elevated TMAO levels in athletes may stem from dietary factors (e.g., eggs, fish) rather than hypoxic training. In conclusion, the developed LC-MS/MS method provides a robust tool for TMAO quantification in clinical and research settings. The findings highlight the potential influence of diet over hypoxia on TMAO levels in athletes, offering new insights into gut microbiota metabolism under physiological stress. Further research is needed to explore the interplay between diet, exercise, and gut microbiota in TMAO production. *The authors marked with an asterisk equally contributed to the work.

P-33-011
Cytokine-based diagnostic test for early detection of infection and rapid determination of its etiology

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Rapid diagnostic tests are essential for controlling the spread of infections, as they enable early detection, patient isolation, and epidemiological tracking. Among these, cytokine-based rapid tests provide a valuable tool for both the early infection identification and the monitoring of disease progression, allowing

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patients to assess their condition personally. This will be especially important for people who travel frequently. One widely used method is the immunochromatography (lateral flow assay), which detects specific cytokines in blood or serum samples, offering a fast and cost-effective diagnostic solution. Our Diagnotrop project aims to develop an innovative rapid test to identify inflammatory proteins associated with infections. The selected biomarkers are carefully chosen to distinguish between infections caused by viruses, bacteria, or parasites. A critical aspect of this project is defining the precise concentration thresholds for these markers, enabling distinction between bacteria, viruses and parasite. To achieve this, we analyze and compare cytokine levels in samples from both healthy and infected individuals. By integrating analytical methods and optimizing detection sensitivity, the project aims to enhance diagnostic precision and accessibility. The final test will provide non-invasive diagnostic tool supportive in early medical treatment decisions. This innovative test has the potential to improve infection management, reduce healthcare burdens, and contribute to mere effective epidemic control strategies. Research funded by National Centre for Research and Development from the State Budget. Funding agreement number: DOB-BIO-12-04-001-2022. Project Title: "Innovative Integrated Field and Stationary Diagnostics System for Parasitic, Bacterial and Viral Invasions in Pmc Areas (Diagnotrop)".

P-33-012
Six-sigma calculation for serum protein markers according to 2025 CLİA total allowable error criteria

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Six-sigma methodology is one of the quality measurement methods used to evaluate laboratory performance. The sigmametric method evaluates internal (IQC) and external quality control (EQC) processes together. Serum proteins, used as biomarkers play critical roles in various biological processes. In this study, we aimed to evaluate the analytical performance of serum protein markers C3, C4, IgG, IgA, IgM by sigmametric method according to the 2025 CLIA Acceptance Limits. The study was conducted Kocaeli University Faculty of Medicine Hospital Central Laboratory, carried out on the Roche Cobas 8000c702 (Roche, Basel, Switzerland) biochemistry auto-analyzer for December 2024 and January 2025. IQC (Cobas PreciControl ClinChem Multi 1 and 2) data used for calculating coefficient of variation (%CV). EQC data obtained from the External Quality Assurance Services (EQAS) and used in %bias calculation. On the sigma scale, sigma values are ≤ 3 low; 3–6 acceptable; ≥ 6 good. According to December 2024 data, Six-sigma values was calculated as for level 1 and 2 IQC materials, respectively C3 (4.23-3.52); C4 (10.93-7.13); IgG (10.23-11.56); IgM (6.13-4.59); IgA (10.99-13.16). According to January 2025 data was calculated as, level 1 and 2, respectively C3 (5.89-7.16); C4 (3.91-5.9); Ig G (6.93-9.66); IgM (2.94-3.8); IgA (5.02-5.23). When the December and January data were examined, IgA showed the highest performance. IgM (Level 1) showed the lowest performance and sigmametric evaluation was below 3 sigma. According to our results, it is seen that the internal and external quality control results evaluation of IgM in both December and January are within the normal range however, the six-sigma performance was found to be low. Different analyses and methods should be used to determine the variables that cause low sigma levels, and more detailed evaluations and arrangements should be made in order to increase the analytical performance of this test. *The authors marked with an asterisk equally contributed to the work.

P-33-013
Precision calculation of labsan trion urine dipstick parameters

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Chemical testing of urine samples (reagent strip method) and identification and counting of particles is performed routinely to identify and monitor diseases of the kidney and urinary tract. Urinary test strip analysis serves as an initial and efficient screening method for reflex testing with accurate quantitative methods. Our aim in this study was to find out the precision of Labsan Trion urine dipstick parameters. Within-run (20 replicates for each level of urine controls in the same batch) and between-run (4 replicates for each level of urine controls for 5 days) precision experiments for urine chemistry were performed with Labsan Urine Chemistry Control (Level 1 and Level 2). Mean, standard deviation and coefficient of variation (CV) values were calculated by using Microsoft Excel v16.0.15831.20098 according to background reflactance values. Level 1 and Level 2 within-run and between-run CV values were calculated as 3.3, 3.4 and 3.9, 3.5; 1.1, 1.0 and 9.7, 9.1; 0.3, 0.5 and 8.0, 6.5; 0.4, 0.4 and 0.8, 0.4; 1.7, 2.0 and 1.6, 1.9; 3.8, 6.1 and 4.6, 7.1; 0.6, 0.5 and 1.9, 1.0; 0.8, 0.8 and 0.9, 1.0; 1.8, 2.4 and 1.8, 2.4 and 2.8, 3.2% for pH, glucose, protein, bilirubin, urobilinogen, blood, ketone, nitrite and leukocyte esterase, respectively. According to European Urinalysis Guideline 2023, allowable total CV values for dipstick chemistry parameters were determined to be $\leq 30\%$. Labsan Trion urine dipstick parameters demonstrated satisfactory performance and can be used in clinical urinalysis.

P-33-014
Prozone effect in flow cytometry in a multiple myeloma patient

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Multiple myeloma (MM) is a malignant lymphoproliferative B-cell disorder characterized by the excessive proliferation of abnormal clonal plasma cells. Normal plasma cells produce immunoglobulins composed of one heavy and one light chain. Malignant clonal plasma cells, however, secrete either excessive amounts of intact immunoglobulins or only a single type of free light chain, referred to as monoclonal proteins. Light chain multiple myeloma constitutes 15% of MM cases, in which clonal plasma cells fail to produce heavy chains and instead secrete only light chains. A 70-year-old male patient presented to the emergency department with back pain and hypercalcemia. His serum free kappa level was measured at 3570 mg/L, and his

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serum free lambda level was 38.40 mg/L. Following a bone marrow aspiration biopsy, he was diagnosed with MM and classified as stage IIIB. The patient underwent autologous stem cell transplantation after lenalidomide treatment. Maintenance therapy was interrupted due to the development of thrombocytopenia. Due to the progression of the disease, a repeat bone marrow aspiration was performed for flow cytometry analysis. In the initial analysis, cytoplasmic kappa and lambda values were both positive. Electrophoresis results revealed kappa monoclonal gammopathy. Since the cytoplasmic kappa value in the flow cytometry analysis was not consistent with the electrophoresis results, a prozone effect was suspected, and the sample volume was reduced by half for reanalysis. In the subsequent analysis, cytoplasmic kappa was evaluated as positive, while lambda was negative. This case highlights the risk of antigen excess in flow cytometry analyses. In some cases, lower signal intensity may be observed at higher concentrations. This can be attributed to antibody aggregation, which prevents binding, the prozone effect leading to reduced immune complex formation in the presence of excess antibodies. To eliminate the prozone effect, sample dilution must be taken into consideration.

P-33-015
Development and optimization of LC-MS/MS method for simultaneous quantitative analysis of pioglitazone, metformin hydrochloride and gliclazide

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Oral antidiabetic drugs are commonly used by people with diabetes to control blood sugar. Sulfonylureas (like gliclazide), meglitinides, biguanides (metformin hydrochloride), thiazolidinediones (like pioglitazone), α-Glucosidase inhibitors, DPP-4 inhibitors, SGLT2 inhibitors, cycloset are commonly used in Diabetes management. This study aimed to establish a reliable method for the simultaneous quantitative analysis of pioglitazone, metformin hydrochloride, and glyclazide in biological matrices by optimizing the separation and measurement of all analytes in a single chromatogram. Briefly, 300 µl of the sample was taken into Eppendorf tubes, 100 µl of d5-tamoxifen (100 ng/mL) as internal standard, and 600 µl of acetonitrile was added. Then, the mixture was vortexed for 30 seconds and centrifuged at 14 000 rpm for 10 minutes. Supernatants were taken into clean glass tubes and dried under nitrogen gas at 40°C. Dried residues were dissolved in a mixture of 300 µl of acn: water (30:70, %v/v). The linear range for pioglitazone, metformin hydrochloride, and gliclazide were found as 1000–0.48 ng/ml and 5000 – 9.7 ng/ml, and r2 values were obtained as r2 = 0.995, r2 = 0.999 and r2 = 0.997 respectively. Accuracy between 94.9–101.8% and precision < 5%. The run time was 15 min. A minimum of 8.06%-7.59%-2.85% and a maximum of 11.36%-14.28%-5.71% were observed as matrix effects. Simultaneous analysis of pioglitazone, metformin hydrochloride, and gliclazide in a single chromatogram by LC-MS/MS was successfully performed. The developed method showed high specificity and sensitivity, enabling accurate and reliable quantitative determination of all three analytes in biological matrices.

P-33-016
Comparison of two serum creatinine assays and their impact on KDIGO staging

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We aimed to compare two serum creatinine (sCr) measurement methods – Beckman (sCrB) and Improgen kit on Snibe analyzer (sCrI) using the kinetic Jaffe procedure – and evaluate their agreement as reflected in eGFR. Precision was assessed per CLSI EP15-A3 via verifymylab application. Method comparison and bias estimation were conducted on 160 serum samples via Passing-Bablok regression and Bland-Altman analysis, per CLSI EP09c with Analyse-It v5.80.2. eGFR was calculated via the CKD-EPI 2021 formula per KDIGO staging, and semi-quantitative comparisons were conducted with Kappa statistics. For the low-level sCr pool (1.1 mg/dL), sCrB within-lab CV% (1.68%) slightly exceeded the UVL (1.53%), while sCrI remained within limits. For the high-level sCr pool (4.1 mg/dL), sCrI within-lab CV% (1.66%) exceeded the UVL (0.95%), whereas sCrB remained within limits. Passing-Bablok regression between sCrB (x) and sCrI (y) results yielded: y = 0.2178 + 0.8699*x. Bland-Altman analysis showed that for < 0.8 mg/dL, sCrI measured higher than sCrB with a mean difference of 13.65% (9.87% to 17.43% 95% CI), supported Passing-Bablok (y = -0.07885 + 1.358*x). For sCr 0.8–1.2 mg/dL, sCrI measured higher, with a mean difference of 17.41% (14.89% to 19.92% 95% CI) (Passing-Bablok (y = 0.1048 + 1.05*x)). For sCr 1.2-2.0 mg/dL, sCrI and sCrB measurements showed results consistent with a difference of 3.36% (1.23% to 5.47% 95% CI), below the allowable limit of 6.3% from EFLM BV database. For sCr > 2 mg/dL, sCrI measured lower, with a mean difference of -8.35% (-9.23% to -7.47% 95% CI), supported by a Passing-Bablok slope of 0.858 (0.846-0.871 95% CI). Semi-quantitative eGFR staging showed high coherence (κ = 0.950). Except for CKD stage G2 (agreement ratio:0.42), the methods showed a high agreement in all other subgroups (agreement ratio > 0.70). The overall agreement in eGFR staging suggests comparable clinical interpretations. Further studies are needed to explain the differences between the methods.

P-33-017
Measurement uncertainity in clinical laboratories

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The accuracy of the results we obtain in the laboratory is important as it affects clinical decisions. Measurement uncertainty (MU) is an approach that assesses the reliability and accuracy of measurements. It characterizes the distribution of

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values that can be attributed to a measurement and is associated with the result. It gives the range of values measured with the same probability as a measurable property of the analyte, from which we can expect a true value. Due to the large number of variables that can affect samples in the medical laboratory, different methods have been introduced to calculate measurement uncertainty. The literature presents two models, bottom-up and top-down, for estimating the MU value. Studies comparing the MU value obtained by both calculations stated that there was no statistical difference between the results. Our study was calculated using internal quality control and external quality control material data in Ankara University Cebeci Central Laboratory between January 2024 and December 2024. Measurement uncertainty of TP, HDL cholesterol, BUN, triglyceride, sodium, and potassium tests was calculated. We calculated the standard uncertainty (u) based on the IQC (internal quality control) results of two levels of (normal: Level 1, pathological: Level 2) control materials performed in the analyzer. We calculated the 12-month mean measurement uncertainty for the TP, BUN, HDL, TG, Na, and potassium tests as 3.67, 8.36, 2.43, 6.52, 1.62, and 0.38, respectively. We compared these measurement-uncertainty values with the maximum expanded allowable measurement uncertainty (MAU) value obtained from the EFLM database. MAU values for TP, BUN, HDL, TG, Na, and potassium were 2.6, 13.9, 5.7, 19.7, 0.5, and 3.9, respectively. We observed that we exceeded the MAU value in sodium and total protein tests. In our other tests, potassium, TG, BUN, and HDL tests, we obtained a value below the MAU value. *The authors marked with an asterisk equally contributed to the work.

P-33-018
A rapid, robust and reliable measurement method for the quantitation of pregabalin

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Gabapentinoids such as gabapentin (GP) and pregabalin (PGL) have been used to treat a wide range of neurological and psychiatric disorders. Pregabalin abuse is a rapidly growing health problem worldwide, and little is known about the effects of prolonged high dose use in patients with pregabalin use disorder. Our aim in this study was to develop a simple, fast and effective mass spectrometric method for the determination of pregabalin levels. 300 µl of sample was taken into Eppendorf tubes and after adding 50 µl of internal standard (2500 ng/mL) and 600 µl of acetonitrile, it was vortexed for 30 seconds and centrifuged at 14000 × g for 10 minutes. Supernatants were taken into clean glass tubes and dried at 40°C under nitrogen gas. The dried residues were dissolved in 200 µl of acetonitrile: water (20:80, %v/v) and 40 µL of the supernatant was injected into the ABSCIEX API 3200 system. The developed method for the determination of pregabalin levels was linear in the range of 0.6-3000 µg/L. Total run time was 5 minutes. The precision values were less than 11.6% and the accuracy results ranged from 99.6% to 110.4%. Extraction recovery ranged from 97.1% to 99.1%, and matrix effect values were less than 20%. A rapid, cost-effective, robust and reliable measurement method has been developed for the quantification of pregabalin.

P-33-019
FIB-4 index in the evaluation of fibrosis in nonalcoholic steatohepatitis

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Nonalcoholic steatohepatitis (NASH) has an important place in chronic liver diseases and is characterised by progressive fibrosis. The gold standard diagnostic method for NASH is liver biopsy. The FIB-4 index is a scoring method used to non-invasively assess liver fibrosis. In this study, we aimed to evaluate the clinical utility of the FIB-4 index in NASH patients. The study included 62 patients diagnosed with NASH in the pathology clinic of our hospital between 2008 and 2024 and a control group consisting of 30 healthy subjects. Age, aspartate aminotransferase (AST), alanine aminotransferase (ALT) and platelet (PLT) values were recorded in the patient and control groups. FIB-4 scores of the patients were calculated. NASH patients were divided into two groups according to fibrosis stages (stage 0-1-2 and stage 3-4). ROC analysis was performed to evaluate the diagnostic value of the FIB-4 index and area under the curve (AUC) was calculated. FIB-4 score showed a significant difference between the patient and control groups ($p < 0.05$). According to fibrosis stages, FIB-4 score was significantly higher in the advanced fibrosis (stage 3-4) group ($p < 0.05$). As a result of ROC analysis, the AUC value of the FIB-4 index was calculated as 0.770, indicating that FIB-4 has a good performance in determining the fibrosis stage. The optimal cut-off value for FIB-4 in detecting NASH was determined to be > 0.6 (95% CI: 0.15–0.76). At this threshold, the sensitivity and specificity were 90.32% and 43.33%, respectively. FIB-4 index shows a significant correlation with fibrosis stages in NASH patients. It was observed that FIB-4 score increased significantly especially in advanced fibrosis cases. Therefore, FIB-4 index can be used as a non-invasive marker in NASH patients. However, the results need to be confirmed by studies in larger patient populations.

LB-R-33-01
Evaluation of total error and sigma metrics in spot urine tests: a quality assessment study

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Allowable total error (TEa) is an analytical quality specification that defines acceptable limits for a test result. It is used to assess test performance through sigma metric evaluation. TE of a test should not exceed its TEa. Spot urine tests are widely used in clinical practice, and ensuring their analytical reliability is essential. This study aimed to calculate the TE of analytes measured in spot urine samples, use TEa values based on biological variation as a target, and evaluate the analytical performance by calculating sigma values. This retrospective study analyzed nine months of internal quality control (IQC) data for eleven spot urine analytes measured with the Cobas 501 biochemistry analyzer in Ankara Training and Research Hospital. Standard deviation and coefficient of variation were

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calculated from IQC results. Bias values were obtained from the external quality control program. TE and sigma values were determined, and sigma performance was classified as excellent (≥ 6), acceptable (3–6), or poor (< 3). TE values are 2.68 for sodium, 3.23 for chloride, 4.22 for magnesium, 4.85 for calcium, 5.17 for uric acid, 5.19 for creatinine, 6.16 for urea, 6.17 for phosphor, 6.98 for total protein and 8.07 for albumin. TEa values are 37.6 for uric acid, 40 for total protein, 40.1 for albumin, 41.5 for sodium, 42 for urea, 46.5 for chloride, 56.4 for creatinine, 60.6 for magnesium, 66.7 for phosphor, 73.7 for calcium. Six sigma values are 11.33 for albumin, 14.76 for uric acid, 16.16 for total protein, 17.61 for urea, 22.75 for creatinine, 27.36 for phosphor, 34.06 for potassium, 34.31 for magnesium, 39.32 for sodium, 40.78 for chloride, 42.71 for calcium. The evaluation of total error and sigma values in spot urine tests demonstrated that all analytes exhibited high analytical performance, with sigma values well above the acceptable range. Regular performance monitoring remains essential to maintain analytical quality in clinical practice. *The authors marked with an asterisk equally contributed to the work.

LB-R-33-02
Combined analytical error in calculated tests: the overlooked detail in the case of low-density lipoprotein (LDL)

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Calculated biochemical tests play an essential role in clinical diagnostics by providing estimates of physiologically important parameters that are either impractical to measure directly or not routinely available. One widely used example is low-density lipoprotein cholesterol (LDL-C), which is typically estimated using the Friedewald equation incorporating total cholesterol (TC), high-density lipoprotein cholesterol (HDL-C), and triglycerides (TG). The formula, expressed as LDL-C = TC – HDL-C – (TG/5). Analytical reliability of calculated tests such as LDL-C must be evaluated through total error (TE), a composite metric that reflects both systematic bias and random imprecision. TE is typically calculated using the formula: TE = -Bias- + 1.65 × CV, where Bias represents systematic deviation from the true value, and CV is the coefficient of variation. For derived tests like LDL-C, the error from each component contributes to the overall uncertainty. To estimate this, the following formula for combined total error is applied: $TE_LDL = \sqrt{(TE_TC^2 + TE_HDL^2 + (TE_TG/5)^2)}$. Based on reported values in the literature, total error ranges between 5.7%–13% for TC, 9.9%–13% for HDL-C, and 10.4%–10.6% for TG. Substituting these into the formula, the estimated combined total error for calculated LDL-C ranges from 11.6% to 18.5%. Although the generally accepted total allowable error (TEa) for LDL-C is ±12%, this threshold is primarily intended for direct measurement methods. Importantly, no specific TEa is defined for LDL-C in the CLIA 2025 specifications. Exceeding the TEa may compromise clinical reliability, particularly when LDL-C values fall near decision thresholds for cardiovascular risk. Such deviations can lead to misclassification and potentially affect treatment strategies. Therefore, awareness of combined analytical uncertainty in calculated LDL-C is crucial for accurate interpretation in both clinical and epidemiological settings.

LB-R-33-03
Comparative evaluation of LDL-C estimation equations at high and very high triglyceride levels

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LDL cholesterol (LDL-C) is a key marker in cardiovascular risk assessment and therapy. The Friedewald formula is widely used but has limited accuracy at high triglyceride (TG) levels. To address this, newer formulas such as the Sampson and Extended Martin formulas were developed to improve LDL-C estimation across varied lipid profiles. Our machine learning-based Yayla formula [LDL-C = TC−HDL-C− ($\sqrt{TG}$) × TC/100] aims to improve accuracy not only across low, medium, and high LDL-C levels, but also in patients with high TG levels. This study compares these three formulas with directly measured LDL-C in individuals with TG > 400 mg/dL. Patient data were retrospectively collected from Trabzon Fatih State Hospital and Akçaabat State Hospital in Turkey. LDL-C was measured using a homogeneous enzymatic assay on the Beckman Coulter AU5800 analyzer. Patients were grouped by TG level: 400–800 mg/dL and > 800 mg/dL. LDL-C was estimated using the Sampson, Extended Martin, and Yayla formulas. Performance was assessed using mean absolute error (MAE), root mean square error (RMSE), mean absolute percentage error (MAPE), concordance correlation coefficient (CCC), and accuracy within ±12% of the enzymatic method. *In the 400–800 mg/dL group (n = 1310)* Sampson: MAE 37.65, RMSE 41.05, MAPE 25.80%, CCC 0.63, ±12% accuracy 11.98% Extended Martin: MAE 22.73, RMSE 26.54, MAPE 15.32%, CCC 0.81, ±12% accuracy 36.26% Yayla: MAE 12.51, RMSE 17.08, MAPE 8.73%, CCC 0.90, ±12% accuracy 81.07% *In the > 800 mg/dL group (n = 106)* Sampson: MAE 66.04, RMSE 71.98, MAPE 25.80%, CCC 0.24, ±12% accuracy 1.89% Martin: MAE 45.64, RMSE 54.56, MAPE 32.33%, CCC 0.45, ±12% accuracy 15.09% Yayla: MAE 24.51, RMSE 33.13, MAPE 20.18%, CCC 0.65, ±12% accuracy 47.17% The Yayla formula consistently achieved the lowest error metrics and highest concordance correlation coefficient across both TG ranges, suggesting it may provide a more reliable LDL-C estimation in patients with high TG.

LB-R-33-04
Serum ceruloplasmin reference limits: an indirect verification and recalculation study based on laboratory data

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The aim of this study is to evaluate the indirect verification of reference limits for ceruloplasmin, with the ReflimR method. Subsequently, for reference limits that were not verified, laboratory-specific reference intervals were calculated using indirect statistical methods (RefineR and non-parametric approaches). Results of 883 men and 1099 women (aged18-65 years) and results were filtered from the Laboratory Information Management System. Ceruloplasmin measurements were

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performed with commercial immunoturbidimetric method (Roche c6000 analyser; Roche Diagnostic, Germany). ReflimR was used for reference interval (RI) verification and RefineR and indirect non-parametric percentile methods were used for RI calculations. For ReflimR and RefineR algorithms, an open-source R package (https://CRAN.R-project.org/package = refineR) was utilized. All statistical analyses were conducted with the Analyse-it Software. The average age of our patients was 38 ± 15 for men and 40 ± 15 for women. Using the ReflimR method, the RI lower (%95 CI) and upper limit (%95 CI) were determined as 0.150(0.141-0.159)–0.299(0.284-0.314) g/L for men and 0.187(0.177-0.197)–0.327 (0.312-0.342) g/L for women. In the verification study, the reference limits for the manufacturer's data were verified for men (0.15-0.30 g/L), while were not verified for women (0.16-0.45 g/L). With the RefineR method, the RI for women was determined as 0.168(0.142-0.191)–0.349(0.315-0.358) g/L. In the indirect non-parametric method, the RI for women was calculated as 0.150 (0.140-0.160)–0.340(0.330-0.340) g/L. It is crucial for laboratories to verify the current reference intervals they use and calculate their own population-specific reference intervals for those that are not verified. Open-access statistical programs facilitate this process for medical laboratory specialists.

LB-R-33-05
Ultrasensitive Mpox-specific detection by loop-mediated isothermal amplification technology

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Mpox is a significant infectious disease of zoonotic origin, and its causative agent is the Mpox virus (MPXV), a member of the *Orthopoxvirus* genus of the *Poxviridae* family. It was previously known to be endemic to the African continent. However, it has recently spread outside endemic regions and has become a global outbreak threat. The World Health Organization (WHO) assessed Mpox disease as a public health emergency of international concern (PHEIC) twice in 2022 and 2024. Due to the epidemic potential and rapid spread risk of Mpox in recent years, the need for effective and rapid diagnostic methods has increased. In this study, a molecular test at the Point-of-care (PoC) level that can be used to diagnose Mpox was developed. Loop-mediated isothermal amplification (LAMP) technique was used as the method. Genetic map matching was performed with *DNA_bacer* and *Blast* programs for target region selection. The *F3L* gene, which differs from other *Orthopoxvirus* genus members in terms of genetic sequence and is the same in both clades of MPXV, was selected as the target region. The *F3L* gene sequence was cloned into pUC57 plasmid and used as template DNA in the reactions. According to the LAMP method's unique primer design principle, two outer primers (F3, B3), two inner primers (FIP = F1c + F2, BIP = B1c + B2) and two loop primers (LF, LB) were designed. LAMP reactions were carried out on qPCR device at constant temperature of 68°C for 30 min, and the results were monitored by fluorescence readout. LAMP assay was optimized for the specific detection of MPXV and showed ultrasensitive with a LoD value of 2.76×10^3 copies/μL at a dilution level of 10. These findings indicate that the developed LAMP assay can be used as an effective diagnostic tool for the early detection of Mpox disease. It can significantly contribute to public health by providing rapid, reliable, and practical molecular diagnostic solutions, especially in regions with limited infrastructure.

LB-R-33-06
Could blood irisin, elabela, endoglin, apelin and ischemia modified albumin (IMA) levels be used as an alternative to high sensitivity troponin-I (HsTn-I) in predicting acute myocardial infarction?

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In this study, in addition to blood high sensitivity troponin-I (HsTn-I) levels – the gold standard for Acute Myocardial Infarction (AMI) pre-diagnosis – the levels of apelin, elebela, endoglin, irisin, and Ischemia Modified Albumin (IMA) in the blood were determined, and the relationships between these parameters explored. Also, their predictive values in the pre-diagnosis of AMI were examined. In a cross-sectional study involving 60 AMI patients [30 ST-elevation myocardial infarction (STEMI) and 30 non-ST-elevation myocardial infarction (NSTEMI), who came to the emergency room within the first 6 hours] and 30 healthy controls, blood biomarker levels were measured by ELISA and routine blood parameters withdrawn from the hospital automation system. The statistical analyses used Kruskal Wallis H test, Spearman Correlation test and Receiver Operating Characteristics (ROC). The blood levels of HsTn-I, CK-MB, endoglin were significantly increased in AMI patients compared to the control group, while irisin and elabela levels were significantly decreased ($p < 0.001$). Specifically, serum HsTn-I exhibited negative correlations with irisin, apelin and, elabela, alongside a positive correlation with endoglin. According to ROC analysis, HsTn-I showed the highest diagnostic accuracy (AUC [95%CI]: 0.996), followed by elabela, endoglin and irisin (AUC [95%CI] above 80%). The significant differences in blood irisin, elabela and endoglin levels during early AMI and their acceptable diagnostic performance suggest their potential as biomarkers to supplement the gold standard HsTn-I. *The authors marked with an asterisk equally contributed to the work.

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LB-R-33-07
Impact of lipemia on ethanol measurement: evaluating natural ultralipemic materials (NULM) and intravenous lipid emulsions (IVLE) interferences according to CLSI guidelines

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Lipemia interference is a prevalent issue in the preanalytical phase that can compromise the accuracy of ethanol measurements. This study aimed to assess the impact of lipemia on ethanol analysis across varying concentration levels (high, moderate, and low) using both natural ultralipemic materials (NULM) and intravenous lipid emulsions (IVLE). NULM was prepared from native serum samples with high triglyceride levels. The influence of lipemia on ethanol measurements was examined using NULM and IVLE solutions. Interference evaluations were conducted following the guidelines set by the Clinical and Laboratory Standards Institute (CLSI) EP7-A2 and C56-A. The serum pools with high (204.0 ± 1.5 mg/dL), moderate (59.0 ± 0.8 mg/dL), and low (15.5 ± 0.2 mg/dL) ethanol results were diluted with both NULM and IVLE stock solutions to create sub-pools containing five different concentrations of triglycerides (TG). Ethanol analysis was performed on a Roche Cobas 6000 analyzer. Statistical analysis was carried out using repeated measures ANOVA. In the NULM interference study: in low and moderate ethanol pools, only the highest TG concentration (2359 mg/dL) caused a statistically significant difference ($p = 0.0118$ and 0.0082); in the high ethanol pool, statistically significant differences were observed at both 1154 mg/dL and 2359 mg/dL TG concentrations ($p = 0.02$ and 0.003, Bonferroni). In the IVLE interference study: a significant difference was observed only in the high ethanol pool at the highest TG concentration (2845 mg/dL) ($p = 0.037$, Bonferroni); no significant interference was observed in low and moderate ethanol pools for IVLE. Lipemia interferes with ethanol analysis in a TG concentration- and ethanol level-dependent manner. Natural lipemic samples showed more pronounced interference, particularly in high TG and high ethanol conditions, while IVLE had a limited impact.

LB-R-33-08
Early prediction of preeclampsia using machine learning: trimester-specific biomarker analysis

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Preeclampsia is a serious pregnancy-specific complication since hypertension happens in addition to multi-organ systems failing, including the liver, kidneys, and lungs. This condition affects approximately 3–5% of pregnancies and significantly increases maternal and neonatal morbidity and mortality. Clinicians still find it hard to identify those at risk. Therefore, this study intends to develop predictive models using machine learning. Retrospective trimester-specific data from 1346 pregnant women followed at the Department of Obstetrics and Gynecology, Ege University, between 2010 and 2024 were analyzed. Preeclampsia was diagnosed based on a systolic blood pressure of ≥ 140 mmHg or a diastolic blood pressure of ≥ 90 mmHg, along with proteinuria (≥ 300 mg in 24-hour urine or a protein/creatinine ratio ≥ 0.3). Supportive diagnostic criteria included elevated liver enzymes, thrombocytopenia, renal dysfunction, and fetal growth restriction. To identify predictive markers, demographic data (age, BMI, blood pressure), routine biochemical markers (complete blood count and 76 serum parameters), and ultrasonographic findings (amniotic fluid index, fetal growth restriction, and uteroplacental artery Doppler) were analyzed. Due to their ability to model nonlinear and complex relationships among variables, the XGBoost and Random Forest algorithms were selected. The models developed were tested using cross-validation to assess generalizability. The results demonstrate that the risk of preeclampsia can be predicted early using routine pregnancy monitoring data, and this approach may contribute to clinical decision-support systems. Nevertheless, the study's single-center design may restrict the broader applicability of the findings.

LB-R-33-09
Machine learning analysis of routine trimester-based biomarkers for early prediction of gestational diabetes mellitus

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Gestational diabetes mellitus (GDM) is a prevalent pregnancy complication marked by glucose intolerance during gestation, leading to significant health implications for both mother and fetus. Although the oral glucose tolerance test (OGTT) established by the International Association of Diabetes and Pregnancy Study Groups (IADSPG) at 24-28 weeks of gestation is the diagnostic gold standard, its limitations in timing, feasibility, and patient compliance underscore the necessity for earlier and more accessible predictive methods. This retrospective cohort study utilized a comprehensive dataset of clinical records from 3196 pregnant women monitored at the Ege University Department of Obstetrics and Gynecology from 2010 to 2024. The aim was to develop machine learning models to evaluate the predictive utility of trimester-specific biomarkers for GDM. We evaluated age, BMI, and blood pressure, trimester-specific biochemical markers including CBC and 83 additional biochemical parameters, and ultrasound findings such as amniotic fluid index, fetal growth restriction, and UtA/PlA Doppler measurements. Our study utilized two advanced machine learning algorithms, XGBoost and Random Forest, which are recognized for their capacity to accurately model intricate, non-linear relationships among biomarkers and have demonstrated considerable success with clinical datasets. To assess the adaptability of the models to diverse samples and their reliability with novel patient data, a thorough performance

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analysis was conducted utilizing cross-validation techniques. The resulting predictive framework highlights the clinical potential of integrating comprehensive biomarker analysis with advanced machine learning techniques for the early detection of GDM. This methodological approach can offer clinicians a valuable decision-support tool, facilitating timely interventions and addressing various limitations of current diagnostic methods.

LB-R-33-10
Reference limits for serum sialotransferrins: a preliminary study on indirect verification and recalculation using laboratory data

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This study aimed to assess the validity of current reference limits (RLs) for various sialotransferrin isoforms (disialo-, trisialo-, tetrasialo-, and pentasialotransferrin) through indirect verification using the ReflimR method. For isoforms whose RLs were not verified, new laboratory-specific reference intervals were estimated using the RefineR algorithm and non-parametric techniques. Data from 943 male patients aged 18–65 years with normal liver function were extracted from the Laboratory Information Management System. Carbohydrate-deficient transferrin (CDT) isoforms were quantified by high-performance liquid chromatography (HPLC) using the IFCC reference method (Shimadzu LC-2052). RL verification was performed using ReflimR based on manufacturer-provided cut-offs. For disialotransferrin and other isoforms that failed verification, reference intervals were recalculated using RefineR (https://CRAN.R-project.org/package=refineR) and indirect non-parametric percentile methods. Statistical analysis was conducted with Analyse-it software. Asialo- and monosialotransferrin levels were below the analytical detection limit. Based on the results of indirect statistical modeling, the recalculated reference intervals were determined as 0.62–1.47% for disialotransferrin, 1.88–5.57% for trisialotransferrin, and 79.4–83.9% for tetrasialotransferrin. The pentasialotransferrin isoform exhibited borderline verification characteristics during analysis, and its reference interval was calculated as 11.8–17.0% Findings indicate that existing RLs for trisialo- and tetrasialotransferrin do not reflect this population. Indirect modeling enables the derivation of more accurate, population-specific reference intervals. Routine verification using open-access tools should be integrated into laboratory quality processes.

LB-R-33-11
Pediatric reference intervals for serum sialotransferrin isoforms: a preliminary indirect study based on sex and age stratified laboratory data

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This study aimed to establish age- and sex-specific reference intervals (RIs) for serum sialotransferrin isoforms (disialo-, trisialo-, tetrasialo-, and pentasialotransferrin) in pediatric populations, using indirect statistical approaches. Both 1–12-month and 1–18-year age groups were analyzed separately for female and male. A total of 21 134 anonymized serum carbohydrate-deficient transferrin (CDT) test results from patients aged 1 month to 18 years were retrospectively extracted from the laboratory information system. CDT isoforms were measured using high-performance liquid chromatography (HPLC; ShimadzuLC-2052) based on the IFCC reference method. Indirect reference interval verification was performed with the ReflimR package, while interval estimation was conducted using RefineR (v1.6.x, Box-Cox model). Non-parametric and bootstrap methods were used in cases with sample sizes below 200. Recalculated RIs varied significantly by age and sex. For female and after male aged 1–18 years, the refined RIs (2.5–97.5 percentiles) were: Disialotransferrin 0.46–1.74% (F)/0.479–1.81% (M); Trisialotransferrin 1.13–8.19% (F)/1.19–7.16% (M); Tetrasialotransferrin 77–82.7% (F)/ 76.2–83.6% (M); Pentasialotransferrin 9.37–18.6% (F)/ 10.4–19.5% (M). In the 1–12 month subgroup, Several results in this group displayed increased variability and require further population-level validation. Our findings indicate that reference intervals for serum sialotransferrin isoforms vary by age and sex in pediatric populations. Indirect estimation methods such as ReflimR and RefineR provide a practical framework for establishing population-specific pediatric RIs, especially where direct sampling is limited. Routine stratification by age and gender is essential for the accurate clinical interpretation of CDT profiles in children.

Biosensors

P-34-001
AI supported single-entity electrochemistry for glycated albumin detection using MPBA-modified silver nanoparticles

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Glycated albumin (GA) is a critical biomarker for short-term glycemic control, offering an alternative to HbA1c for diabetes monitoring. Traditional GA detection methods such as ELISA and HPLC are limited by high costs, lengthy processing times, and relatively low sensitivity. In this study, we present a novel single-entity electrochemistry (SEE) approach coupled with machine learning (ML) models for the highly sensitive and rapid detection of GA and glucose. 4-mercaptophenylboronic acid (MPBA)-modified silver nanoparticles (AgNPs, 60 nm) were used to enhance the selective interaction with GA, generating characteristic electrochemical signals. Baseline correction via Savitzky-Golay filtering and Wavelet Denoising significantly improved signal quality, eliminating drift and high-frequency noise while preserving critical electrochemical peaks. Peak detection and collision frequency analysis using the Find Peaks Algorithm enabled precise identification of GA-related oxidation events. A linear relationship ($R2 = 0.986$) between collision frequency and GA concentration ([GA]) was observed and modeled. Additionally, nanoparticle size estimation using Faraday's Law confirmed the stability of AgNP behavior during sensing. To further enhance detection accuracy and enable glucose-GA differentiation, we implemented a machine learning model (Random Forest and Support Vector Machine, SVM)

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trained on electrochemical features such as peak amplitude, charge transfer, and frequency distribution patterns. The ML model demonstrated high classification accuracy (>95%) in distinguishing GA from glucose oxidation events and predicting GA concentrations based on electrochemical responses. This integrated SEE-ML approach offers a rapid, label-free, and real-time alternative to traditional GA and glucose assays.

P-34-002
Design and development of Cherry-Body and Cherry-CAR biosensors targeting hypoxic tumor microenvironment

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Solid tumors pose significant challenges to current CAR T cell therapies. A critical aspect of effective immunotherapy for solid tumors is understanding the tumor microenvironment, often characterized by hypoxia. This study focuses on hypoxia to develop novel biosensors named Cherry-Body and Cherry-CAR. Cherry-Body is a split antibody, and Cherry-CAR is a split chimeric antigen receptor (CAR), both featuring the mCherry marker protein. These constructs utilize a split intein system that enables selective protein assembly under defined conditions. Cherry-Body consists of half-cherry N- and C-terminals fused to split intein N- and C-terminals, respectively. The split inteins serve as adaptor proteins, facilitating the assembly of full mCherry constructs from the half-cherry components. Similarly, Cherry-CAR represents a next-generation CAR T cell platform that includes a half-cherry N terminal fused with split intein N terminal, designed to integrate with the chimeric antigen receptor on T cells. Cherry-Body and Cherry-CAR interact synergistically within the hypoxic tumor microenvironment. The Cherry-Body and Cherry-CAR heads (half-cherry N-Intein N terminal) and Cherry-Body tail (Intein C-half-cherry C-terminal–Fc fusion) were produced in *Escherichia coli*, purified using nickel affinity columns under mild solubilization conditions. Cherry-CAR was expressed in Jurkat cells as Intein C–half-cherry C-terminal–Fc fusion constructs via electroporation, selected with antibiotics, and presented alongside the Cherry-Body tail to half-cherry heads under reductive conditions that mimic the hypoxic tumor microenvironment. The engineered split inteins demonstrated the capability to selectively fuse Cherry-Body and Cherry-CAR components under hypoxic conditions, assembling fluorescent proteins detectable through fluorescence microscopy. Future studies will explore the interactive functionality of these advanced biosensors for solid tumor detection.

P-34-003
Development of amyloid fibril-graphene oxide composite biomaterial for simultaneous detection of heavy metals

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Increasing environmental pollution, urbanization, and industrial activities lead to the spread of toxic heavy metals, posing serious health risks due to their accumulation in tissues, membrane and DNA damage, disruption of enzyme and protein activity. Therefore, detecting the levels of highly toxic heavy metals such as cadmium, lead, arsenic, and mercury in real human samples is crucial for public health and environmental risk assessment. Amyloid fibril (AF) and graphene oxide (GO) structures have recently gained attention in biosensor applications. AFs are protein aggregates consisting predominantly of β-sheets, exhibiting a highly ordered structure and having high mechanical strength, stability, and biocompatibility, while GO, a graphene derivative, provides a large surface area, high ionic and electrical conductivity, and functional groups that enhance biomolecule immobilization and electron transfer. A hybrid AF-GO composite was designed as a promising option for selective and simultaneous heavy metal detection. AFs were synthesized using a sustainable approach from β-lactoglobulin which was partially purified from whey. β-lactoglobulin purity was determined by SDS-PAGE electrophoresis and the formation of AF from purified protein was evaluated by Congo Red staining using red shift of its characteristic absorbance spectrum peak from 490 to 512 nm. GO was synthesized by modified Hummer's method, Graphene papers containing mixtures of AF-GO were obtained and the electrical conductivity of the obtained paper was achieved by thermal reduction. The SWV method was employed to anodically strip electrodeposited heavy metals in buffered HCl (pH 2) by scanning electrode potential from -1.0 to -0.2 V. Distinct stripping peaks at different potentials allowed simultaneous quantification of multiple metals using calibration curves. The study's findings hold potential for the development of biosensors that can identify heavy metals in actual human samples like blood and serum.

P-34-004
Development of novel immunodiagnostic tools through the integration of leukocytes into gold nanoparticles (AuNP)-based paper biosensors

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The rapid evaluation of leukocyte activation in infectious diseases offers significant potential for enhancing diagnostics and healthcare. Leukocytes, essential immune system cells, play a central role in defending against pathogens, and monitoring their activation can provide insights into the immune response during infection. A promising method involves using paper-based biosensors (oPBs) with gold nanoparticles (AuNPs) to detect membrane biomarkers on leukocytes, enabling real-time immune response detection. However, integrating cells into paper biosensors presents challenges due to the complex interactions between cells and paper substrates. This study explored how different Whatman paper strips with varying pore sizes affect leukocyte behavior, aiming to identify factors influencing cell interaction with paper. HL-60 neutrophils, granulocytes, monocytes, and lymphocytes were tested for retention on paper strips, with the results showing differences in retention based on pore size. Whatman W41 paper proved most effective at retaining HL-60 cells and granulocytes while filtering lymphocytes and monocytes. The study also examined the ability of AuNPs to detect CD66b, a membrane antigen on granulocytes. The results showed successful detection of CD66b

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in just 15 minutes, with W41 paper yielding minimal non-specific binding, making it ideal for biosensor applications. The findings highlight the feasibility of integrating leukocytes into paper biosensors, though challenges remain in understanding the interactions between cells, AuNPs, and paper substrates. The results suggest that colorimetric tests based on AuNPs can enable real-time immune response monitoring, offering a promising tool for rapid diagnostics, especially in resource-limited settings. This research lays the foundation for further development of decentralized diagnostic tools for infectious diseases, improving patient care and healthcare outcomes.

P-34-005
Dual functionality: a new benzimidazole based compound with cytotoxic and imaging capabilities

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Fluorescence-based compounds represent a powerful tool for noninvasive bioimaging of living systems in real time. Most pH biosensors measure a change in fluorescence intensity based on a change in the protonation state of the fluorophore. This study explores the therapeutic potential of a new benzimidazole-based molecule specifically designed for targeting live cells. Cancer treatment today is highly advanced and typically involves a multidisciplinary approach tailored to each patient's specific diagnosis and needs. Detection of specific biomarkers is an important way to recognize or confirm the presence of cancer in the body. The fluorescent N-heteroatomic compounds are known to accumulate in different parts of biological tissue. For example, uorophores with N-heterocyclic moiety playing an essential role due to their exceptional optoelectronic properties and biocompatibility. In our studies, the chemical synthesis of the benzimidazole derivative-4-(2-(1H-benzo[d]imidazol-2-yl)-2-isocyanovinyl)-N,N-diphenylaniline was carried out using a three-step synthesis, where the most critical step, in terms of reaction efficiency, was the Knoevenagel condensation. Optical characterization was performed using UV-Vis spectrometers, which revealed interesting properties of the compound depending on the molecule's environment, including polarity and pH. The compound's cytotoxic potency was evaluated through a series of *in vitro* SRB assays, demonstrating significant inhibitory effects on glioma cell proliferation. Additionally, as the small molecule exhibited fluorescence properties, it allows for effective imaging of glioma cells, which enhances its utility in both therapeutic and diagnostic applications. These findings suggest that the newly synthesized compound holds promise as a dual-function biosensor, capable of not only reducing glioma cell viability but also facilitating real-time imaging, thereby contributing to improved strategies for glioma diagnosis and treatment.

P-34-006
Modification of PVA based-polymeric matrices for the detection of environmental pollutants

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Pollution is one of the leading causes of premature deaths, bringing to the development of eco-friendly and economic system for its identification. This context led to a strong drive to the development of colorimetric sensors based on functionalized polymeric matrix, able to create complexes with pollutants allowing their easy and unquestionable detection in trace elements. Using the solvent casting method, different poly-vinyl alcohol (PVA) based-polymeric matrices were produced functionalizing them with quercetin. This functionalization makes them suitable for the identification of metallic and organic (crystal violet, phenol red) pollutants in distilled water and in natural water-spiked samples and helps to overcome the problem of PVA solubility in water. The samples were able to detect ppm of elements in solution, resulting in a colour change easily detectable with visible spectroscopy and colorimetric analysis. Fourier transform infrared spectroscopy (FTIR), colorimetric assays, thickness analyses, solubility and mechanical assays were performed to characterize the samples. The functionalization of the polymer matrix with quercetin results in a decrease in its solubility. FTIR analysis reveals that the compounds are dispersed rather than creating new bands characterized by the formation of covalent bonds. The crystallinity index of the starting material also decreases, as shown by the changes in the bands at 1138 cm^{-1} (due to shoulder stretching of C—O, representative of the sequence with crystalline structure), 1083 cm^{-1} (due to the stretching of C=O and the bending of –OH, representative of the sequence with amorphous structure). Overall, the functionalization with quercetin creates a cheap sensor with low detection limits that can be easily detected even with the use of a colorimeter. This work has been partially funded by the European Union (Next Generation EU), through the MUR-PNRR project SAMOTHRACE (ECS00000022). *The authors marked with an asterisk equally contributed to the work.

P-34-007
Utilization of baicalein as a sensor for the identification of metal and organic pollution of water environment

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Baicalein is a flavonoid belonging to the class of flavone, isolated from the Lamiaceae family. It shows a wide range of biological properties (1,2). Polyvinyl alcohol bioplastic was modified by baicalein addition (0.25, 0.5 and 1.0 mM), leading to the creation of sensor able to detect metals and organic pollutions in aquatic environment. In particular, the new materials obtained detect crystal violet up to 0.8 mg/mL or phenol red up to 0.08 mg/mL in solution, resulting in the creation of red-violet coloration of the bioplastic. Moreover, the color change is directly correlated to bacalein concentration. The new materials were also able to detect ppm of elements (Cu^{2+}, Zn^{2+}, Fe^{2+}, Hg^{2+} and Ni^{2+}) in

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solution. Regarding biocompatibility, we tested the leaching liquid assay, demonstrating that red blood cells incubated with leaching liquids from the functionalized materials do not induce cell damage, as shown by trypan blue coloration and by haemoglobin release, compared to the control group. Fourier transform infrared spectroscopy showed that the modification of the starting polymeric matrices with increasing concentrations of baicalein does not induce the formation of covalent bound eventually revealed by the appearance of new bands, indicating a dispersion of utilized flavonoid. The functionalization of bioplastics with flavonoids creates a sensor with low limits of detection, easily achievable without complex sample preparation techniques with environmentally eco-friendly procedures. This work has been partially funded by the European Union (Next Generation EU), through the MUR-PNRR project SAMOTHRACE (ECS00000022). References: 1. Islam MN et al (2011) Metabolomics 7, 446-453. 2. Nik Salleh NNH et al (2020) Molecules 25(23), 5677. *The authors marked with an asterisk equally contributed to the work.

P-34-008
Polymeric aptamer-imprinted surface for immuno-capture of cancer cells for biosensor applications

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Non-invasive approaches in cancer diagnosis are important for increasing cancer survival rates. Circulating tumor-derived components play an important role in early cancer diagnosis, prevention of metastasis, and prognosis. Circulating tumor cells are often associated with advanced metastasis and cancer-related morbidity. Biosensors are promising strategies in this field due to their significant advantages such as high sensitivity, high specificity, and rapid analysis. The use of polymeric surfaces is a rational approach to enhance the analytical performance of biosensors. The surface immobilization of capturing biomolecules is generally performed by functional polymers decorated on the surfaces. Nucleic acid aptamers are widely studied in immune affinity-based strategies due to their high specificity and low cost. In the study, an innovative aptasensor design is proposed by imprinting thiol-tailed aptamer on a PGMA-PPDS block copolymer-decorated glass surface. First, the PGMA-PPDS block copolymer was decorated on APTES-modified glass surfaces by the reaction of amines of APTES and glycidyl groups of PGMA block. The thiol-tailed Anti-nucleolin aptamer (AS1411) was then imprinted on the surface by the reaction between the thiol of aptamer and the pyridyl disulfide of PPDS block. The human breast cancer cell capture ability of the designed polymer-based aptasensor surface was tested using fluorescence microscopy.

P-34-009
Development of a test system based on dual-priming isothermal amplification combined with multicomponent hybridization probes for molecular genetic diagnostics of lower respiratory tract infections

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Rapid diagnostics of lower respiratory tract infections (LRTIs) are important due to their potential for complications and high mortality, especially in vulnerable groups. Traditional methods are time-consuming, delaying treatment or not providing complete information. A promising approach is dual-priming isothermal amplification (DAMP) combined with multicomponent hybridization probes. DAMP enables DNA amplification at a stable temperature, while multicomponent probes enhance accuracy and reduce false positives. The aim of this work is to explore DAMP with multicomponent probes for the molecular genetic diagnostics of LRTIs, creating fast, accurate, and affordable diagnostic methods to improve medical care. Specific genomic regions of common pneumonia pathogens (*S. pneumoniae*, *H. influenzae*, *S. agalactiae*, *K. pneumoniae*, *P. aeruginosa*) were selected, and DAMP primers were designed. The reaction was optimized at 60°C for 45 minutes, achieving a sensitivity of no higher than 100 genomic equivalents per pathogen with no cross-reactivity. Hybridization probes with a DNAzyme core were manually designed to complement target fragments and F-sub reporter molecules [previously published in: Filatov et al. (2024) JoVE 204, e66461]. Detection involved mixing probes with the analyte, F-sub, and reaction buffer, followed by incubation at 55°C for 60 minutes. Results were evaluated via fluorometry at 495/525 nm. The project developed a DAMP-based test system combined with detection by multicomponent hybridization probes for the detection of LRTI pathogens, demonstrating high analytical sensitivity and specificity against the host and other commensal organisms. This work was supported by St. Petersburg State University (project 125021902561-6) and RSCF (project 25-16-20127).

P-34-010
Development of a novel platform for *in situ* analysis of dsDNA

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In situ analysis of biomarkers is highly desirable in molecular pathology because it allows the examination of biomarker status within the histopathological context of clinical specimens. Immunohistochemistry (IHC) and DNA *in situ* hybridization (ISH) are widely used in clinical settings to assess protein and DNA biomarkers, respectively, but clinical use of *in situ* dsDNA analysis is rare. We propose a novel platform, the Multifunctional DNA Machine for dsDNA analysis (MDMD), for *in situ* analysis and

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validation of single nucleotide variations (SNV) based on dsDNA ISH method. Single-molecule visualization in individual cells will be achieved through the use of a novel probe design strategy and a hybridization-based signal amplification system to simultaneously amplify signals and suppress background. We will take advantage of recent developments of DNA nanotechnology to design a multifunctional platform that enables (i) unwinding of a specific dsDNA analyte, (ii) specific recognition of a targeted fragment, (iii) enhanced delivery of a signal-producing substrate to a target-activated catalytic sensor, and (iv) signal amplification by catalysis. This hybridization-mediated signal amplification scheme is similar to the branched DNA method, but the MDMD design strategy should ensure superior background control because it is highly unlikely that a nonspecific hybridization event will juxtapose a DZa strand along an off-target sequence. We tested a series of MDMD platform and validated its selectivity using PCR amplicons, genomic DNA from cell lines, and PCR amplicons containing SNVs. The optimized MDMD platform demonstrated excellent selectivity for single-base differences. Current efforts focus on optimizing the FISH protocol for enhanced performance. The project is funded by St. Petersburg State University (project 125021902561-6) and RSCF (project 25-16-20127).

P-34-011
Cell interactions with modified graphene foams and fibers

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In this study, it was demonstrated that laser-induced graphene (LIG) foams and fibers (LIGF) can be used for cell growth. LIG has attracted significant attention as a promising material for biomedical applications such as biosensors due to its exceptional properties such as exceptional conductivity, large surface area, and ease of processing. The research focused on the production of graphene and graphene fiber matrices using laser printing techniques with various parameters. Comprehensive characterization of chemical, electrochemical, and morphological properties of these materials was carried out using analytical methods including scanning electron microscopy (SEM), Raman spectroscopy, X-ray diffraction (XRD), and X-ray photoelectron spectroscopy (XPS). Afterwards, LIG and modified LIG were sterilized and BEAS-2B (epithelial cell line) cells were seeded on them. Viability was then measured by MTT viability test. Cells adhered to graphene within 24 h and increased in number at 48 and 72 h. The vitality of the cells was also checked with a confocal microscope by performing a live/death assay. All the cells were alive. These surfaces, which do not harm cells, have the potential to be used as biosensors in the future.

P-34-012
A miniaturized SPR-based nanoMIP sensor for the selective detection of bisphenol A in drinking water: a step towards enhanced environmental and public health safety

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Bisphenol A (BPA) is an endocrine-disrupting chemical that can pose serious health risks when present in drinking water. In particular, prolonged exposure to BPA may lead to hormonal imbalances, have adverse effects on reproductive health, and trigger metabolic disorders. Therefore, the rapid, selective, and reliable detection of BPA in drinking water is of great importance for public health. Molecularly imprinted polymers (MIPs), also known as artificial antibodies, have become a novel tool in sensor studies. In this study, BPA-imprinted nanoMIPs were used as a miniaturized surface plasmon resonance (SPR)-based sensor. The morphological and physicochemical properties of the BPA-imprinted nanoMIPs were characterized using atomic force microscopy (AFM), transmission electron microscopy (TEM), and dynamic light scattering (DLS) analyses. The average size of the nanoMIPs was determined to be 77.5 ± 5.3 nm. The developed nanoMIP sensor achieved a detection limit of 1.46 nM for BPA across a broad concentration range in bottled water samples from eight different brands. Selectivity studies using control MIPs showed a selectivity coefficient of 10.29. Kinetic studies revealed that BPA adsorption was consistent with the Langmuir-Freundlich model. In conclusion, the developed nanoMIP sensor provides a sensitive and selective method for the detection of BPA in drinking water, contributing to environmental biosafety efforts. However, further large-scale epidemiological and clinical studies are needed to better understand the impact of this technology on public health.

LB-R-34-01
Design and characterization of a biosensor for the detection of CTX and PINP in biological fluids

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Biochemical markers, such as the C-terminal telopeptide of type I collagen (CTX) and the procollagen type I N-terminal propeptide (PINP), are widely recognized as reliable indicators of bone turnover and are clinically valuable for the management of metabolic bone disorders. In this study, we developed and characterized a novel electrochemical biosensor platform for the sensitive and selective detection of CTX and PINP in biological fluids. The biosensor was fabricated by functionalizing a gold electrode with 3-mercaptopropionic acid (3-MPA), followed by the covalent immobilization of specific monoclonal antibodies using carbodiimide (EDC/NHS) coupling chemistry. The electrochemical performance was evaluated through cyclic voltammetry (CV) and electrochemical impedance spectroscopy (EIS), confirming successful surface modification and efficient biomarker recognition. Fourier-transform infrared spectroscopy (FTIR) revealed characteristic peaks associated with the formation of carboxyl and amide bonds to further validate the surface modification steps. Scanning electron microscopy (SEM) and atomic force microscopy (AFM) demonstrated distinct morphological and topographical changes, with increased surface roughness observed after each modification layer. Contact angle measurements supported changes in surface wettability, consistent with successful functionalization. The biosensor exhibited high sensitivity, broad linear detection ranges, and strong selectivity in buffer solutions and diluted human serum, saliva, and urine samples. These findings underscore the potential of this biosensing platform as a clinically relevant, non-invasive diagnostic tool for monitoring bone metabolism and facilitating

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the follow-up of treatment of conditions such as osteoporosis and fractures. Gazi University Scientific Research Projects Unit supported this study with the code TDK-2024-9725.

LB-R-34-02
Evaluation of the DNA platform addition effect on the hybridization kinetics and nucleic acid detection characteristics of the 4-way junction (4WJ) DNA sensor

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Nucleic acid recognition utilizing fluorescent DNA probes is a promising method of point-of-care diagnostics due to their specificity for single nucleotide polymorphisms (SNP), associated with antimicrobial resistance, and simple experimental design not requiring any special conditions. 4-Way junction (4WJ) DNA nanosensor, a facile system of 2 analyte-specific strands and a molecular beacon (UMB) for signal generation, shows promising results in sensitivity and specificity, as well as high hybridization speed to the target analyte. However, it was demonstrated that additional DNA scaffold can improve the limit of detection (LOD) for such probes [previously published: in Kolpashchikov D. M. (2019) Acc. Chem. Res. 52(7), 1949–1956]. In this research two 4WJ probes were modified by an additional DNA platform of different lengths (~20 and ~40 nucleotides). *Mycoplasma genitalium* 23S rRNA gene fragment with A2072G substitution and *Neisseria gonorrhoeae* penA gene fragment with G936T substitution were selected as research objects for their clinical relevance as antibiotic resistance associates. The aim of the research was to evaluate the effect of DNA platform on sensitivity, hybridization time and SNP specificity of 4WJ nanosensors. Experiments were conducted at room temperature in a buffer containing 50 mM $MgCl_2$, 50 mM Tris-HCl. Fluorescence intensity was measured at 480/525 nm excitation/emission. Research showed LOD improvement from ~6.5 nM to ~807 pM, selectivity factor (S_F) increased from 19.73% to 99.73%, and reliable detection time ($\Delta t1.5$), time interval by which signal is higher than background by 50%, was lowered from 275 to 65 seconds for *M. genitalium*. *N. gonorrhoeae* demonstrated the same trends: LOD was lowered from ~4.05 nM to ~188 pM; $\Delta t1.5$ – from 55 seconds to 10 seconds and S_F increased from 20.78% to 94.39%. The authors of the study are grateful to the Ministry of Education and Science of the Russian Federation No. FSER-2025-0019 for financial support. *The authors marked with an asterisk equally contributed to the work.

Drug development

P-35-001
Odorant-binding protein 7 – a new compound of potential applications in red biotechnology

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Insect immunity relies heavily on antimicrobial peptides (AMPs), that offer a promising therapeutic avenue for developing new medicine based on their broad activity against insects and human pathogens. Here, we identified and characterized *Galleria mellonella* odorant-binding protein 7 (*Gm*OBP7), which was detected in the hemolymph of insects following infection with *Pseudomonas entomophila* (*Pe*). Its role in the immune response of *G. mellonella* was previously unknown. Gene expression analysis revealed that *Gm*OBP7 mRNA levels in the gut and fat body significantly increase with infection dose. This was correlated with temporal protein profiling in hemolymph and gut tissue which showed that *Gm*OBP7 levels varied with infection dose of *Pe*, route of infection (oral or injection), and time after infection. Notably, similar profiling patterns were observed upon infection with human pathogens such as *Escherichia coli*, *Pseudomonas aeruginosa*, *Staphylococcus aureus*. Functional assays showed that *Gm*OBP7 exhibits antimicrobial activity against insect and human pathogens, atomic force microscopy (AFM) revealed *Gm*OBP7-induced surface alterations on bacterial cell membranes, which prompted further investigation of its membrane-perforating capabilities. The investigated protein caused perforation of bacterial membrane, resembling the action of antimicrobial peptides, which are called alternative antibiotics or antibiotic of third generation. These findings establish *Gm*OBP7 as a critical player in insect immunity and propose new perspectives as a new compound with potential use in biotechnology. *Pseudomonas entomophila* is a kind gift from dr. Frideric Boccard (France), whose group isolated this strain in Guadeloupe. The work was financed by the National Science Centre, Poland, project number 2020/37/B/NZ6/00167.

P-35-002
In vitro and *in silico* analysis of novel steroidal oxime and azine derivatives: binding affinity for estrogen, androgen, and glucocorticoid receptors and inhibition of aldo-keto reductases

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Steroid hormones regulate various biological processes, and their structurally modified analogs have found extensive use in

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medicine as anti-inflammatory and anticancer agents by targeting steroid receptors. Steroid derivatives were proven to be efficient drugs against several types of cancer, particularly hormone-dependent cancers. Recently, enzymes from the aldo-keto reductase (AKR) superfamily have also gained attention as important targets in anticancer drug discovery. These enzymes play a crucial role in the metabolism of xenobiotics, steroids, and carbohydrates and in the development of cancer and drug resistance, making their inhibition a focus for therapeutic research. This study aimed to evaluate the biological activity of newly synthesized steroidal oxime and azine derivatives including *in vitro* screening of their binding affinity for estrogen (ER), androgen (AR), and glucocorticoid receptors (GR) and inhibition of AKR1C3, as well as *in silico* prediction of molecular interactions between targets and ligands identified in the preliminary screening. Methods applied included a fluorescent assay in yeast for identifying and quantifying steroid receptor-ligand binding, fluorescence spectroscopy to identify AKR1C3 inhibitors, and molecular docking to explain the molecular basis of protein-ligand interactions. Among tested compounds, one compound showed a high selective binding affinity for the ligand-binding domain of ERβ. Several compounds were identified as potent GR ligands, with binding affinities higher than prednisolone, while no ligands were detected for ERα and AR. In the enzymatic assay, all tested steroidal oxime derivatives and two azine derivatives exhibited potent inhibitory effects against AKR1C3, higher than ibuprofen. These results encourage future studies to discover mechanisms behind the action of these steroid derivatives and may serve as a starting point for the design of new anticancer drugs or adjuvants.

P-35-003
The impact of rhBMP6 on osteochondroprogenitor cell recruitment and differentiation in bone regeneration

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Osteogrow-C is a novel bone regeneration device composed of recombinant human bone morphogenetic protein 6 (rhBMP6) in autologous blood coagulum (ABC) with synthetic calcium phosphate (CaP) ceramics. The aim of this study was to elucidate how the addition of rhBMP6 to implants affects the host cellular response to the implantation of ABC containing ceramic particles. Rats were implanted with CaP ceramics mixed with ABC (N = 5) or CaP ceramics added to ABC-containing rhBMP6 (Osteogrow-C; N = 5), and were euthanized 72 hours after implantation. Single-cell suspensions were labeled with anti-rat granulocyte-FITC, anti-rat CD11b-APC, anti-rat CD3-PE, anti-rat CD45RA-PECy7, anti-rat CD45-PE, anti-rat erythroid cells-PE, anti-rat CD31, purified anti-rat CD140a, anti-rat CD24-PECy7, and anti-rat CD90-APCCy7. In the second step, goat anti-rabbit IgG-AF488 was used. Frequencies of myeloid lineage cells among single, live hematopoietic (CD45+) cells were comparable across experimental groups. In contrast, frequencies of cells bearing early osteochondroprogenitor (CD90, CD24) and committed osteochondroprogenitor (CD140a) markers among single, live, non-hematopoietic (CD45–CD31–Ery–) cells were significantly higher in implants containing rhBMP6. CD90+ cells were further subdivided based on their expression of CD24. Frequencies of both the earliest multipotent progenitors (CD90+CD24+ cells) and adipogenic progenitors (CD90+CD24– cells) were significantly higher in implants with rhBMP6. In conclusion, the addition of rhBMP6 to ceramic implants significantly enhances the recruitment and differentiation of osteochondroprogenitor cells. This suggests that rhBMP6 plays a critical role in modulating the host cellular response, favoring the presence of multipotent progenitors, which may contribute to more effective bone regeneration. These findings support the potential of Osteogrow-C as a promising approach to improving bone healing and regeneration.

P-35-004
Novel aminoquinoline derivatives with potent anticholinesterase activity as potential symptomatic treatment for Alzheimer's disease

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Alzheimer's disease (AD) is the leading cause of dementia in the world, predominately affecting population over 65. The most prominent hallmarks of AD include deficiency of the acetylcholine and deposition of amyloid-β plaques and hyperphosphorylated neurofibrillary tangles in the brain. Treatments currently approved by FDA act towards alleviating symptoms of cognitive dysfunctions or target amyloid plaque build-up in the brain. Since the number of AD cases is forecasted to triple in the next 30 years due to general aging of human population, new possible treatments for AD are extensively researched. In this study, we have evaluated a series of 20 novel aminoquinoline derivatives with variable length of linker between aminoquinoline ring and terminal amino group with different substituents as potential inhibitors of human acetyl (AChE) and butyrylcholinesterase (BChE), as well as BACE1, a β-secretase involved in the production of self-aggregating amyloid peptides. All 20 compounds reversibly inhibit both cholinesterases, with inhibition constants ranging from 1 nM up to 1 μM. *In silico* calculated physicochemical properties of compounds proved that 7 compounds comply with Lipinski's values determined for orally bioavailable drugs, and 6 more compounds violate only one of recommended values, and therefore could be considered as potentially orally bioactive compounds. According to the pkCSM model, all compounds are predicted to be well-absorbed in human intestines (84–92%). Most potent cholinesterase inhibitors were tested as BACE1 inhibitors, but they did not display inhibitory activity higher than 6%. However, considering the remarkable anticholinesterase activity of certain compounds in subnanomolar concentrations, this series of aminoquinoline derivatives can be considered as promising scaffold for development of compounds to be used in symptomatic treatment for AD. Supported by CSF, Grant No. HrZZ-2020-02-9343 and the ERDF project KK.01.1.1.02.0007 "ReC IMI".

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P-35-005
Fluorinated selenoesters as novel compounds that inhibit the staphylococcal bifunctional enzyme AAC/APH which is essential for aminoglycoside-resistance

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Staphylococcus aureus infections can no longer be routinely treated with aminoglycosides due to high levels of antimicrobial resistance (AMR). Adjuvant therapy attempts to counter AMR by combining an antibiotic with a resistance enzyme inhibitor. The bifunctional AAC/APH enzyme fusion protein, comprising aminoglycoside acetyltransferase (AAC) and aminoglycoside phosphotransferase (APH), is a key player in *S. aureus* resistance to aminoglycosides, and so we assessed the ability of selenoesters (74 compounds) to inhibit this enzyme and reverse the resistance to aminoglycosides. Preceding high-throughput screening of potential adjuvants uncovered the synergic effect of selenoester compounds with tobramycin against MRSA clinical isolate *in vivo*. To confirm their specificity for the AAC/APH enzyme, an *E. coli* strain expressing AAC/APH was prepared by genetic engineering methods. A structure-activity relationship (SAR) study of the selenoesters was performed on the purified enzyme to determine their main features. Enzymological methods were used to study the mechanism of interaction between the enzyme and the most effective substance – fluorinated nitryl of selenoester. This inhibitor was revealed to be a competitive inhibitor of both AAC/APH domains with K_I in low micromolar range. Protein-ligand docking of selenoesters onto the protein was performed to investigate the accessibility of the binding site, aided by funnel-metadynamics simulations. Both domains of the enzyme were also separated to reveal the crystal structures with bound inhibitor. Thus, our results suggest that selenoesters are promising compounds for the adjuvant therapy of aminoglycoside-resistant staphylococcal infections and could even bring these antibiotics back into play for clinical practice. The project was financed by the National Institute of Virology and Bacteriology (EXCELES Program, Project ID No. LX22NPO5103) – Financed by the European Union – Next Generation EU.

P-35-006
Rubus ulmifolius fruits extract as potential multitarget agent for type 2 diabetes

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Type-2 diabetes (T2D) is characterized by persistent hyperglycemia, primarily resulting from impaired glucose metabolism due to enzymatic degradation of carbohydrates. This study explores the hypoglycemic effect of *Rubus ulmifolius* Schott (Rosaceae family) fruits extract, an evergreen shrub known for its traditional medicinal uses, both *in vitro* and *in vivo*. The antioxidant properties and inhibitory effects of the 80% ethanolic fruits extract on key enzymes involved in glucose metabolism, α-glucosidase and α-amylase, were investigated, with the extract proving to be significantly more effective than the commercial drug acarbose against α-glucosidase. Additionally, amyloid plaques formed by the aggregation of islet amyloid polypeptide (IAPP) are found in the pancreatic β-cells of over 90% of T2D patients. IAPP, normally released with insulin in response to elevated blood glucose, can form toxic fibrils under pathological conditions like T2D, leading to β-cell death. In this context, *R. ulmifolius* fruits extract demonstrated a strong capacity to inhibit IAPP aggregation, representing a novel therapeutic strategy for T2D management. Cellular studies revealed that the extract is non-cytotoxic and reduces reactive oxygen species (ROS) levels in Caco-2 cells. Furthermore, the extract was successfully incorporated into a liposomal system for *in vivo* testing, which showed a reduction in glucose levels in rats. Notably, the nanoformulation enhanced its hypoglycemic effect even at low doses. This study highlights *R. ulmifolius* as a promising natural therapeutic agent for T2D management, demonstrating not only significant enzyme inhibitory activity but also protective effects against β-cell dysfunction and oxidative stress, making it a valuable candidate for complementary therapy in T2D management and paving the way for the development of new strategies to improve glycemic control while preserving pancreatic function.

P-35-007
Assessing the therapeutic potential of *Phlomis crinita* extract-loaded nanoparticles for improved wound healing

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Skin injuries are a common health issue often requiring advanced wound care solutions. In this regard, the potential of plant extracts encapsulated in nanoparticles presents a promising approach for enhancing skin regeneration. This study focuses on assessing the therapeutic efficacy of *Phlomis crinita* extract-loaded nanoparticles, aiming to contribute to the development of improved wound healing strategies. Poly(lactic-co-glycolic acid)-polyethylene glycol nanoparticles (PLGA-PEG NPs) were synthesized using the solvent displacement method and underwent physicochemical and biopharmaceutical characterizations. These assessments involved the study of the release kinetics, the biomechanical properties and biocompatibility of the prepared formulations. The *in vitro* wound healing efficacy was validated through the scratch assay on 3T3-L1 cells. The PLGA-PEG NPs exhibited a highly negative zeta potential, indicating good stability, with a relatively small particle size. The release kinetics of PLGA-PEG NPs were characterized by a hyperbolic profile. Our data revealed that 3T3-L1 cells and HaCaT cells were able to tolerate the PLGA-PEG NPs formulation as no toxicity was observed along the experiment, proving the cytocompatibility of NPs formulation

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with skin cells. The nanoformulations revealed a remarkable increase in the wound closure percentage, after 24 h at low concentration, compared to control and free extract. After applying the nanoformulations on human skin, a decrease in transepidermal water loss (TEWL) and skin hydration (SCH) were observed, without compromising skin integrity. Moreover, there were no visible signs of skin irritation when PLGA-PEG NPs were applied on the volunteers' skin. The results obtained in this work suggest that the prepared PLGA-PEG NPs have great potential for dermal drug delivery showing no cytotoxic potential on skin cells and displaying an enhanced wound healing effect.

P-35-008
Binding, bridging, building: biparatopic antibody multi-format panel generation and binding characterisation against a soluble target antigen

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Biparatopic antibodies (bpAbs), a subset of bispecific antibodies, have emerged as a promising therapeutic modality, due to their unique ability to bind two distinct, non-overlapping epitopes of a target antigen. In this work, a panel of multi-format biparatopic antibody formats were generated and their biophysical parameters and early developability parameters evaluated. The developability of 1 + 2 and 2 + 2 format biparatopic antibodies is relatively understudied, with anticipated complexity in the analytical evaluation of these more complex formats. A large panel of > 100 biparatopic antibodies was successfully generated in 1 + 1, 1 + 2, and 2 + 2 stoichiometric formats, alongside the parental monospecific antibodies. These antibodies were analysed using liquid chromatography-mass spectrometry (LC-MS), analytical size exclusion chromatography (aSEC), and capillary gel electrophoresis (CGE), revealing challenges in confirming heterodimerisation for larger, tetravalent molecules and variable purity due to increased half-body presence. Surface plasmon resonance (SPR) revealed that changing stoichiometric format retains equivalent affinity to the epitope of the soluble antigen target. Furthermore, mass photometry and negative-stain electron microscopy (NS-EM) showed a diverse array of in-trans immune complex structures, a unique feature of biparatopic antibodies which cannot be achieved using monospecific antibodies alone or in combination. This research provides valuable insights into the potential of biparatopic antibodies as a novel therapeutic strategy and the utility of biophysical and structural analytical techniques to guide biparatopic antibody panel engineering.

P-35-009
Insights into the inhibition of ADAM10 and ADAM17 via *in-silico* methods

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ADAM10 and ADAM17 are type-I transmembrane proteases that regulate neuronal differentiation, immune responses, and synaptic plasticity via ectodomain shedding. Their targeted inhibition shows therapeutic promise in oncological and inflammatory disorders. Numerous X-ray crystallographic structures of ADAM17 incorporating inhibitor configurations exist. Conversely, the absence of inhibitor-bound X-ray structures for ADAM10 complicates *in-silico* analyses. To elucidate ADAM10 inhibition, the binding modes of the selective ADAM10 inhibitor GI254023X and the ADAM17 inhibitor TAPI-2 in both ADAM10 and ADAM17 were examined. The crystal structures of ADAM10 (PDB ID: 6BE6) and ADAM17 (PDB ID: 3EDZ) enzymes were prepared with Maestro 11.8. GI254023X and TAPI-2 were minimized with Avogadro. Docking studies were performed using AutoDock Vina. As a result of docking studies, it was determined that GI254023X showed bidentate binding to Zn^{2+} at its first position and was located at the S1′, S1 and S3 regions of the active site, unlike TAPI-2. The S1′ pocket of ADAM10 is notably deeper, favoring the accommodation of hydrophobic substituents. In contrast, the S1′ site of ADAM17 is narrower, making it more selective for aliphatic moieties (1). Hence, the phenyl ring of GI254023X positioned in the S1' region as expected. Its ethyl group confers sufficient conformational flexibility to facilitate its accommodation. It forms hydrogen bonds with residues E384 and A650, and polar interactions with H383, H393 (zinc-binding residues), and G329. Effective binding to ADAM17 requires Zn^{+2} binding capability, hydrogen bond acceptors for forming hydrogen bonds with the L348 and G349, and occupying S1' and S3′ binding regions (2). Unlike TAPI-2, GI254023X does not interact with Zn^{2+} or L348 at its first binding position. They both position at S1′ and S3′ region. References: 1. Seegar TC et al. (2017) Cell 171(7), 1638-48 2. Mazzola Jr RD et al. (2008) Bioorganic & medicinal chemistry letters 18(21), 5809-5814.

P-35-010
Antioxidant and antibacterial activity of Caucasian endemic medicinal plants

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Even though 700 species have been used for centuries in Georgian traditional medicine, the chemical composition and the antibacterial properties of Caucasian endemic medicinal plants remain unknown. In this study, antioxidant and antibacterial activities of leaf and root extracts of Caucasian endemic medicinal plants *Paeonia daurica* subsp. *mlokosewitschii* (Lomakin) D. Y. Hong, *Sempervivum transcaucasicum* Muirhead and *Thymus tiflisiensis* Klokov & Des.-Shost. were investigated. The antioxidant activity of plant extracts was determined using DPPH and total antioxidant capacity (TAC) assays. The highest DPPH scavenging activity and TAC were revealed for *Paeonia daurica* leaf extract. The highest phenol content was also found in *Paeonia daurica* leaf extract, while flavonoid content was the highest in *Paeonia daurica* and *Thymus tiflisiensis* leaf extracts. The analysis of the content of water-soluble antioxidants revealed the highest content of reduced glutathione in *Paeonia daurica* leaves and *Paeonia daurica* and *Sempervivum transcaucasicum* roots. The highest content of ascorbate was detected in *Paeonia daurica* leaf extract. The antibacterial activity

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of leaf and root extracts against *Escherichia coli* ATCC 25922 strain was investigated by disc diffusion method, and minimal inhibitory concentration (MIC) values were determined. The highest antibacterial activity was revealed for *Paeonia daurica* leaf and root extracts (MIC 2 mg/ml and 3 mg/ml, respectively). The antibacterial activity was lower for *Thymus tiflisiensis* leaf and root extracts (MIC 4 mg/ml and 6 mg/ml, respectively). The antibacterial activity against *E. coli* ATCC 25922 was not revealed for *Sempervivum transcaucasicum* leaf extract, while antibacterial activity was detected for the root extract (MIC 5 mg/ml). The results of the study demonstrate the potential of Caucasian endemic medicinal plants as natural antioxidant and antimicrobial agents. *The authors marked with an asterisk equally contributed to the work.

P-35-011
Synthesis and antiproliferative evaluation of isatin-derived fluorescence anticancer compounds

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Cancer continues to be an important global health problem with an increasing prevalence worldwide. According to WHO data for 2022, breast cancer, which has the highest prevalence worldwide, is also a crisis in Türkiye. An aggressive breast cancer type, triple-negative human breast cancer (TNBC) cell line MDA-MB-231 is resistant to hormone therapies as it is negative for estrogen, progesterone and HER2 receptors. In order to investigate the anticancer effects in 231 cell lines, new compounds with fluorescence and anticancer properties were synthesized. The synthesis was based on the conjugation of isatin and its derivatives, used as pharmacophores in many approved drugs, with 1,8-naphthalimide. All compounds in each reaction were characterized using TLC, FTIR, ^{1}H-NMR, ^{13}C-NMR spectroscopic techniques. In the first step of the study, the *in vitro* activity of the synthesized compounds was evaluated in the MDA-MB-231 cell line. Antiproliferative activities were evaluated using MTT [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide] assays to measure cell viability and IC_{50} values were determined. According to our first-stage data, methylisatin (54.09 μM), bromoisatin (68.55 μM), iodoisatin (69.90 μM) and chloroisatin (77.31 μM) derivatives were the compounds with the lowest to highest IC_{50} values at 24 h. At 48 h, methyloisatin, chloroisatin, bromoisatin and iodoisatin derivatives showed significant antiproliferative effect with IC_{50} values of 59.72 μM, 59.72 μM, 77.37 μM and 82.35 μM. The localisation of the substances with the highest anticancer effect in the cell was visualised by confocal microscopy. The new anticancer agents have the potential to be further developed as a therapeutic agent for TNBC. These findings suggest that targeting cell cycle regulation and apoptosis in the next steps of the study could be an effective strategy for the treatment of aggressive breast cancers.

P-35-012
Evaluation of the effects of sulfamethoxazole on the regenerative properties of human endothelial cells

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Sulfamethoxazole (SMX) is a cost-effective sulfonamide-derived antimicrobial drug used for both prevention and treatment of many bacterial infections for indications such as urinary tract infection, acute otitis media, treatment of shigellosis, approved by the FDA in combination with trimethoprim. Human umbilical vein endothelial cells (HUVECs) are derived from human umbilical cord and widely used in angiogenesis studies. Endothelial cells make it possible for newly formed tissues to grow and survive due to their special role in providing blood flow to tissues. Disruption of this condition negatively affects healing. The HUVEC is useful to study the effects of new drugs on human endothelium. In this study, cell viability and migration ability of the HUVEC cell line were examined *in vitro* following the administration of different concentrations of SMX followed by PCR assays. To examine the effects on cell viability, the HUVEC cell line was incubated with SMX at 1 μM, 10 μM, 50 μM, 100 μM, 250 μM, 500 μM and 1 mM doses for 24 hours. At the end of the period, MTT assay was performed to determine the effect on viability. An increase in viability ranging from 3% to 10% was observed at all doses compared to the control group. The migration ability of HUVEC cells after SMX administration was examined using 1, 50 and 100 μM doses. After 24 h, while 22% closure was observed in the control, approximately 38% (1 μM), 30% (50 μM), and 32% (100 μM) closure was observed in study groups. The evaluation of the effect of SMX (50 μM and 100 μM) on *COL1A1* gene expression resulted in down-regulation of *COL1A1* gene expression. This study was conducted to determine the effects of SMX on endothelial cells, which play an important role in wound healing process, in addition to its routine indications in clinic. It is important to elucidate the antimicrobial effects of SMX as well as its effects on cells in the same environment, in order to evaluate the safety profile of the drug.

P-35-013
Assessment of CMKLR1-mediated endosomal escape of cell-penetrating peptide conjugates

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Targeting of intracellular proteins as drivers of a variety of currently undruggable diseases is a major goal in modern drug delivery. However, these targeting approaches must be cell-selective to limit potential side effects. In the case of macromolecular drugs like peptide and proteins, bypassing the cell membrane is crucial to deliver these otherwise membrane-impermeable compounds[1]. Upon binding of their respective ligands, some G protein-coupled receptors undergo receptor-mediated endocytosis and can be utilized to address specific cells, which express the receptor, while cell-penetrating peptides (CPP) appear to be sufficient tools to overcome the endosomal membrane. Nevertheless, most CPPs are usually not cell-selective and must be modified to prevent unspecific internalization via

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the plasma membrane[2]. The cyclic chemerin-9 ligand (cChem9) is a proteolytically stable, potent ligand of the chemerin chemokine-like receptor 1 (CMKLR1), which was previously developed in our research group[3]. By combining cChem9 which a modified variant of the CPP Tat (trans-activator of transcription), we aim to construct a CMKLR1-selective shuttle for different peptide cargoes using solid-phase peptide synthesis. To assess the performance of our candidates, we investigate their capacity for CMKLR1-mediated endosomal escape by fluorescence microscopy, fluorescence correlation spectroscopy and a methotrexate-based cell viability assay in a HEK293 cell-model overexpressing the CMKLR1 and determine the efficacy of our CPP-masking approach. References [1] Li et al. (2025), J. Drug Target., 1–16. [2] De Jong et al. (2020), RSC Chem. Biol. 2020, 1 (4), 192–203. [3] Fischer et al., (2021), Cancers, 13 (15), 3788.

P-35-014
Allele-specific silencing of a dominant-negative BAG3 gene in myofibrillar myopathy 6

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Myofibrillar myopathies are group of rare genetic disorders characterized by impairment of striated muscle and neuron-muscle connections, leading to impaired muscle growth, movement difficulties, and potentially life-threatening complications. These conditions can be caused by mutations in genes essential for autophagy, resulting in the accumulation of protein aggregates in muscle cells, which disrupts cellular function. A key protein in this process, BAG3, plays a crucial role in degrading misfolded proteins. The p.P209L mutation (c.626C>T) in the *BAG3* gene leads to myofibrillar myopathy type 6 (MFM6), a fatal, ultra-rare disease affecting approximately 30 people worldwide. Symptoms include muscle weakness, respiratory difficulties, and heart complications, with no effective treatment currently available. Since the *BAG3* (c.626C>T) mutation is dominant, allele-specific therapy is needed to selectively target the mutated allele without affecting the healthy one. In our study, we used allele-specific siRNAs to silence the mutated allele in patient-derived fibroblasts. As the patient's fibroblast typically do not exhibit disease-related phenotypes, we induced protein aggregate formation by inhibiting the proteasome. While no protein aggregates were formed in control cells after proteasome inhibition, the BAG3 protein formed aggregates in the cells of MFM6 patients. In cells treated with siRNAs, BAG3 aggregates did not form. We confirmed that our designed siRNAs specifically silenced the mutated allele while preserving the function of the healthy one. Additionally, protein fraction analysis revealed a reduction in aggregated BAG3 after siRNA treatment. These findings demonstrate that allele-specific silencing of the BAG3 gene effectively reduces protein aggregation, offering new hope for patients with this rare disorder.

P-35-015
Boroxazolidones: dual enzyme inhibitors with antihypertensive potential – *in vitro* and *in vivo* evaluation

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Systemic arterial hypertension is a chronic health condition and a major risk factor for cardiovascular diseases (CVDs), including heart failure, coronary artery disease, and myocardial infarction. Current treatments primarily involve angiotensin-converting enzyme (ACE) inhibitors such as captopril, enalapril, and lisinopril. However, these medications often cause adverse effects, including persistent cough, skin reactions, and angioedema, highlighting the need for alternative therapeutic options. Building on recent findings from our research group regarding the antihypertensive potential of boroxazolidones, this study aimed to design and synthesize a series of boroxazolidone derivatives from α-amino acids and assess their inhibitory activity against ACE and acetylcholinesterase (AChE) through *in silico*, *in vitro*, and *in vivo* studies. Although boron-containing compounds were once considered toxic in medical applications, they are now widely explored for their therapeutic potential, including their use as antibiotics, antiseptics, and anticancer agents. Three promising boroxazolidone derivatives (BXZ-Lys, BXZ-His, and BXZ-Arg) were identified through *in silico* screening as potential ACE and AChE inhibitors. Following their synthesis, *in vitro* assays confirmed their inhibitory activity at micromolar concentrations. These compounds were then further evaluated *in vivo* using spontaneously hypertensive rats, both in acute and chronic administration protocols. BXZ-Lys and BXZ-Arg demonstrated a significant reduction in systolic, diastolic, and mean arterial blood pressure, exhibiting greater potency than the commonly used ACE inhibitor captopril. These findings highlight the potential of boroxazolidones as novel antihypertensive agents. Further studies are required to elucidate their precise mechanism of action and explore their broader therapeutic applicability.

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P-35-016
Discovery and biological validation of a novel small-molecule inhibitor of Drp1-mediated mitochondrial fragmentation

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Mitochondria are crucial organelles involved in energy production, apoptosis, mitophagy, calcium homeostasis, and redox signaling. They dynamically alter their morphology via tightly regulated fusion and fission mechanisms essential for adapting to cellular stress. Dysregulation of mitochondrial dynamics, particularly increased fission mediated by dynamin-related protein 1 (Drp1), significantly contributes to various pathologies, including cancer, diabetes, and neurodegenerative disorders. Elevated Drp1 expression promotes mitochondrial fragmentation, enhancing apoptosis resistance, proliferation, and therapeutic resistance in cancer cells. Similarly, altered Drp1 levels correlate with disease progression in diabetes and neurodegeneration. In this study, we discovered a novel small-molecule inhibitor targeting the Drp1-GTPase interaction, aiming to prevent mitochondrial fission. Approximately 3.6 million compounds were screened virtually, and selected candidates were validated experimentally. The inhibitory effects were evaluated in A549 cells (cancer), 1.1B4 cells (diabetes), SH-SY5Y cells (neurodegenerative diseases), and BEAS-2B cells as a healthy control. Confocal and fluorescence microscopy analyses confirmed the inhibitor's effectiveness in reducing disease-associated mitochondrial fragmentation without negatively affecting healthy cells. Functional analyses included mitochondrial membrane potential, oxygen consumption rates, reactive oxygen species production, cell viability, and apoptosis assays. Additionally, protein-protein interaction analyses revealed the binding kinetics of the inhibitor with Drp1. Our findings highlight the therapeutic potential of specifically targeting the Drp1-GTPase interaction to modulate mitochondrial dynamics, positioning this inhibitor as a promising candidate for treating conditions characterized by excessive mitochondrial fragmentation, including cancer, diabetes, and neurodegenerative diseases.

P-35-017
A novel thromboxane receptor antagonist, Ctkf, mitigates vascular injury-induced thrombosis and restenosis

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Neointima formation and atherosclerosis are proliferative vascular diseases driven by dysregulated vascular smooth muscle cell (SMC) proliferation. Thromboxane receptors (TP) play a key role in platelet activation, endothelial dysfunction, vasoconstriction, and SMC proliferation, all contributing to cardiovascular diseases. Increased TP expression has been observed in the intimal layer of blood vessels in individuals at high cardiovascular risk. In this study, we identified a novel natural benzimidazole derivative, Ctkf, which effectively blocks U46619-induced platelet aggregation, suggesting that Ctkf may serve as a new TP receptor antagonist. Furthermore, we investigated whether Ctkf also offers protective effects against thrombosis and restenosis following vascular injury. To evaluate the cardiovascular protection potential of Ctkf, we examined its protective effects in FeCl –induced vascular injury and balloon injury-induced neointima formation animal models. Ctkf significantly inhibited the TP agonist U46619-induced platelet aggregation and reduced fluorescent dye-induced platelet-rich thrombus formation in mesenteric venules. Unlike aspirin, Ctkf did not prolong bleeding time, suggesting a safer antithrombotic profile. Moreover, Ctkf effectively suppressed thrombus formation and neointima development in vascular injury models. Our findings demonstrate that Ctkf, a novel TP receptor antagonist, is a promising therapeutic candidate for thrombosis-related diseases, including atherosclerosis, stroke, and restenosis.

P-35-018
Enhancing MC4R selectivity of optimized setmelanotide variants by structure-activity relationship studies

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The melanocortin 4 receptor (MC4R) has a crucial role in the leptin-melanocortin pathway, regulating energy balance by suppressing food intake and promoting energy expenditure upon activation [1]. However, dysfunction of this pathway, primarily due to insufficient MC4R activation, significantly contributes to obesity-related health issues, frequently manifesting in severe early-onset obesity [2]. In 2020 the U.S. Food and Drug

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Administration granted approval for setmelanotide, a peptidic MC4R agonist, to counteract insufficient MC4R activation caused by genetic disorders upstream in the pathway. In contrast to the endogenous ligands, setmelanotide selectivity activates the $G\alpha_{q/11}$ pathway and the $G\alpha_s$ pathway with high potency [2]. This distinct agonistic behavior seems to promote the therapeutic effect in facilitating weight loss and reducing hunger levels in patients [2]. However, long-term treatment with setmelanotide leads to skin darkening and hyperpigmentation, which is attributed to its affinity at the melanocortin 1 receptor (MC1R) [3]. The lack of subtype selectivity of setmelanotide presents challenges for patient acceptance, particularly in young children. Hence, we aim to develop setmelanotide variants with enhanced MC4R-selectivity and $G\alpha_{q/11}$ bias. Combining solid phase peptide synthesis and structure activity relationship studies, we identified key positions in the peptide that influence selectivity toward either MC1R or MC4R. Moreover, mutagenesis studies allowed to systematically analyze differences in the binding pockets of both receptors and to investigate unique interaction points for subtype selectivity. These findings provide deeper insights into MCR subtype selectivity and support the refinement of setmelanotides signaling portfolio for improved therapeutic outcomes. References: [1] Walley AJ et al, Nat Rev Genet, 2009, vol. 10, 431–442. [2] Clément K et al, Nat Med, 2018, vol. 24, 551–555. [3] Kanti V et al, Skin Pharmacol Physiol, 2021, vol. 34, 307-316.

P-35-019
Synthesis and biological evaluation of new coumarin-phthalonitrile derivatives as monoamine oxidase inhibitors

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Monoamine oxidase-A (MAO-A) and monoamine oxidase-B (MAO-B) are enzymes targeted in the treatment of some neurodegenerative diseases such as Alzheimer's (AD) and Parkinson's diseases (PD). AD and PD involve complex pathological mechanisms characterized by neuronal degeneration and specific protein aggregates. AD is primarily marked by the presence of amyloid plaques in the spaces between nerve cells, whereas PD is characterized by decreased dopamine (DA) levels in neuronal synapses. In this study, a series of coumarin-phthalonitrile derivatives were synthesized, characterized, and evaluated for their inhibitory activity on MAO-A and MAO-B for treatment of AD and PD. Among the investigated compounds, 4-[7-oxy-3-phenylcoumarin]phthalonitrile (K1) selectively inhibited the MAO-B activity with an IC_{50} value of 80 nM (MAO-B specific Selectivity Index (SI) is 1250). Additionally, the mechanism and the reversibility of this inhibition were further analyzed. Molecular docking studies were performed to predict the binding modes of the most active compounds. The results suggest that coumarin-phthalonitrile derivatives could serve as promising candidates for further development of selective MAO-B inhibitors targeting neurodegenerative disorders, including AD and PD.

P-35-020
Antiviral efficacy of essential oils and their constituents

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The spreading of novel viruses and increasing environmental awareness have put plant-based extracts, such as essential oils (EOs), and their components in the focus of alternative antiviral agent research. EOs are hydrophobic liquid concentrates of plant secondary metabolites, mainly terpenes and phenylpropanoids. The antiviral efficacy of most EOs has not yet been extensively investigated, with most research being focused on their action against enveloped viruses. Since most EO components are volatile, these substances have the potential to be applied in the vapor phase, an approach only scarcely studied. In this work, the antiviral properties of eight EOs (those of lemon myrtle, lemongrass, palmarosa, lavender, coriander, mandarin, lemon verbena, and tea tree) as well as their principal components were investigated. The yeast *Saccharomyces cerevisiae* virus L-A (ScV-L-A), belonging to the Orthototiviridae family, was utilized as a safe non-enveloped virus model system. The composition of EOs was analyzed by GC-MS, the antiviral effect of tested substances in their liquid and vapor phases was evaluated by observing the synthesis of radio-labeled viral transcripts by affected ScV-L-A viral particles. Citral-rich EOs were determined to exert the most potent antiviral effect in both liquid and vapor phases. Mandarin EO and its major constituent, limonene, displayed a pronounced effect only in the vapor phase. In most cases, EOs were found more efficacious than their pure primary components. Additionally, TEM imaging was employed to investigate the effect of selected substances on ScV-L-A viral particle integrity. The results of this study add to the growing knowledge of the antiviral properties of EOs and their constituents, which are necessary for the effective practical application of these substances for combating viral threats. This study has received funding from the Research Council of Lithuania (LMTLT), agreement No S-MIP-24-55.

P-35-021
CNS-active, uncharged bis-oximes afford neuroprotection in mice exposed to organophosphate compounds

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Cationic pyridinium oximes are used as antidotes in cases of organophosphorus (OP) nerve agent and pesticide poisoning, reactivating inhibited acetylcholinesterase (AChE) and butyrylcholinesterase (BChE). OP compounds induce neurotoxicity by phosphorylating the catalytic Ser, which prevents neurotransmitter acetylcholine catalytic hydrolysis and causes overstimulation of cholinergic receptors. Oximes were identified as possible OP poisoning antidotes in the 1950s when their nucleophilic potential that enables displacement of the

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phosphoryl moiety was discovered. However, pyridinium-based oximes used in clinical practice worldwide are ineffective in recovering activity of the synaptic AChE in the brain, as permanently charged compounds have difficulty penetrating the blood–brain barrier (BBB). The goal of this research was to determine *in vitro* and *in vivo* antidotal potential of uncharged but ionizable oximes, equilibrated between the neutral species that traverse BBB, and charged species that act as reactivator. The tested bis-oximes exhibited efficient *in vitro* reactivation comparable or better than the standard oxime 2-PAM, for both AChE and BChE inhibited by nerve agents, sarin, cyclosarin, VX, paraoxon, and insecticide fenamiphos. Cytotoxicity assays showed no impact of acetamide bis-oximes on the viability of human neural (SH-SY5Y) and hepatic (HepG2) cell lines up to 1.6 mM concentration within 24 hours. Compared to 2-PAM, the bis-oximes demonstrated improved protective indexes and less acute toxicity in a mouse model. One of the bis-oximes restored up to 70% of AChE activity in the blood and brain of mice exposed to nerve agent sarin. Therefore, our results emphasize zwitterionic bis-oximes as a promising new therapeutic alternative to pyridinium oximes, which possess capacity to reactivate brain AChE and improve treatment in cases of OP poisoning. This research was supported by the CounterACT Program, NIH OD, and the NINDS (Grant Number 1R21NS120884-01A2).

P-35-022
Protein denitration by tyrosine-containing peptides is a new strategy to treat intractable diseases including depression, epilepsy, hyperammonemia, cognitive impairment, and acute kidney injury

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Studies have frequently reported that key regulatory proteins lose their functions in many intractable diseases because of tyrosine (Tyr, Y) nitration, which can cause other diseases or worsen the existing disease. Many attempts have been made to identify druggable substances to restore Y-nitrated proteins to denitrated forms and thus produce active proteins, but no remarkable result has been found. Based upon our recent findings that chronic stress decreased glutamine synthetase (GS) activity via Y-nitration and that Y supplementation restored its activity, we determined whether Y and Y-containing peptides had a denitration effect on several regulatory proteins including GS, manganese-superoxide dismutase, copper/zinc superoxide dismutase, catalase, and HSP60. After confirming the denitration effect of Y and Y-containing peptides, we evaluated the clinical availability using well-established intractable disease mouse models of depression, mild cognitive impairment, epilepsy, hyperammonemia, and acute kidney injury. Interestingly, Y or tyrosyl glutamine (YQ) treatment showed remarkable therapeutic effects on each disease. Although the disease-causing nitrated proteins were different for each disease, Y or YQ treatment showed denitrative effects on such proteins and activated enzymes, resulting in therapeutic efficacy. These results, to the best of our knowledge, are for the first time in drug development history and will make a way to overcome intractable diseases.

P-35-023
Coumarin-phthalonitrile derivatives as potent inhibitors of D-amino acid oxidase

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D-amino acid oxidase (EC 1.4.3.3, DAAO) is a flavo-dependent oxidative stereospecific enzyme that is involved in the oxidative deamination of D-amino acids such as D-serine and D-alanine. These D-amino acids act as potential co-agonists of the N-methyl-D-aspartate (NMDA) receptor and stimulate the NMDA receptor by binding to the glycine binding site. Thus, D-amino acid oxidase regulates the function of this receptor through these D-amino acids, and since the NMDA receptor is associated with various diseases such as schizophrenia and Alzheimer's disease, the discovery of new DAAO inhibitors is an active field of research. Coumarins are synthetic or natural compounds with the benzo-α-pyrone ring system and are generally known for their low toxicity and high biological activity. Phthalonitrile is an organic benzene derivative that is white and crystalline at room temperature. By derivatizing these structures, it is possible to synthesize molecules with a wide range of effective biological activity potential. In this study, four coumarin-phthalonitrile derivatives were synthesized and characterized by their spectral data. The effect of these compounds on porcine kidney D-amino acid oxidase was spectrofluorimetrically investigated using D-serine as substrate and Amplex Red® as fluorophore. Amongst the studied compounds, 3-[7-oxy-3-phenylcoumarin] phthalonitrile showed the highest inhibitory activity with an IC_{50} value of 10.18 ± 0.356 μM. The reversibility and mechanism of the inhibition by this derivative was also investigated. The results suggest that coumarin-phthalonitrile derivatives could be novel scaffolds for the discovery of new DAAO inhibitors.

P-35-024
Evaluation of compounds as multitarget therapeutics in neurological disorders

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Alzheimer's disease is a neurological disorder which according to cholinergic hypothesis is described with low levels of neurotransmitter acetylcholine leading to cognitive decay. However, Alzheimer's disease is a complex condition involving various factors as the accumulation of Aβ plaques, oxidative stress, tau protein hyperphosphorylation, NMDA receptor

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overstimulation, and elevated MAO-B enzyme activity, that can contribute to neuron loss and connectivity issues in the brain together with acetylcholine accumulation. This complexity calls for the development of multitarget-directed ligands to address multiple aspects of the disease. In our study, we investigated heterostilbene derivatives as potential drugs for the central nervous system, focusing on their ability to inhibit cholinesterases. These compounds were designed as analogs of resveratrol, a polyphenol known for its antioxidant, anti-inflammatory, and neuroprotective properties, which have shown promise in treating various mental disorders including major depressive disorder, bipolar disorder, and Alzheimer's disease. Among the tested compounds, 10 showed potential as selective inhibitors of cholinesterase enzymes, with dissociation constants (Ki) ranging from 7 to 68 μM. Furthermore, we evaluated the effects of these compounds on pathways related to oxidative stress and cell death in neural and hepatic cell lines. Remarkably, the tested compounds did not induce significant toxicity in cells up to 200 μM within 24 hours. This allowed us to assess their antioxidant capacity by measuring their ability to modulate induction of reactive oxygen species in *in vitro* conditions. With this research we aimed to identify promising candidates for further development of more effective drugs to treat advanced stages of Alzheimer's disease. This research was supported by University of Zagreb, the Croatian Science Foundation (IP-2022-10-6685), ERD Fund (KK.01.1.1.02.0007), Next Generation EU (Class: 643-02/23-01/00016).

P-35-025
Design, synthesis and biological evaluation of novel proteolysis targeting chimeras (PROTACs) targeting human D-aspartate oxidase (hDASPO) based on the structure of olanzapine

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D-Aspartate oxidase (DASPO) plays a key role in breaking down D-amino acids, particularly D-aspartate (D-Asp), which can activate *N*-methyl-D-aspartate (NMDA) receptors. Impaired NMDA receptor-mediated neurotransmission is linked to several psychiatric disorders, including schizophrenia. Therefore, inhibiting DASPO could elevate D-Asp levels in the brain, potentially enhancing NMDA receptor function and offering therapeutic advantages. Recently, the antipsychotic drug olanzapine was identified as a mild DASPO binder (IC_{50} = 23.4 μM), leading to increased extracellular D-Asp levels in the prefrontal cortex [1]. Based on these findings, we aimed to apply proteolysis targeting chimeras (PROTAC) technology to selectively degrade DASPO using bifunctional molecules derived from the olanzapine structure [2]. PROTACs function by promoting the proteasomal degradation of target enzymes within cells, thereby increasing intracellular D-Asp concentrations. This novel approach could provide a potential therapeutic strategy for conditions associated with D-Asp deficiency. A series of new bifunctional compounds were designed using the olanzapine core, incorporating aliphatic, amide, or polyethylene glycol (PEG)-ylated linkers, with lenalidomide and VHL ligands as E3 ligase binders. The compounds were efficiently synthesized via simple Click chemistry techniques, and initial biological evaluations revealed that the first PROTAC, MGA26, exhibited potent DASPO inhibition (IC_{50} = 4.13 μM) and influenced cellular DASPO levels. References: [1] Sacchi *et al.* [(2017) Sci. Rep. 7(1), 46288. [2] Wang *et al.* [(2022) Eur. J. Med. Chem. 235, 114290.

P-35-026
The inhibitory effect of gaylussacin, a stilbene glycoside, on the development of chronic obstructive pulmonary disease in mice

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Chronic obstructive pulmonary disease (COPD) is one of the main causes of human death worldwide. Emphysema is among the main symptoms of COPD, which is characterized by an abnormal enlargement of airspaces and widespread destruction of lung parenchyma, resulting in a significant reduction in gas exchange. Currently available therapies for treating COPD mainly offer symptomatic relief and preservation of lung function, whereas curative treatment options are limited. Therefore, it is necessary to develop novel therapeutic and/or preventive agents for COPD. This study demonstrates the inhibitory effect of the stilbene glycoside gaylussacin on elastase-induced emphysema in mice. Administration of gaylussacin significantly reversed elastase-induced destructive changes in the lungs of mice, which was accompanied by alleviation of elastase-induced matrix metalloproteinase activity and apoptosis in the lungs. Moreover, gaylussacin displayed minimal cytotoxicity in normal cell lines derived from various organs and caused no significant changes in body weight or serum aspartate aminotransferase and alanine aminotransferase levels in mice. These results indicate the novel biological activity of gaylussacin with respect to suppression of elastase-mediated pulmonary emphysema with no overt toxicity and highlight the potential utility of gaylussacin as a preventive or therapeutic agent for the treatment of COPD.

P-35-027
Dual action of CSA-13 in combating bacterial infection and suppressing inflammation during acute pyelonephritis – implications for urinary tract infection therapy

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Pyelonephritis, often originating from ascending urinary tract infections (UTIs), presents significant challenges due to its progression to the kidneys and associated collecting systems. *Escherichia coli*, a primary pathogen in acute pyelonephritis

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(APN), contributes to severe cases leading to urosepsis and increased mortality rates despite antibiotic treatment. The spreading of multidrug-resistant *E. coli* strains further complicates therapeutic options. While antibiotics administration remains the primary treatment, anti-inflammatory adjunctive agents are necessitated due to an excessive inflammatory response in APN. This study assessed the potential of ceragenins, particularly CSA-13, in ameliorating inflammation associated with APN. Preliminary, minimum inhibitory concentration (MIC) and minimum bactericidal/fungicidal concentration (MBC/MFC) assays were conducted against clinical strains of *E. coli*, *Klebsiella pneumoniae*, and *Candida glabrata* to assess the antimicrobial potential of CSA-13 against UTI-causing pathogens. To assess the antimicrobial and anti-inflammatory properties of ceragenins in a murine model of APN induced by *E. coli*, female NUDE mice were intravesically inoculated with *E. coli* and subsequently treated intravenously with fluorescent-labeled CSA-13 (CSA-13-IRDye800CW). Microbial load in urine and kidneys, visceral index, hematological examination, and serum cytokine analysis were also performed. CSA-13 displayed significant antimicrobial activity and reduced renal inflammation in APN, demonstrating high renal tissue affinity. The efficacy of CSA-13 in attenuating renal inflammation in APN using a murine model is demonstrated, shedding light on its therapeutic potential and suggesting novel treatment avenues.

P-35-028
Spatial immunolocalization of key markers in Osteogrow-C implants with different size ranges of calcium phosphate ceramic particles

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Osteogrow-C is a novel device for bone regeneration, consisting of recombinant human bone morphogenetic protein 6 (rhBMP6), and autologous blood coagulum as a carrier with the addition of calcium phosphate synthetic ceramic particles. Bone tissue engineering involves understanding cellular and molecular interactions between biomaterials and host tissues. In this study, immunohistochemical analyses were performed to investigate the spatial expression of key signaling and cell surface markers in different formulations of Osteogrow-C implants containing synthetic ceramic particles of varying sizes (small: 74-420 μm; medium: 500-1000 μm; large: 1000-4000 μm), with and without rhBMP6. Different formulations of Osteogrow-C were implanted subcutaneously in the axillary region of rats. Tissue samples were collected at 3- and 5-days post-implantation and analyzed for Smad1/5/8, CD90, PDGFR, Syndecan, GDF5, CD45, CD44, CD24, LeptinR, Smad2, FGF2, and NCAM1 expression. Heatmap visualization of histological sections allowed for spatial comparison of marker expression within distinct implant regions: periphery, inter-particle spaces, and ceramic surfaces. The most notable findings included significantly higher expression of Smad1/5/8, CD90, PDGFR, and Syndecan in the inter-particle spaces and on the surfaces of small ceramic particles compared to medium and large particles, suggesting differential cell recruitment and signaling activation. The presence of rhBMP6 influenced marker distribution, further emphasizing its role in early osteogenic differentiation. These findings highlight the importance of ceramic particle size and signaling environment in modulating early tissue responses, which may inform future biomaterial design for enhanced bone regeneration.

P-35-029
Dose optimization of MO-2097, a novel chiral-free HIF-1α inhibitor, against BALB/c mice xenograft model using MATLAB-based integrated pharmacokinetics-pharmacodynamics (PK-PD) model

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An integrated pharmacokinetics-pharmacodynamics (PK-PD) model is useful for predicting and optimizing doses and administration intervals to produce the drug's efficacy. Recently, MO-2097, a novel chiral-free HIF-1α inhibitor derived from moracin-O as a natural product, was found to have efficacy and biocompatibility for inhibiting accumulation of hypoxia-inducible factor HIF-1α, by suppressing the hnRNPA2B1 protein in a BALB/c mouse xenograft model. A One-compartment model in a PK aspect, the tumor growth in BALB/c mouse xenograft model was explained by a three-compartment transit model. This model assumed that the MO-2097's efficacy against tumor growth inhibition can be indicated by the constant parameter, 1.01 L/mg/day of k2, which is an indicator of drug efficacy, and systemic exposure (AUC), which act as proportional factors to reduce tumor growth. Tumor cell death through three stages influenced by the rate constant 1.13/day of k1, which represents the dynamics of cell death. Among various dosage regimens simulated by the developed PK-PD model of MO-2097, dosage regimen of MO-2097 at 100 mg/kg, administered intraperitoneally once daily for 2 weeks was suggested considering pharmacological efficacy with little systemic accumulation. These results might contribute significantly to MO-2097's treatment strategy and new drug development.

P-35-030
Carborane-tagged peptidomimetics as potent inhibitors of cysteine cathepsins: a 3D aromatic approach to targeted drug design

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The closo-1,2-C2B10H12 carborane was used as a 3D aromatic icosahedral building block with an unusual electron distribution governed by the presence of the outer hydridic BH and acidic CH vertices, resulting in an unusually large dipole moment. This carborane cage was attached via a B-S bond to an active-site peptidomimetic ligand of cysteine cathepsin proteases. The inhibitory potency of the carborane-tagged compound was compared to its 2D aromatic conventional phenyl analog. Enzymological data showed that the carborane cage at the P3 position enhanced the inhibitor efficacy against several cathepsin B- and L-type enzymes, with a prominent contribution to the

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inhibition of SmCB1, an established cathepsin B1 drug target in the *Schistosoma mansoni* parasite. X-ray crystallography of the inhibitor-SmCB1 complex, complemented by quantum mechanical computations revealed that in addition to a dense network of non-polar contacts, the carborane tag provides a favorable interaction between the positive end of the carborane dipole moment and the negatively charged S3 subsite in the enzyme's active site. Finally, the increased affinity of the carborane-tagged inhibitor for SmCB1 resulted in specific anti-schistosomal activity. In conclusion, the presented functionalization of the carborane cage can be exploited for the tagging various bioactive peptidomimetics.

P-35-031
Serum paraoxonase-1 activities and their relationship with serum oxidized high-density (oxHDL) and low-density (oxLDL) lipoprotein levels in subjects with dyslipidemia

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Dyslipidemia is one of the most important risk factors for atherosclerosis. While oxidized low-density lipoprotein (oxLDL) shows a close relationship with development of atherosclerosis, high-density lipoprotein HDL shows anti-atherogenic properties mainly with its reverse cholesterol transport mechanism and antioxidant enzyme activities including the paraoxonase-1 (PON-1). In the present study, we aimed to investigate the relationship between serum PON-1 levels and its enzyme activities and serum oxidized HDL (oxHDL) levels in subjects with dyslipidemia. The study group consisted of 160 subjects with normolipidemia (n = 40) and with dyslipidemia (hypercholesterolemia; n = 40, hypertriglyceridemia; n = 40 and mixed dyslipidemia; n = 40). OxLDL, oxHDL and PON-1 levels were determined by ELISA. PON-1 enzyme activities including paraoxonase, lactonase and arylesterase were measured by spectrophotometric methods. OxLDL and oxHDL concentrations were found to be at the highest level in mixed dyslipidemia ($p < 0.05$). The arylesterase enzyme activity was found to increase with total cholesterol ($p < 0.05$). In the total study group (n = 160), oxLDL levels were positively correlated with total cholesterol (r = 0.52, p = 0.0001), triglyceride (r = 0.35, p = 0.0001), low density lipoprotein (r = 0.40, p = 0.0001) and oxHDL (r = 0.24, p = 0.003) levels. In hypercholesterolemic subjects, oxHDL levels were negatively correlated with specific paraoxonase enzyme activity (r = -0.46, p = 0.0001), specific arylesterase enzyme activity (r = -0.62, p = 0.0001) and specific lactonase enzyme activity (r = -0.54, p = 0.0001). As a result, HDL-C, PON-1 levels and PON-1 enzyme activities of hypertriglyceridemic individuals were more significantly affected than those of hypercholesterolemic subjects. It was concluded increased oxHDL, decreased PON-1 levels and its activities may be associated with HDL dysfunction, and these resulted in increased serum oxLDL concentrations in dyslipidemia. It may be related to development of atherosclerosis in dyslipidemia. *The authors marked with an asterisk equally contributed to the work.

P-35-032
Monobody-based antivirals for broad-spectrum RNA virus neutralization: screening for potential cytotoxicity and off-target effects

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The emerging need to overcome the shortcomings of current antiviral therapeutics prompts the need to develop innovative strategies, such as protein-based biopharmaceuticals, that can be effective against broad spectrum of viruses to prevent and respond to viral pandemics. With that in mind, we are developing a platform for the discovery, development, production and validation of evolvable and rapidly adaptable protein-based antivirals under the Horizon project EvaMobs. As a proof of concept, this platform will be applied to four RNA viruses with high societal impact: SARS-CoV-2, RSV, influenza A and Zika. This platform uses computational methods to design monobodies, which are small proteins (~90 amino acid residues) derived from the fibronectin type III domain, tailored to bind to viral surface proteins and neutralize a given viral agent. To ensure their safety and drugability, we conducted a comprehensive preliminary screening for potential cytotoxicity and off-target effects of monobodies designed for SARS-CoV-2 and influenza H3N2. Cytotoxicity was tested in HepG2, SH-SY5Y, HEK293, PBMC cells after 24 h exposure. Off-target interactions were evaluated following acetylcholinesterase activity (synaptic enzyme known to interact with peptide-based xenobiotics), while the immune response was analyzed using PBMC cytokine release assays. Preliminary results indicate that the tested monobodies did not influence significantly any of the parameters evaluated in the *in vivo* application relevant range (approx. nanomolar to low micromolar). However, buffer composition for monobodies preparation played a significant role for establishing reliable testing on cells, and HEPES was pointed as the buffer of choice. These findings confirm the favorable safety profile of computationally designed monobodies as antiviral candidates and provide a solid foundation for their further development as next-generation broad-spectrum antivirals.

P-35-033
Exploring piperazine-based compounds as potential radioprotective agents

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Radiotherapy is a crucial tool in both cancer treatment and medical imaging; however, exposure to ionizing radiation is not limited to clinical settings. Radiation risks also arise from nuclear accidents and radiation-based weaponry, posing

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significant threats to human health. A major challenge in these scenarios is mitigating radiation-induced damage to healthy tissues, driving the need to develop effective radioprotective agents. This study focuses on the design, synthesis, and evaluation of novel piperazine derivatives as potential radioprotective agents to overcome the limitations of current countermeasures. A series of 1-(2-hydroxyethyl)piperazine derivatives was synthesized and their cytotoxicity tested for in various human cell lines. Their radioprotective properties were further assessed in the MOLT-4 lymphoblastic leukemia cell line and peripheral blood mononuclear cells (PBMCs) exposed to gamma radiation. To quantify their ability to reduce DNA damage, the dicentric chromosome assay (DCA) and apoptosis detection were employed. Among the synthesized compounds, compound 6 demonstrated the most potent radioprotective effects *in vitro*, with minimal cytotoxicity. Compound 3 also exhibited notable efficacy, particularly in reducing dicentric chromosomes, suggesting its potential in mitigating DNA damage. Both compounds showed enhanced safety and effectiveness compared to amifostine, highlighting their promise as improved radioprotective agents. Based on the obtained findings, the mentioned compounds served as molecular templates for the synthesis of a new series of substances which show enhanced radioprotective properties. Acknowledgements: This work was supported by grant project NU23-08-00256 of the Ministry of Health.

P-35-034
Unlocking PD-1/PD-L1 axis in non-small cell lung cancer: *in silico* design of tetrahydroquinoline-based small-molecule immune checkpoint inhibitors

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The programmed cell death protein-1 (PD-1)/PD-L1 immune checkpoint axis is a critical therapeutic target in non-small cell lung cancer (NSCLC); however, treatment resistance and high costs continue to limit the effectiveness and accessibility of current therapies. Development of small-molecule inhibitors targeting the PD-1/PD-L1 interaction offers a promising approach to enhancing efficacy and accessibility. Tetrahydroquinoline (THQ) derivatives, known for their immunomodulatory and apoptosis-inducing effects, have emerged as compelling candidates for dual checkpoint inhibition due to their broad biological activity and role in modulating key cellular processes, making them an attractive scaffold for anticancer drug development. This study employs a structure-based drug design approach to develop novel THQ-based PD-1/PD-L1 inhibitors. Molecular docking (Schrödinger 2020-3, Glide XP) and MM-GBSA calculations identified five leads with superior ΔGbind values ranging from (PD-L1: -28.04 to -49.82 kcal/mol; PD-1: -3.08 to -46.11 kcal/mol), significantly outperforming BMS-200 (ΔGbind = -2.42 for PD-1; -7.54 for PD-L1). Key interactions involve hydrogen bonding with PD-L1's Lys124 and hydrophobic contacts near Tyr56, alongside analogous interactions with PD-1, reinforcing the dual affinity of THQ derivatives. Guided by these computational insights, chemical synthesis of selected THQ derivatives is currently in progress using optimized synthetic methodologies. To further assess their therapeutic potential, future studies will focus on ADME profiling, molecular dynamics simulations, kinetic and thermodynamic evaluations of PD-1/PD-L1 inhibition, and *in vitro* validation of lead compounds in NSCLC cells (A549). This work aims to advance the development of next-generation small-molecule immune checkpoint inhibitors, bridging the gap between computational drug discovery and translational oncology. *The authors marked with an asterisk equally contributed to the work.

P-35-035
Identification of potent inhibitors of Zika and Dengue viruses proteases from small-molecule compound libraries

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Zika virus (ZIKV) is a flavivirus transmitted by *Aedes* mosquitoes, responsible for outbreaks of zika fever, a disease that can result in severe neurological complications such as congenital Zika syndrome and Guillain-Barré. Despite its re-emerging potential, there are no approved antivirals or vaccines against the virus. The NS2B-NS3 protease (NS2B-NS3^{pro}) plays a critical role in processing the viral polyprotein and is a promising drug target. Here, we conducted a high-throughput screening (HTS) of 12 000 compounds from three small-molecule libraries (ReFRAME, HGL1, and HGL2) against the recombinant ZIKV NS2B-NS3^{pro}. Eleven compounds inhibited the enzyme by > 80% at 10 μM and six exhibited a concentration-dependent effect with IC_{50} values in the micro to submicromolar range. Those compounds were further evaluated against the viral replicon and three showed antiviral activities resulting in selectivity indices up to 5.2. Additionally, differential scanning fluorimetry (DSF) revealed varied thermal stability effects, supporting direct protein-ligand interactions. To assess cross-inhibition potential, the most potent ZIKV protease inhibitors were tested against the NS2B-NS3^{pro} from Dengue virus (DENV) serotypes 1 to 4. Four compounds inhibited DENV1 protease, while Compound 1 was active against all tested serotypes. Our findings provide valuable insights for antiviral drug discovery targeting flavivirus proteases, with Compound 1 emerging as a promising lead for further optimization.

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P-35-036
GalNAc-siRNA targeting CNNM4 as a novel therapy for liver disease

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Dysregulation of magnesium homeostasis plays a crucial role in liver diseases such as metabolic dysfunction-associated steatotic liver disease (MASLD), alcoholic liver disease (ALD), fibrosis, and cholangiocarcinoma (CCA), contributing to mitochondrial dysfunction and endoplasmic reticulum (ER) stress. CNNM4, a key magnesium transporter, has been implicated in these pathologies, regulating metabolic activity and cellular stress responses. Targeting CNNM4 represents a promising strategy to restore liver homeostasis. We developed a GalNAc-conjugated siRNA against CNNM4 (GalNAc-siRNA-CNNM4), designed for liver-specific delivery to efficiently silence CNNM4 expression. Our preclinical models demonstrate that GalNAc-siRNA-CNNM4: restores magnesium balance, improving mitochondrial respiration and ATP production; reduces ER stress, preventing UPR activation and hepatocyte apoptosis; attenuates fibrosis, decreasing collagen deposition and hepatic stellate cell activation; suppresses tumor progression in CCA, enhancing sensitivity to chemotherapy. Our findings, based on previous studies in MASLD (J Hepatol, 2021; PMID: 33571553), drug-induced liver injury (DILI) (Nat Commun, 2022; PMID: 36433951), ALD (Hepatology, 2024; PMID: 39641635), and CCA (Gut, accepted), highlight CNNM4 as a key regulator in liver disease. The combination of GalNAc-siRNA technology enables highly specific hepatic targeting, optimizing therapeutic efficacy while minimizing off-target effects. This study establishes CNNM4 silencing via GalNAc-siRNA as a novel approach to treat chronic liver diseases by modulating mitochondrial function and ER stress.

P-35-037
Self-associating supramolecular Evans blue as drug nanocarrier in immunotargeting, which interacts with model drug doxorubicin and model immune complexes

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Supramolecular ribbon-like structures (SN) interact with proteins by penetrating transiently accessible regions due to structural rearrangements related to the protein's function, enhancing irreversible (e.g., immune complexation) processes. This study explores an SN-based drug carrier using Evans blue (EB) bound with model immune complexes. doxorubicin (Dox) binds to EB via intercalation, enabling targeted transport and release. Heat-aggregated immunoglobulins (HAI) served as a model system to examine SN-protein interactions. In HAI, heating at 63°C induces structural distortions similar to those in antibodies upon antigen binding, enabling SN binding. The aims were: 1) to describe the possibility of binding EB-Dox to HAI; 2) to determine the carrier-drug ratio at which the drug is completely bound and the carrier retains supramolecular properties and is completely bound to the model immune complex; 3) to describe the possibility of effective releasing drug from EB-Dox. Electrophoresis, chromatography, spectrophotometry, and dialysis were used for the analysis of EB and EB-Dox binding to HAI. The results showed: 1) that the higher the concentration of EB, the larger the ribbon aggregates formed; 2) the binding of free EB and EB-Dox to HAI depending on the concentration. The optimal proportion of HAI-EB-Dox complex formation was selected. The results were compared with the previously studied SN system: CR and a weaker binding of EB to HAI than CR was observed, which, however, may be beneficial for efficient drug release. The drug binding site in the HAI-EB complex was identified. An analysis of the release of Dox bound via the carrier to the immune complex model was also carried out. The presented research is essential for the investigation of optimum concentration for the application of EB-Dox in immunotargeting techniques on cell cancer models. Funding sources: project (no. U1C/P04/NO/02.24) in the strategic program Initiative of Excellence at the Jagiellonian University. *The authors marked with an asterisk equally contributed to the work.

P-35-038
TRPA1 channel activation prevents pro-fibrotic responses in human lung fibroblasts

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Lung fibroblasts are one of the main players triggering airway remodeling in the course of many chronic lung diseases. They respond to various humoral (primarily growth factors, cytokines, and chemokines) and mechanical factors, as well as certain extracellular matrix proteins, which are secreted in diseased lung tissue. Under the influence of the above, lung fibroblasts switch to a profibrotic phenotype manifested primarily by increased proliferation, migration, contraction, and fibroblast to myofibroblast transition (FMT). Limiting pro-fibrotic responses of lung fibroblasts is currently one of the most interesting directions in the development of new drugs for respiratory diseases. Recent studies indicate that the transient receptor potential ankyrin 1 (TRPA1) channel may be an interesting molecular target in airway remodeling therapy. However, it remains unclear whether activation or inhibition of this channel is better in terms of anti-fibrotic effects. The aim of our work was to investigate the effect of three TRPA1 channel activators (two novel triazine-based agonists – compound I and II as well as the reference agonist – compound ASP-7663) on transforming growth factor type β (TGF-β)-induced pro-fibrotic responses in human lung fibroblasts (MRC-5 cell line). All tested TRPA1 activators enhanced calcium fluxes in MRC-5 cells. They were also able to diminish TGF-β-induced FMT as well as lung fibroblasts contraction. We demonstrated a limiting effect of the tested TRPA1 agonists on TGF-β-induced profibrotic genes expression, increased level of selected myofibroblast markers and collagen gel contraction in MRC-5 cultures. The obtained results

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indicate that the use of TRPA1 channel agonists may be useful in limiting several lung fibroblast pro-fibrotic responses. This confirms that TRPA1 channel activation represents an interesting direction for the development of novel drugs for airway remodeling in respiratory diseases.

P-35-039
Rational design of green-inspired biomedical nanoplatforms stabilized by novel sorbitan esters with polyunsaturated fatty acid (PUFA) chains as an effective tool in delivery issues

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The goal of current experiments on the border of pharmacy, colloidal chemistry, and the chemical industry is to implement strategies for the research and development of more sustainable products with specific features and functionality. In the present study, a process has been designed and optimized to obtain novel green surfactants based on sorbitan esters with polyunsaturated PUFA acids in the promising design of biocompatible nanoplatforms of high colloidal stability with significant pharmaceutical application potential as delivery systems for active substances. Extensive structural analysis was carried out, including elemental analysis and spectroscopic techniques such as nuclear magnetic resonance (NMR) and mass spectrometry (MS). Chromatographic methods, i.e. Flash chromatography, thin-layer chromatography (TLC), were used to obtain high-purity compounds. To explore the application potential of the desired nanoplatform, advanced "soft" colloidal nanocarriers of emulsion type based on the obtained "green" sugar esters with a varied structure of PUFAs were characterized by dynamic and electrophoretic light scattering techniques. Significant differences in nanoemulsion droplet size and polydispersity with predicted effects on stability were observed. This, combined with the biodegradable and biocompatible nature of the proposed emulsifiers, represents a novel and prospective group in the targeted delivery and controlled release of active substances in nanomedicine. This research represents an important and promising scientific innovation in the effective stabilization of "soft" colloidal nanocarriers with biomedical and pharmaceutical potential and is a groundbreaking alternative in the constantly growing interest in modern, bioavailable, non-toxic surfactants as the next generation of industrial "cleavable" emulsifiers and solubilizers offering low environmental impact. Research supported by WUST.

P-35-040
Investigation of antioxidant and antidiabetic activities of propolis obtained from different locations of artvin

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Diabetes is a common chronic disease in the society and studies are being carried out to find active substances that can be used in its treatment. This study was carried out to investigate the antioxidant properties and antidiabetic activities of Artvin propolis. Ethanol extracts of propolis obtained from six different regions of Artvin (Center, Bağlıca, Alabalık, Kabaca, Şavşat, Ardanuç) were prepared by the maceration method. Total polyphenol content of the extracts was determined by the Folin-Ciocalteu method, total flavonoid content by the aluminum chloride colorimetric method and antioxidant capacity by iron reducing power determination and DPPH (2,2-diphenyl-1-picrylhydrazyl) methods. In the antidiabetic activity method, IC_{50} values of alpha amylase and alpha glucosidase enzyme inhibition were determined as mg/mL. Total polyphenol contents, total flavonoid contents, iron reducing power and DPPH, alpha glucosidase enzyme inhibition, alpha amylase enzyme inhibition IC_{50} values, when compared with other samples in the world, show that the propolis ethanol extracts of the studied region have high bioactivity. At the same time, there are differences between the propolises of the Artvin region in terms of these parameters. It was determined that the antioxidant and antidiabetic activities of propolis changed in six different regions, and the highest values in all analyzes were observed in the propolis obtained from the Artvin center. It is thought that the difference observed in the results is due to the different flora of the regions where the propolis samples were collected, the specific locations of the hives and the propolis accumulation period of the bees. It is also known that there are more than 300 different compounds detected in propolis. The high bioactivity of the propolises of the Artvin region, which has a flora with a high endemic plant content, is due to the valuable polyphenolic compounds in its content.

P-35-041
Repurposing SP600125 as a novel inhibitor of proneural-mesenchymal transition in glioblastoma through connectivity map analysis

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Cancer drug development is costly, slow, and has a low success rate. Repurposing existing drugs offers a promising alternative by finding anti-tumor activity in approved compounds, reducing preclinical/safety expenses. This study aimed to identify anti-glioblastoma agents via repurposing, targeting the proneural-mesenchymal transition (PMT), a key regulator of tumor invasion. RNA sequencing data from patient tumors (TCGA-GBM) and glioblastoma cells (GSE192710) were reanalyzed. WGCNA, gene set variation analysis (GSVA), and differential expression analysis identified 160 genes linked to PMT. These genes were used to construct a protein–protein interaction network, identifying 30 hub genes critical for PMT regulation based on six centrality criteria: MCC, Degree, MNC, EPC, Closeness, and Radiality. The Connectivity Map platform identified 120 compounds inhibiting hub gene expression. Chemoinformatic analysis (PreADMET, AlzPlatform, ADMETlab, SwissADME, LiverTox) identified five compounds – SP600125, dibenzoylmethane, FG-7142, vemurafenib, fensuximide – as PMT inhibitors predicted to cross the blood–brain barrier and avoid P-glycoprotein efflux. Network pharmacology analysis revealed the strong connectivity of SP600125 within the hub gene network. *In vitro* experiments confirmed PMT-inhibitory activity of SP600125. SP600125 dose-dependently reduced viability in U87, U118, and EPNT-5

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glioblastoma cells. SP600125 pretreatment (20 μM) inhibited U87 cell migration in wound healing assays and suppressed TGF-β1 or CoCl2-induced PMT. This was evidenced by reduced morphological changes, decreased transwell migration, and lower expression of mesenchymal markers (fibronectin, N-cadherin, Slug). Taken together, SP600125 is a promising PMT inhibitor to suppress glioblastoma aggressiveness and enhance therapy effectiveness. Research funded by Russian Science Foundation (RSF) grant No. 23-14-00374.

P-35-042
Novel terpene-based inhibitors of glial-mesenchymal transition as promising anti-glioblastoma drug candidates

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Glioblastoma multiforme (GBM) is a highly malignant cancer with an extremely poor prognosis. The 2-year post-treatment survival rate of GBM patients does not exceed 15% due to the ability of GBM cells to undergo glial-mesenchymal transition (GMT), which promotes diffuse tumor cell growth, increased clonogenicity, and the development of drug resistance. In this study, the pharmacological potential of two series of chemical derivatives of isoalantolactone (IAL) and 18βH-glycyrrhetinic acid (GLA) against key GMT-related processes was evaluated. *In vitro* screening of IAL derivatives identified blood–brain barrier (BBB)-permeable pat651, which effectively inhibited clonogenicity, adhesion, motility and invasiveness of U87 glioblastoma cells and showed high synergy with temozolomide. Network pharmacology analysis revealed that the anti-GMT potency of pat651 was associated with its direct interaction with Hsp90α. Among GLA derivatives, soloxolone *para*-methylanilide (SMA) was found to effectively inhibit transforming growth factor-beta (TGF-β)-induced GMT both *in vitro* and *in vivo* through direct interaction with TGF-β type I and type II receptors. SMA inhibited morphological changes, motility, invasion, and expression of mesenchymal markers in TGF-β-induced GBM cells and xenograft GBM tumors and significantly inhibited the stemness of glioblastoma cells. Taken together, the developed terpene-based small inhibitors of GMT showed promising potential as novel components of combination therapy of GBM. This work was supported by the Russian Science Foundation (Grant No. 23-14-00374).

P-35-043
Development of novel P-glycoprotein inhibitors based on pentacyclic triterpenoids

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Multidrug resistance (MDR) remains one of the most significant challenges in cancer chemotherapy, primarily due to the overexpression of ATP-binding cassette (ABC) transporters, which pump chemotherapeutic drugs out of tumor cells. Among these, P-glycoprotein (P-gp) plays a crucial role in reducing drug sensitivity, making it a key target for MDR reversal. However, the clinical application of P-gp inhibitors is limited by their toxicity and side effects, necessitating the search for novel alternatives. Pentacyclic triterpenoids have emerged as a promising class of bioactive compounds due to their structural diversity and biocompatibility. In this study, we identified and characterized two novel P-gp inhibitors: N,N-dimethylamine-containing soloxolone amide (1) and *meta*-pyridine-containing oxadiazole of 18βH-glycyrrhetic acid (2). Molecular docking simulations revealed their strong binding affinity to the P-gp transmembrane domain. Functional *in vitro* assays in P-gp overexpressing human cervical carcinoma KB-8-5 and murine lymphosarcoma RLS40 cell lines demonstrated that these compounds significantly increased intracellular accumulation of rhodamine-123 (Rho123) and doxorubicin (DOX), thereby enhancing the cytotoxic effect of chemotherapeutic agents. Importantly, these effects were not associated with downregulation of P-gp expression. Further mechanistic studies suggest that compound 1 function through competitive and non-competitive inhibition of P-gp mediated DOX and Rho123 efflux, leading to drug retention. The ability of these compounds to enhance drug accumulation without affecting normal cellular function highlights their potential as effective MDR modulators. So, our findings pave the way for the development of pentacyclic triterpenoid-based inhibitors as adjunctive agents in chemotherapy, offering a novel strategy for overcoming drug resistance in aggressive malignancies. This research was supported by the Russian Science Foundation, grant No. 23-14-00374.

P-35-044
Advancing 3-substituted quinuclidine quaternary ammonium compounds (QACs): mechanisms and biodegradability

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Quaternary ammonium compounds (QACs) derived from 3-substituted quinuclidine serve as an alternative platform for next-generation antibacterial agents developed according to the principles of natural product synthesis. Recent research has focused on optimizing their structure-activity relationships (SAR) to increase potency, reduce bacterial resistance, and improve biodegradability and safety profile. We reported several series of QACs derived from the heterocyclic quinuclidine scaffold containing different substituents at the C3 ring position. Antibacterial potency increased with alkyl chain length, with some candidates exhibiting bactericidal activity exceeding that of commercial QACs. The highest efficacy was observed with structurally optimized 3-aminoquinuclidine QACs, where increased hydrophobicity and a protonated amino functional group effectively mimicked a second positive charge, enhancing bacterial membrane interactions. In addition, these novel QACs exhibited reduced cytotoxicity against animal cells, highlighting their potential for therapeutic applications. In contrast to conventional QACs, which primarily disrupt bacterial membranes, the reported "soft" QACs showed a dual antibacterial mechanism combining bacteriostatic effects with

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inhibition of protein synthesis. Molecular dynamics simulations revealed that these compounds adopt "hook-like" conformations that restrict deep penetration into membranes, reducing cytotoxicity while maintaining antibacterial efficacy. Future research will focus on new biodegradable "soft" QACs through amide bond introduction and subsequent hydrolysis to reduce persistence of these derivatives in the environment. These results position 3-substituted quinuclidine QACs as next-generation antimicrobials with improved efficacy, safety and sustainability.

P-35-045
Preparation and biophysical characterization of lipid nanoparticles encapsulating mRNA for vaccine delivery applications

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Lipid nanoparticles (LNPs) are currently the leading delivery platform for mRNA-based vaccines and therapeutics due to their ability to protect and efficiently deliver nucleic acids into target cells. In this study, we designed and characterized LNPs encapsulating mRNA (LNP-mRNA), using a lipid composition based on Moderna's Spikevax® mRNA vaccine. LNPs were prepared by thin-film hydration of lipids followed by extrusion to achieve uniform nanoscale particles. Incorporation of mRNA was performed during the hydration step in an acetate buffer. The resulting LNPs were characterized by dynamic light scattering and electrophoretic light scattering to determine their hydrodynamic size and zeta potential. The average diameter of empty LNPs was approximately 114 nm, while mRNA encapsulation increased the size to 134 nm, with both formulations exhibiting a zeta potential of + 32 mV, indicating good colloidal stability. The structural organization and membrane dynamics of LNPs were further evaluated by electron paramagnetic resonance spectroscopy using spin-labeled fatty acids, which confirmed preserved bilayer fluidity and stability after mRNA incorporation. Additionally, fluorescently labeled LNP-mRNA nanoparticles were prepared to enable future cellular uptake studies. These results demonstrate the successful formulation of stable mRNA-loaded LNPs with biophysical properties suitable for further therapeutic development and functional testing. Authors are thankful for the financial support by the Ministry of Science, Technological Development and Innovation, Contract numbers 451-03-137/2025-03, 451-03-136/2025-03, and the Science Fund of the Republic of Serbia, PROMIS REDIRECT, Contract number 11132. *The authors marked with an asterisk equally contributed to the work.

P-35-046
The anti-mitotic potential of vincamine targeting spindle microtubules

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Natural compounds isolated from plants are commonly used as drugs to improve health or to treat diseases. Indole alkaloids isolated from *Vinca* species, like vinblastine and vincristine, are currently used in anti-cancer therapy approaches as they interfere with the formation of microtubules and thus mitotic spindle assembly. Another *Vinca* alkaloid, vincamine, is currently used as a food supplement due to its antioxidant and antihyperlipidemic effects. Recent studies, however, attributed some anti-proliferative activity to vincamine as well. We hence sought to test whether vincamine, like vinblastine, also interferes with microtubule dynamics and mitotic spindle assembly using *in silico* docking simulations, *in vitro* reconstitution assays and *in vivo* tests on mitotic RPE1 cells. While docking simulations suggest a strong interaction between vincamine and tubulin, we show that vincamine might have a stabilizing effect on reconstituted microtubules *in vitro* as well as spindle microtubules *in vivo*, leading to spindle deformation and defects in mitosis. These data underline the emergent anti-proliferative and anti-mitotic potential of vincamine and warrants further studies that will have to test whether vincamine induced spindle defects are sufficient to induce apoptosis in cancer cell lines.

P-35-047
Investigation of the potential impacts of probiotic administration on drug metabolism and therapeutic efficacy in C57BL/6 mice

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Probiotics are live microorganisms proven to have numerous positive effects on human health and are commonly consumed as beneficial aids and extensively used as nutritional supplements. They are also advised for the treatment or prevention of certain disorders. Consequently, their concurrent administration with other medications is occasionally indispensable. Literature concerning the impact of probiotics on the efficacy or safety of medications is limited. In this context, this study investigates the capacity of a novel probiotic *Liquorilactobacillus nagelii* (LN), isolated from turnip juice, on the hepatic drug metabolizing enzymes. The LN probiotic was intragastrically administered to 8–10-week-old female C57BL/6 mice at 108 CFU for 21 days. The expression at the mRNA level was then assessed by RT-PCR in the liver tissues of control and LN probiotic-administered mice. The expression levels of genes that play role in drug metabolism such as CYP3A11, CYP2C37, CYP2D9, and CYP2E1 were determined. We have observed that the expression levels of these genes were reduced 2.08, 1.62, 1.62, and 1.79 times respectively in the probiotic-administered group compared to control. In addition, CYP3A4 enzyme activity levels further support this finding that CYP3A11 dependent erythromycin N-demethylase activity was reduced 25.23% in the probiotic-administered group. Although further studies are required, these preliminary findings indicate that the LN probiotic, which belongs to the *Lactobacillus* genus, may affect the pharmacokinetics and pharmacodynamics of commonly utilised drugs through drug metabolizing enzymes.

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P-35-048
Exploring affibodies as a strategy against β2-microglobulin aggregation

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Beta-2 microglobulin (β2m) is a small protein essential for immune system regulation, serving as the invariant subunit of the Major Histocompatibility Complex I. While monomeric β2m remains stable under normal conditions, elevated local concentrations or specific mutations can cause protein misfolding, leading to amyloid fibril formation. The prolonged accumulation of these fibrils contributes to the onset and progression of various chronic and autoimmune diseases. Therefore, stabilizing the native state of β2m could be a crucial step in preventing disease progression and promoting recovery. To achieve this, small, customizable affinity proteins known as affibodies are employed. These affibodies, derived from the scaffold of *Staphylococcus aureus* protein A, are highly soluble and environmentally stable. In this study, we analyzed the binding capability of three affibodies specifically designed to target β2m, along with a fourth affibody targeting a different protein as a control. Alphafold3 simulations suggested that one affibody successfully binds β2m, while results for the other two remain inconclusive, and no binding is expected for the control affibody. Following expression and purification, β2m and affibodies were incubated to facilitate complex formation. Initial binding assessments were conducted using size-exclusion chromatography, followed by confirmatory analyses through isothermal titration calorimetry and microscale thermophoresis. The results revealed dissociation constants in the nanomolar to micromolar range for two of the complexes. Additionally, thioflavin T (ThT) assays were performed to assess whether affibody binding could reduce β2m aggregation. A significant reduction in aggregation was observed upon incubation with one of the affibodies. Overall, these findings indicate that affibodies can bind β2m *in vitro* with high affinity and effectively reduce its aggregation rate.

P-35-049
Human trypsin-isoenzyme inhibitors from Finnish cyanobacteria

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Proteases play important roles in all living organisms. About one third of the over 500 known human proteases are serine proteases. These include trypsins, which are among the best characterized proteases. Among three different human trypsin-isoproteases, trypsin-3 appears to play an important role in the metastatic spread of cancer. Recently selective targeting of trypsin-3 has been shown to reduce the cancer cell invasion. However, due to high sequence and functional similarity, it is difficult to produce selective trypsin-3 inhibitors. Cyanobacteria produce potent inhibitors of trypsins. Here we tested the selective inhibition of three human trypsin-isoproteases using crude extracts of 505 strains, belonging to 19 different genera of cyanobacteria isolated from Baltic Sea and Finnish lakes. Extracts of 88 strains (17.4%) showed strong inhibition of one or more trypsin-isoproteases. The strains producing these inhibitors belonged especially to *Microcystis*, *Nodularia*, *Nostoc* and *Planktothrix* genera. Extracts of 13 strains showed selective inhibition activity. Of these, 3 preferentially inhibited trypsin-2 and -3 and 10 trypsin-1 and -2. Up to now, we have isolated and characterized trypsin inhibitors among aeruginosin family of cyanobacterial peptides (suomilide and varlaxins) and a dipeptide called radiosumin C. The most potent of these inhibited human trypsins with subnanomolar IC_{50} values. Interestingly, these peptides showed different selectivity profiles for inhibiting different human trypsin-isoproteases. Suomilide also inhibited the invasion of aggressive and metastatic PC-3M prostate cancer cells without effecting the cell proliferation. These results show that cyanobacteria are rich source of protease inhibitors with interesting selectivity profiles. The inhibitory peptides we have isolated and characterized may serve as leads for development of selective and potent trypsin inhibitors.

P-35-050
Improving the bioavailability of silymarin using beta-glucan particles

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Silymarin, a polyphenol complex with significant pharmacological effects, suffers from low bioavailability, a standard limitation of many natural compounds. One practical approach to enhance its delivery is using drug carriers like β-glucans, which can improve silymarin's cellular uptake. Due to specific receptors on the surface of monocytes, β-glucans can effectively penetrate cells, making them an ideal carrier for silymarin. The anti-inflammatory, antioxidant, and cytotoxic effects of β-glucan carriers encapsulating silymarin and propolis were assessed and compared to the activity of the pure extracts. Cytotoxicity was evaluated on embryonic kidney cells (HEK 293) and human macrophages (THP-1) using the resazurin assay. Anti-inflammatory effects were measured by quantifying IL-6, NO, and TNFα release in RAW 264.7 macrophages via ELISA and Griess reagent assay. The antioxidant effect was assessed by monitoring cellular oxidative activity in RAW 264.7 cells, while the antimicrobial potential was tested against *Staphylococcus aureus* and *Candida albicans*. Our findings indicate that the β-glucan carriers significantly enhanced the anti-inflammatory effects of both silymarin and propolis while also improving the antioxidant potential of propolis. However, a slight increase in cytotoxicity, particularly in monocytes, was observed. The non-toxic glucan particles inhibited IL-6 release and demonstrated strong inhibitory properties against the microorganisms tested. This study highlights the potential of β-glucan carriers in improving the bioavailability and therapeutic effectiveness of silymarin and propolis. This work was funded by the Grant Agency of the Czech Republic (GACR), No. 25-15246S.

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P-35-051
Bismuth-based metal-organic frameworks for drug delivery: biocompatibility and brain endothelial cell uptake studies

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Neurodegenerative disorders pose a major challenge due to the difficulty of delivering therapeutics across the blood–brain barrier (BBB), emphasizing the need for effective drug carriers. Metal-organic frameworks (MOFs) have emerged as promising candidates due to their tunable porosity, stability, and drug-loading capabilities. In this study, a bismuth naphthalenedicarboxylate (BiNDC) MOF and a europium-doped BiNDC (EuBiNDC) MOF were synthesized and functionalized with polyethylene glycol (PEG 8000) or fructose 1,6-bisphosphate (FDP) to enhance colloidal stability. Structural and elemental analyses confirmed the successful formation of these MOFs, and scanning electron microscopy (SEM) revealed nanometric, rod-like morphologies. To evaluate their drug-loading potential, Rhodamine B adsorption studies were performed, showing that BiMOF-FDP exhibited a higher adsorption capacity, whereas BiMOF-PEG demonstrated greater cumulative release over time, suggesting different drug delivery profiles. The europium-doped MOF was further investigated for its cellular uptake in human brain microvascular endothelial cells (HBMECs) using confocal microscopy. Imaging confirmed the internalization of EuBiNDC MOFs within the cytoplasm without nuclear penetration, supporting their potential for safe intracellular drug delivery. Additionally, the biocompatibility of BiMOF-PEG and BiMOF-FDP was assessed via MTT assays at concentrations ranging from 5 to 75 μg/ml over 24, 48, and 72 hours. Both MOFs were well tolerated at lower concentrations, but a dose- and time-dependent reduction in cell viability was observed at higher doses, indicating the need for further optimization. These findings highlight the potential of bismuth-based MOFs as promising drug delivery platforms for neurodegenerative disease treatment. *The authors marked with an asterisk equally contributed to the work.

P-35-052
The WOW-Effect in nanomedicine: towards engineering of biocompatible water-in-oil-in-water (W/O/W) emulsions for advanced drug delivery

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Theragnostic approaches integrate diagnostics and therapy, offering solutions for various cancers. This study investigates water-in-oil-in-water (W/O/W) emulsions as stable nanocarriers for simultaneous drug and marker encapsulation, enabling both therapeutic delivery and bioimaging. The aim is to improve emulsion stability and examine how phase ratios and surfactant concentrations influence physicochemical properties. To assess the internal environment and structural integrity, fluorosolvatochromic dyes were used as polarity-sensitive probes and fluorescent agents. For long-term colloidal stability, we employed nonionic surfactants POE (15) cocoamine and Sorbitan esters (e.g., Sorbitan monooleate), ensuring a well-preserved multiphasic nanostructure. Dynamic (DLS) and electrophoretic (ELS) light scattering confirmed nanoscale particle size, narrow distribution (polydispersity index < 0.3), and stable zeta potential. Adjusting system parameters – organic to aqueous phase ratio (1:4, 1:5, 1:6), stabilizer type, and concentration (0.5%, 1%) – demonstrated a clear impact on physicochemical properties (e.g. size and surface charge), enabling precise nanocarrier tailoring and use W/O/W emulsion as a base formulation for various applications. Using fluorosolvatochromic dyes with varying hydrophobicity as a model cargo, UV-Vis spectroscopy verified successful solubilization and allowed real-time micropolarity monitoring within the emulsion system. This insight provided structural validation of the integrity of the nanoplatform and confirmed its potential for solubilization of both diagnostic and therapeutic agents. Due to its multiphasic W/O/W architecture, the nanoplatform enables controlled bioactive compound release while reducing toxicity and enhancing efficacy. These findings establish the foundation for further research in nanomedicine, including the encapsulation of biomolecules for targeted therapies. The work was supported by the project Minigrants for doctoral students of the WUST.

P-35-053
Drug repositioning for kinase inhibition: preventing inflammatory responses

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Receptor-interacting serine/threonine-protein kinase 1 (RIPK1) is a master regulator of the cellular decision between survival and death as well as inflammation in response to a broad set of stimuli. The inhibition of RIPK1 kinase activity has shown efficacy in animal models of human diseases including autoimmune diseases and neurodegenerative diseases. The ability to develop selective small-molecule kinase inhibitors of RIPK1 are the major factors that have contributed to RIPK1's prominence as a therapeutic target, due to the presence of a unique kinase-regulating allosteric pocket. Nevertheless, there is still no therapeutic inhibitor of RIPK1 to date. Therefore, we screened a collection of FDA-approved drug and ongoing clinical trials drug to identify structurally like Necrostatin-1 (Nec-1), which has been widely used to inhibit the kinase activity of RIPK1. This led to the discovery of Phensuximide as a potent inhibitor of RIPK1. The activation of RIPK1 as indicated by the phosphorylation of serine 166 of RIPK1, was abolished by Phensuximide. Furthermore, we show that Phensuximide can protect against systemic inflammatory response syndrome (SIRS), which is an animal model of sepsis, involving RIPK1 kinase activity, Phensuximide (SIRS) RIPK1.

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P-35-054
Pore-forming peptides: approaches to overcome antibiotic resistance

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The management of bacterial infections is becoming a major clinical challenge due to the rapid evolution of antibiotic-resistant bacteria. Pore-forming antibiotics have shown significant efficacy against pathogens resistant to conventional antibiotics, but the mechanisms underlying their action remain a matter of debate. To examine the toxicity of pore-forming peptides melittin, mastoparan, and alamethicin, derived from bee and hornet venoms and fungi, respectively, we used tightly coupled rat liver mitochondria as a simple and readily available test system. Mitochondria were employed as biosensors to measure transmembrane currents induced by pore-forming peptides. Based on experimental data, for studied pore-forming peptides two mechanisms of their self-association in the mitochondrial inner membrane differing in limiting stages of pore formation and impact on the transmembrane potential were proposed. The peptides were divided into two groups: moderately and radically affecting the transmembrane potential. Most likely that transmembrane potassium current induced by mastoparan and melittin was limited by self-association of monomers into a non-conducting pre-pore, while the current induced by alamethicin was limited by pore formation. We also proposed that the pore formed by low-oligomeric alamethicin in the inner mitochondrial membrane may be toroidal, containing dimerized peptide and lipid. The size of pores formed by peptides was also determined. Thus, using the test system applied it was possible to quickly assess the toxicity of these compounds, recommend the use of non-toxic concentrations and get closer to understanding their conformation and mechanism of action. *The authors marked with an asterisk equally contributed to the work.

LB-R-35-01
Physical surface modification of inhalable dry powder for nose-to-brain delivery based on design of experiments (DOE)

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Nose-to-brain (N2B) drug delivery provides a promising alternative for bypassing the blood-brain barrier (BBB), facilitating direct transport of therapeutic agents to the central nervous system (CNS). The intranasal administration mechanism facilitates efficient deposition on the nasal mucosa, with drug absorption occurring via the olfactory and trigeminal nerve pathways, enabling rapid and direct access to the brain. In particular, physically surface modified inhalable dry particles including surface coated and/or porous particles have emerged as promising carriers for nose-to-brain (N2B) delivery due to their physicochemical properties and enhanced drug absorption efficiency. Several biocompatible carbohydrate-based excipients are extensively used as a matrix material for inhalable dried powder. This study focuses on the development of a dry powder inhaler (DPI) formulation of model drug optimized for N2B delivery. Utilizing jet milling technology, the formulation achieves precise particle size distribution (PSD) and improved flow properties through the incorporation of lactose as a base excipient and lubricants such as magnesium stearate (MgSt) and L-leucine (L-leu). In addition, this study aims to optimize porous particle fabrication using spray drying by employing a design of experiments (DOE). Comprehensive characterization of the formulation was performed using analytical techniques including PSD, differential scanning calorimetry (DSC), thermogravimetric analysis (TGA), Fourier transform-infrared (FT-IR) spectroscopy, dynamic vapor sorption (DVS) and screening of penetration enhancers to enhance nasal drug permeability. The results demonstrated optimal particle characteristics, enhanced thermal stability, and minimal moisture uptake, supporting the feasibility of this DPI system. The results demonstrated optimal particle characteristics, enhanced stability and nasal membrane permeability, supporting the feasibility of this DPI system for effective N2B delivery.

LB-R-35-02
The effects of empagliflozin on angiogenesis

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Empagliflozin (EMPA) is a selective sodium-glucose cotransporter 2 inhibitor used as antihyperglycemic agent. Evidence also suggests its cardiovascular protective effects. In cardiovascular diseases, therapeutic angiogenesis is vitally valuable as it promotes the growth of new blood vessels from pre-existing ones to transport oxygen and nutrients to tissue. Diabetic neuropathies are important complications of diabetes and angiogenesis is required for wound healing. However, studies about the effects of EMPA on angiogenesis are limited. Our preliminary study showed that EMPA induces hydrogen sulfide (H_2S) formation, which is an angiogenic gasotransmitter [Papapetropoulos A et al. (2009) Proc Natl Acad Sci USA 106, 21972–21977]. Therefore, this study aimed to investigate the effects of EMPA on angiogenesis via CAM assay. Fertilized chicken eggs (n = 8 per concentration) were obtained and incubated. Whatman filter disks with vehicle or EMPA (5, 10, and 100 μM) were applied to CAMs on day 8. Prior to application, 4 mL of albumen was removed on day 5, and shell windows were created to expose the CAMs. After 24-hour incubation, vascularity was scored (0-4) in a blinded manner. Non-viable or abnormal embryos were excluded. One way ANOVA results showed that EMPA has a biphasic, concentration-dependent effect on angiogenesis, with inhibitory effects at low concentrations (5 μM) and stimulatory effects at high concentrations (10 μM and 100 μM) compared to control. The findings highlight the therapeutic potential of EMPA by promoting vascularization in vascular pathologies, beyond its metabolic effects. As both VEGF and H_2S are important

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angiogenic stimulators and it is known that EMPA stimulates VEGF expression and our preliminary result found that it induces H_2S formation, H_2S/VEGF pathway may be potential mechanism for angiogenic effect of EMPA. However further molecular studies are required to elucidate the mechanisms underlying these effects. *The authors marked with an asterisk equally contributed to the work.

LB-R-35-03
The effects of acute and chronic donepezil administration at different doses on experimental convulsive seizure activity

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Epilepsy is a complex neurological disorder characterized by recurrent seizures that significantly impact patients' health and quality of life (Mohammed HS et al. 2025 Brain Res 12, 1858). In this study, the effects of donepezil, a cholinesterase inhibitor effective in the central nervous system, on seizure threshold and severity were investigated. Experimental and clinical studies on the role of cholinergic signaling in epilepsy have shown varying results. In this study, donepezil was administered at doses of 2.5, 5, and 10 mg/kg both acutely and chronically for 21 days. At the end of treatment, seizures were induced with a single dose of pentylenetetrazol [60 mg/kg]. Seventy adult male Wistar rats were divided into ten groups: Acute and Chronic groups, each consisting of Sham, Control, and 2.5, 5, and 10 mg/kg donepezil subgroups. The effects of donepezil on seizure susceptibility were evaluated by measuring seizure onset, severity, and duration. Additionally, to comprehensively assess donepezil's effects on seizure activity, serum levels of oxidant/antioxidant markers including NO, MDA, and GSH will be measured in all groups. To evaluate inflammation, biochemical analyses of BDNF, cytokines (TNFα, IL-1β, IL-6, IL-13), and periostin levels will be conducted. The results showed that seizure onset was significantly delayed in the Acute 10 mg/kg and Chronic 2.5mg/kg donepezil groups compared to their respective control groups. Seizure severity and duration were significantly reduced in the Acute 10 mg/kg group and all Chronic donepezil groups (2.5, 5, and 10 mg/kg). In acute administration, only the high dose was effective, whereas in chronic administration, all doses were effective, with the low dose showing the strongest reduction in seizure activity. Including biochemical serum analysis enhances understanding of donepezil's role in epileptogenesis and may aid in developing more effective treatments for neurodegenerative diseases. *The authors marked with an asterisk equally contributed to the work.

LB-R-35-04
Preclinical assessment of a novel peptide triple agonist with potential application in the treatment of obesity

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The rapid pace of urbanization and socio-economic development has improved living standards but contributed to a more stressful and sedentary lifestyle, leading to unhealthy dietary habits. As a result, obesity and type 2 diabetes have escalated into a global health crisis, with the World Obesity Atlas 2022 projecting that by 2030, over 1 billion people worldwide will be living with obesity. These alarming trends highlight the urgent need for novel and effective therapies. Recent research suggests that coordinated stimulation of GLP-1R, GIPR, and GCGR incretin receptors could provide superior therapeutic benefits. The DILOC2 project aimed to address this unmet need by developing innovative triple agonist peptides. This study evaluated the nonclinical safety and efficacy of one designed candidate, DIL01. Toxicity was assessed in Wistar rats following subcutaneous administration of DIL01 at 200 or 600 μg/kg daily for five days. Efficacy was tested in Zucker obese rats, which received DIL01 every other day at identical doses, for a total of 15 administrations. Body weight was recorded daily, and blood samples were collected before the first and after the final dose for biochemical analysis. No treatment-related adverse events were observed in the toxicity study. In the efficacy study, DIL01 at 600 μg/kg significantly reduced body weight gain in obese rats compared to vehicle controls (34.9% vs 62.7% gain). Additionally, DIL01 reduced elevated levels of triglycerides, GLDH, and α-amylase associated with obesity. These results support the safety and therapeutic potential of DIL01 as a triple agonist peptide in the treatment of obesity and related metabolic disturbances. The study was financially supported by NCBiR, Poland (the "Smart Growth" Operational Program 2014-2020 – Measure: R&D projects of enterprises; Competition 3/1.1.1/2020 Fast Track 3_2020). *The authors marked with an asterisk equally contributed to the work.

LB-R-35-05
Encapsulation of antifungal compounds in cationic solid lipid nanoparticles for enhanced activity against *Candida*

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The incidence of *Candida* infections has risen significantly in recent decades, driven by increased immunosuppression, medical device usage, and the emergence of multidrug-resistant strains. Solid lipid nanoparticles (SLNs) have emerged as promising nanocarrier systems for antifungal drug delivery, offering advantages such as improved drug solubility, controlled release, enhanced bioavailability, reduced systemic toxicity, and the ability to overcome drug resistance mechanisms. The incorporation of citral, a monoterpenoid with broad-spectrum antimicrobial activity, and anidulafungin, a potent echinocandin, into SLNs can address their poor water solubility, improve systemic delivery and enable combined therapy. The aim of this work was to develop and characterize the incorporation of citral and anidulafungin, both individually and in combination, into cationic SLNs, and to evaluate their antifungal activity against *Candida albicans*. Following synthesis, the suspensions of SLNs were ultrafiltrated and characterized by size, polidispersity index (PDI), and zeta potential. They were subsequently filtered through 0.2 μm or 0.45 μm pore filters. In addition to assessing loading capacity and encapsulation efficiency, the stability of

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SLNs over time was evaluated and the morphology of SLNs were analyzed by transmission electron microscopy. The antifungal activity of these SLNs was analyzed *in vitro* against *Candida albicans* isolates. The results indicate that the obtained SLN suspensions exhibit favorable characteristics as citral- and anidulafungin-delivery systems, supporting their use in combination. All the types of SLNs developed were stable and presented similar characteristics in terms of size, PDI and zeta potential. Overall, these results highlight the potential of SLNs as a versatile strategy for developing novel antifungal therapies. This work was supported by MCIN/AEI/10.13039/501100011033 (PID2020-116495RB-I00) and the Basque Government (IT1578-22 and IT1607-22).

LB-R-35-06
C-MST: a coumarin-30-based microscale thermophoresis assay for profiling tubulin ligands with conventional and novel binding sites

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Microtubules are among the most important targets for anti-cancer chemotherapy. Although several inhibitors of tubulin polymerization are clinically successful, the resistance of cancers to existing drugs urges the development of novel tubulin-binding substances that can inhibit microtubule dynamics. The development of such drugs heavily relies on screening methods. Sensitive and accurate detection of tubulin–ligand interactions can be greatly facilitated by fluorescent probes, which are usually binding-site-specific and have been developed for only a handful of conventional binding sites on tubulin. Here, we show that coumarin-30, a widely available laser dye, binds to the colchicine site of tubulin and inhibits microtubule dynamics and cancer cell division at submicromolar concentration. Combining coumarin-30 as a fluorescent probe with microscale thermophoresis offers a fast, accurate, and cost-effective C-MST (coumarin-30-based microscale thermophoresis) assay for detecting tubulin–ligand interactions within and beyond the colchicine site of tubulin. The assay allows sorting of colchicine-site binders from other ligands. Using the assay, we identify several potent tubulin polymerization inhibitors targeting the colchicine site and validate them through *in vitro* microtubule dynamics and cell cycle assays in cancer cells. Next, we show that the C-MST assay is capable of detecting ligands targeting a novel binding site on tubulin, recently established through cocktail crystallography. We confirm detection of a small-molecule ligand targeting that site, and we further design and characterize a series of its analogs. The ability of the C-MST assay to detect tubulin binders regardless of their binding site or their effect on microtubule dynamics opens new avenues for developing unconventional modulators of tubulin-tubulin and tubulin-effector interactions. *In vitro* work in this study was funded by the Russian Science Foundation (project # 25-74-20014).

LB-R-35-07
Identification of small molecules that modulate the activity of chaperonin CCT and characterization of their antitumor potential

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Molecular chaperones are key elements for the regulation of cell homeostasis. In recent years, a direct link has been established between chaperone dysregulation and cancer (up-regulation) or neurodegenerative diseases (down-regulation). Our goal is to investigate the pharmacological modulation of the eukaryotic chaperonin CCT (chaperonin containing TCP1), a ~1MDa complex with 8 different subunits arranged in two stacked rings. CCT participates in the folding of 5-10% of newly synthesised proteins, the cytoskeletal proteins actin and tubulin being its main substrates. CCT is overexpressed in different types of cancer, and its expression levels are associated with poor disease prognosis; however, to date it has not been extensively studied as a drug target. We screened ~1700 small molecules from chemical libraries to evaluate their effect over CCT stability using differential scanning fluorimetry (DSF). We calculated the K_D of the compounds that decreased the CCT T_m, ranging between 0.38 μM and 250 μM. The five compounds with better properties were assayed in cell proliferation assays using two breast cancer cell lines, MCF-7 and MDA-MB-231, and the breast epithelial cell line MCF-10A. Two of these molecules showed a selective toxicity over the tumour cells: Inhibitor 1 displayed an IC_{50} value of 2.6±1 and 1.3±0.5 M, respectively, in the tumour lines, and 410±22 M in MCF-10A; Inhibitor 2 showed selectivity for MDA-MB-231 with an IC_{50} of 8±2 M vs. 235±54 M in MCF-10A. Wound-healing assays in MDA-MB-231 cells showed a significant effect of both inhibitors in reducing cell migration (86 and 85% wound area vs. 75% in untreated cells at 24 h). Immunofluorescence and super-resolution STED microscopy confirmed that actin filaments were altered upon treatment with the inhibitors, suggesting an effective inhibition of CCT that results in defects in actin polymerisation. These results set the basis for the development of novel cancer therapies targeting CCT.

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LB-R-35-08
Isolation of inhibitory substances for survivin-HBXIP complex formation from *Lygodium japonicum* extract using the yeast two-hybrid method

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Apoptosis plays a key role in maintaining organismal health by eliminating abnormal cells. In many tumor cells, however, apoptosis is suppressed, leading to uncontrolled proliferation and resistance to anticancer drugs. Survivin and HBXIP, proteins often overexpressed in tumors, are known to form a complex that inhibits caspase-9, a key executor of apoptosis. Targeting this complex may lead to novel therapeutic strategies for refractory cancers. In this study, we established a yeast two-hybrid system to evaluate the interaction between survivin and HBXIP and screened herbal compounds that exhibit inhibitory activity against this complex (hereafter referred to as the S/H complex). cDNA was synthesized from mRNA extracted from human hepatocellular carcinoma cells (HepG2), and the ORF of survivin-α and HBXIP were cloned into the pACTII and pAS1 vectors, respectively. These constructs were introduced into *Saccharomyces cerevisiae* strain BY3913 to develop an evaluation system that could detect the inhibitory effects based on β-galactosidase activity. By using this system, we screened several hundred herbal extracts available in our laboratory. Ten of these extracts exhibited inhibitory effects on the S/H complex, with *Lygodium japonicum* showing a remarkable inhibition of over 70%. After confirming apoptosis induction in tumor cells, we focused on *Lygodium japonicum* and carried out liquid-liquid partitioning with hexane and water, followed by separation via thin-layer chromatography (TLC). Individual spots were extracted with acetone, dissolved in DMSO, and evaluated for inhibitory activity. As a result, the sample derived from the hexane fraction showed strong inhibitory effects on the S/H complex. Based on GC-MS and HPLC analyses, two fractions with inhibitory activity were identified. Structural analysis of these fractions is currently underway, with the aim of identifying novel anti-tumor agents effective against apoptosis-resistant tumors. *The authors marked with an asterisk equally contributed to the work.

LB-R-35-09
Evaluation of the anti-melanogenic effects of herbal compounds on B16 melanoma cells

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Melanin is synthesized by melanocytes located in the basal epidermal layer. Excessive production leads to hyperpigmentation disorders such as age spots and dark patches. Thus, inhibiting melanogenesis is considered an effective strategy for preventing these conditions. In this study, we screened herbal extracts for their inhibitory effects on melanin synthesis using B16 melanoma cells and to identify bioactive compounds capable of inhibiting melanin synthesis through the regulation of multiple target mechanisms. Confluent B16 melanoma cells were treated with 30 herbal extracts and cultured for two days. After incubation, the cells were lysed with sodium hydroxide, and melanin content was quantified using a calibration curve. The result showed that Eight extracts—*Juncus effusus* var. decipiens, *Anemarrhena asphodeloides, Allium bakeri, Illicium verum, Arctium lappa, Artemisia princeps, Cordyceps sinensis, Sparganium simplex* Hudson showed the same inhibitory effects as the positive control, kojic acid. Liquid-liquid extraction with hexane, ethyl acetate, and aqueous was performed to separate *A. asphodeloides* extract. The inhibitory effects of each fraction on melanin production were evaluated using B16 melanoma cells. The results suggested that the hexane fraction may contain compounds capable of suppressing melanogenesis. Further investigations were conducted to determine whether the active components in the hexane fraction act as tyrosinase inhibitors. The results indicated that although the active components in the hexane fraction of *A. asphodeloides* possess melanogenesis-inhibitory activity, they do not inhibit tyrosinase.

LB-R-35-10
Screening of fungal tip growth inhibitors in Chinese herbal extracts by vesicle transport evaluation system

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In recent years, invasive fungal infections have increased among patients whose immune systems are compromised by chemotherapy or organ transplantation. However, existing antifungal agents are limited and face problems such as the emergence of resistant strains and side effects on human cells, therefore, the development of new antifungal drugs are required. Our laboratory has established a vesicle transport visualization system by transforming the *Saccharomyces cerevisiae* INVSc1 strain with a vector encoding a fusion protein of β-glucanase (BGL2) and enhanced green fluorescent protein (EmGFP). Utilizing this evaluation system, we performed screening assays on herbal medicine extracts to identify inhibitors of vesicle transport. In this report, we present herbal extracts identified as potential candidates for novel antifungal agents. *S. cerevisiae* INVSc1/pYES2-BGL2-EmGFP was grown in SC-Ura medium with 2% raffinose to mid-log phase, adjusted to 2.0×10^7 cells/mL, and was cultured in the medium with or without herbal extracts at 30°C for 5 h with or without herbal extracts. Cells were then induced in SC-Ura medium containing 2% galactose and 1% raffinose at 30°C for 2 h, and vesicle transport to the fungal tip was observed. As the results, in the control, 86% of budding cells exhibited GFP fluorescence at both the apex and the budding site (fluorescent individuals). Of 186 extracts tested, 12 yielded fewer than 30% fluorescent individuals, 16 yielded 30–60%, and 158 yielded ≥ 70%. The 12 extracts with < 30% localization are thus candidates for antifungal activity via tip-growth inhibition. *The authors marked with an asterisk equally contributed to the work.

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LB-R-35-11
Biophysical characterization and antitumor activity of Congo red:OSI-774 (CR:OSI-774) complexes in pancreatic cancer cells

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A key challenge in modern cancer therapy is developing drug delivery systems that enable selective targeting while reducing systemic toxicity. Supramolecular carriers like Congo red (CR), which self-assemble into ribbon-like structures and show strong intercalative properties, offer a promising approach. The ability of CR to form stable complexes with anticancer agents can improve solubility, bioavailability, and release kinetics, enhancing therapeutic outcomes. This study aimed to investigate the formation of supramolecular complexes between Congo red (CR) and the tyrosine kinase inhibitor OSI-774, and to evaluate their *in vitro* effects on pancreatic cancer cell lines (PANC-1, BxPC3). Individual research tasks included biophysical characterization of CR–OSI-774 interactions, optimization of molar ratios using dynamic light scattering (DLS), assessment of cell viability using the MTT assay, determination of IC_{50}, assessment of apoptosis using flow cytometry and analysis of cancer cell migration and invasion. DLS analysis confirmed the formation of stable CR: OSI-774 complexes and allowed for identification of the optimal molar ratio. All tested compounds inhibited pancreatic cancer cell proliferation in a dose- and time-dependent manner. Notably, flow cytometry revealed enhanced apoptosis induction by the CR:OSI-774 complex compared to the free drug. Furthermore, the complex significantly impaired the migratory and invasive potential of both cancer cell lines. These results highlight the potential of supramolecular carriers such as Congo red to enhance the efficacy and selectivity of anticancer agents. This approach may represent a promising avenue for the development of targeted therapies, particularly in aggressive malignancies like pancreatic cancer. Our research was funded by the Polish Ministry of Science and Higher Education from state budget resources as part of the "Student scientific groups create innovations" program (grant number: SKN/SP/601857/2024).

Endocrinology

P-36-001
Impact of sampling time on thyroid-stimulating hormone, free thyroxine, and free triiodothyronine levels

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The timing of sample collection is critical for analytes with a circadian rhythm. Although blood samples are typically collected in the morning, they are often obtained in the afternoon as well. In this extensive study, involving large sample groups, we aimed to investigate hourly changes in serum thyroid-stimulating hormone (TSH), free thyroxine (fT4), and free triiodothyronine (fT3) levels and assess their clinical significance. Measurements were derived from 216 278 patients attending our outpatient clinics over a two-year period (2021–2023). After applying exclusion criteria, 69 141 patient samples collected between 8 am and 5 pm were analyzed in 1-hour intervals. Measurements were performed using the Cobas e801 analyzer (Roche Diagnostics, Penzberg, Germany) with the electrochemiluminescence immunoassay method. Statistical significance was assessed using the standard normal deviation test (Z-test), while clinical significance was determined based on bias percentages calculated from biological variation data provided by the European Federation of Clinical Chemistry and Laboratory Medicine (EFLM). TSH levels demonstrated statistically and clinically significant variations in all hourly intervals between 11 am and 5 pm compared to the reference group. In contrast, no significant differences were found for fT4, and fT3 levels remained stable except for a minor variation in one group. These findings highlight that sampling time has a considerable impact on TSH measurements, underscoring the importance of standardizing sampling protocols to prevent misinterpretation in clinical practice.

P-36-002
Effect of intermittent fasting on the kynurenine pathway

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Intermittent fasting (IF) is based on the principle of intermittent or continuous fasting at various times of the day. During non-fasting periods, either caloric intake is normal or caloric intake is reduced. It has been suggested that activation of the tryptophan kynurenine pathway (KP) is a physiological reaction to stressors such as inflammation, infection, metabolic disorders and aging. The aim of this study was to investigate the effect of intermittent fasting on the KP. Blood samples of 114 healthy individuals before and after intermittent fasting were collected, and KP was measured by liquid chromatography-tandem mass spectrometry (LC-MS/MS). Statistical analysis was performed using IBM SPSS Statistics 26.0. There was a significant decrease in serum tryptophan, kynurenine, 3-hydroxy kynurenine, kynurenic acid, 3-hydroxy anthranilic acid, and qualinic acid levels after intermittent fasting compared to before intermittent fasting ($p = 0.000, 0.000, 0.000, 0.000, 0.000, 0.000, 0.001$, respectively). To the best of our knowledge, this is the first study to comprehensively investigate KP levels in intermittent fasting. A significant decrease in the levels of metabolites in the KP was observed with intermittent fasting. Research in this area is still ongoing and more studies are needed to understand the full effects of IF on KP. *The authors marked with an asterisk equally contributed to the work.

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P-36-003
A rare late-onset Langerhans cell histiocytosis: a case report

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A 23-year-old male presented at the emergency department with complaints of headache, vomiting. Upon examination, the following laboratory values were noted: sodium: 117 mmol/L, glucose: 55 mg/dL, and spot urine sodium: 119 (reference range: 135-146 mmol/L). After administering 300 cc of hypertonic NaCl in the emergency department, the patient was diagnosed with hypovolemic symptomatic hyponatremia and was admitted to the internal medicine department. Hormonal assessments showed the following: ACTH: 7, GH: 0.1, IGF-1: 37.7, cortisol: 0.5, TSH: 0.1, prolactin: 1.6, progesterone < 0.15, estradiol: 6.1, LH: 1.3, and FSH: 2.8. Based on these results, a diagnosis of panhypopituitarism was suspected, and an endocrinology consultation was requested. The patient was started on hormone replacement therapy. Pituitary imaging in MRI was detected in normal physiology. However, a nodular lesion of 8.5 mm in diameter was noted in the right half of the pituitary gland, raising suspicion for a microadenoma. The patient was also scheduled for a thoracic CT scan as part of the screening process. The report revealed limited paraseptal emphysema in both upper lobes of the lungs and multiple thin-walled cavitary lesions, the largest measuring 6 mm, scattered throughout all lobes. A diagnosis of Langerhans cell histiocytosis (LCH), specifically the pediatric form with potential pituitary and lung involvement, was considered. For histological diagnosis, flexible fiberoptic bronchoscopy was performed by the pulmonology team, and biopsy samples were obtained. Langerhans cell histiocytosis is a rare disease characterized by abnormal progression of histiocyte cells. Approximately 50% of cases may involve pituitary gland involvement. Peak onset for the multifocal unisystem type is between 2-10 years of age. In our case, the older age, absence of symptoms such as exophthalmos, lytic bone lesions, multiple organ involvement, polyuria, and polydipsia made the diagnosis more challenging.

P-36-004
Mutations in growth hormone-releasing hormone receptor (GHRHR) affect GHRH-GHRHR dependent cAMP signaling pathway

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Isolated growth hormone deficiency (IGHD) is a medical condition of insufficient or lack of production of growth hormone (GH) and it occurs with an incidence between 1/4000 and 1/10 000 live births. Most of cases are sporadic and idiopathic but 5-30% of growth hormone deficiency (GHD) has a genetic etiology. IGHD is caused by mutations in *GH-N* gene or the mutations in *GHRHR* gene. We determined five missense (p.K264E, p.S317T, p.S330L, p.G369V, p.T257A9), one insertion (c.333_334insC, p.C112Lfs*10) and one nonsense (p.E72X) mutations in the Turkish population but their functions are not shown in GHRH-GHRHR signaling. The purpose of our research was to determine the function of mutations in *GHRHR* gene for GHRH-GHRHR signaling pathway. The QuikChange® Site-directed Mutagenesis kit was used to generate mutations in *GHRHR* gene. Mutations in *GHRHR* gene were confirmed by DNA sequencing. At same time, CRE-luc reporter was prepared by us in order to show function of mutations in GHRHR. Wt and mutant GHRHR plasmids were cotransfected with Cre-luc reporter in CHO cells and expressed. Then the cells were treated with GHRH for 5 hours and their luciferase activity were measured. Five missense mutations (p.K264E, p.S317T, p.S330L, p.G369V, p.T257A9) one insertion (c.333_334insC) and one nonsense mutation (p.E72X) were created in wt *GHRHR* gene. The mutations in the *GHRHR* gene were confirmed by sequencing and CRE-luc reporter plasmid was successfully generated. Wt and mutants GHRHR were coexpressed with the CRE-luc reporter in CHO cells and the luciferase reporter assay results showed that p.K264E, p.S330L, p.G369V, p.S317T ve p.T257A mutations significantly reduced reporter activity. The cells expressing p.E72X and c.333_334insC mutations did not show any reporter activity. Mutations in GHRHR affect GHRH-GHRHR dependent cAMP signaling pathway.

P-36-005
Cystathionine-lyase deficiency exacerbates choline-deficient high-fat diet-induced metabolic dysfunction-associated steatotic liver disease

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Metabolic dysfunction-associated steatotic liver disease (MASLD), formerly known as non-alcoholic fatty liver disease (NAFLD), is a leading contributor to liver-related morbidity and mortality. Recent evidence has demonstrated that MASLD is associated with the transsulfuration pathway. Cystathionine γ-lyase (CSE, a transulfuration enzyme) plays a critical role for biosynthesis of cysteine and thus cysteine-derived glutathione (GSH), the most abundant antioxidant molecule in cells. However, the pathogenesis of MASLD remains unclear. In this study, we investigated the role of CSE in MASLD development and progression using *Cse* gene-deleted (*Cse*–/–) and wild-type (*Cse*+/+) mice. Mice were fed a choline-deficient high-fat diet (CDHFD) to induce MASLD for 4 weeks. CDHFD increased plasma alanine aminotransferase and aspartate aminotransferase levels, hepatic triglyceride levels, vacuolization, and F4/80-positive cell infiltration. Gene deletion significantly augmented these increases compared with normal. CDHFD reduced CSE expression in Cse+/+ mice and cysteine/GSH levels in the liver of either mice, where the declines were much greater in Cse–/– mice. Cse deletion accelerated CDHFD-induced increases in glutathione disulfide (GSSG)/total GSH ratios and 4-hydroxynonenal/malondialdehyde levels in the liver. In addition, Cse deletion inhibited CDHFD-induced increases in glutathione synthetase and γ-glutamyl transpeptidase expression in the liver. These results demonstrate that CSE deficiency augments CDHFD-induced impairment of the cysteine–GSH antioxidant axis, suggesting that CSE plays a protective role against MASLD progression.

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P-36-006
Unexplainable serum thyroid-stimulating hormone elevation: a case report

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Macro-TSH is suspected when serum thyroid-stimulating hormone (TSH) levels are persistently elevated without explainable/supportive clinical or laboratory findings. Various mechanisms have been linked to assay interference, with circulating antibodies being one of them. This phenomenon can lead to clinical confusion and unnecessary diagnostic procedures if not correctly identified. In this report, an elevated TSH level was discovered during routine testing in a 7-year-old girl. A thorough assessment, including medical history, physical examination, and laboratory workup, showed no signs of underlying illness. To evaluate potential assay interference, the sample underwent different analytical methods, such as polyethylene glycol (PEG) precipitation, linearity testing using serial dilutions, measurement on an alternative platform, and adding a commercial blocking agent. The initial TSH levels were 25.7 μIU/mL on the Roche Cobas E801 system. After PEG precipitation, TSH dropped to 3.82 μIU/mL, indicating a recovery of 16.5%. Linearity testing across dilutions remained consistent, with TSH levels ranging from 26 μIU/mL to 22μIU/mL, supporting the suspicion of macro TSH. Further analysis on the Abbott Alinity platform showed TSH at 14.2 μIU/mL. Analysis with a Heterophilic Blocking Tube (HBT) showed persistent TSH levels at 25 μIU/mL. No underlying disease was identified, and the elevated TSH was attributed to macro-TSH interference. High TSH levels without clinical symptoms can lead to unnecessary, costly, and invasive diagnostic workups. Recognizing macro-TSH is crucial to avoid misdiagnosis. When TSH results appear clinically incongruent, interference testing, including HBT analysis, PEG precipitation, or measurement on alternative platforms, should be part of the diagnostic approach to ensure accurate interpretation and patient management. *The authors marked with an asterisk equally contributed to the work.

P-36-007
Assessing nutritional status in diabetes mellitus type 2 patients

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In patients with type 2 diabetes mellitus (T2DM), malnutrition was recognized as a serious health problem. The aim of the study was to evaluate the relationship between Prognostic Nutritional Index (PNI), Controlling Nutritional Status (CONUT) score, and HbA1c and CRP in T2DM hospitalized (KCUS) and ambulatory patients (HC). In this retrospective study, PNI was applied on 171 consecutive patients using the PNI = 5× absolute lymphocyte count (10^9/L) + 10× serum albumin (g/L), and CONUT (a validated scoring system based on concentrations of albumin, cholesterol and total lymphocyte count). Of these, 67 subjects aged 24–89 years ($\bar{x}$ = 62.5) were HC patients, 104 subjects aged 21-90 years ($\bar{x}$ = 67.9) were KCUS patients. The results showed KCUS patients had statistically significantly elevated CONUT (M_{CONUT} = 2.5 (IQR 1.0-5.0), and low PNI (M_{PNI} = 38.0 (IQR 32.1-40.0). HC patients had statistically significantly better median M_{CONUT} = 1.0 (IQR 0.0-2.0), and M_{PNI} = 42.0 (IQR 38.3-44.0) ($p < 0.0001$). The results for the HbA1c showed a statistically significant difference between HC and KCUS patients. The median HbA1c for KCUS patients was M_{HbA1c} = 9.3% (IQR 7.4%-11.4%), while M_{HbA1c} of HC patients was 6.3% (IQR 6.0%-7.3%). A statistically significant difference ($p < 0.0001$) was found between the studied groups for CRP. Median for CRP was M_{KCUS} = 8.8 mg/L and M_{HC} = 1.5 mg/L. Correlation analysis for patients showed a statistically significant and positive correlation of CONUT with HbA1c ($p = 0.0113$) and CRP ($p < 0.0001$). Better correlation of CONUT with CRP was found ($\rho = 0.534$; 95% CI = 0.40-0.65) while the CI for HbA1c was $\rho = 0.206$ (95% CI = 0.05-0.35). Statistically significant ($p = 0.0005$), but weak and negative correlation was found between PNI and HbA1c (ρ = -0.279; 95% CI = -0.42-0.13). Better statistical significance ($p < 0.0001$) and better negative correlation was found between PNI and CRP (ρ = -0.548; 95%CI = -0.66-0.42). Our study showed significant difference in nutritional status in hospitalized and ambulatory patients diagnosed with T2DM.

P-36-008
Well-being after total thyroidectomy: is selenium the missing element?

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Several studies indicate that approximately more than 15% of patients taking levothyroxine who undergo thyroidectomy experience symptoms of hypothyroidism or a decline in quality of life compared to their pre-surgery state, despite having serum TSH (thyrotropin) levels within the normal range. Thus, this study sought to explore the potential role of selenium status and oxidative stress in contributing to this phenomenon. This cohort study involved 44 patients who had undergone total thyroidectomy. Measurements were taken for serum levels of thyroid hormones (i.e., TSH, fT3, fT4), selenoprotein P (SelP), and plasmatic glutathione peroxidase (GPx3) to evaluate selenium status and oxidative stress in such situations. A validated questionnaire was administered to all patients to assess self-perceived quality of life. A statistically significant direct correlation was observed between SelP and GPx3 levels. Conversely, no correlation was found between thyroid hormone levels and either SelP or GPx3. Notably, a significant direct correlation was identified between SelP and tiredness, as well as between GPx3 and tiredness. This study suggests that selenium status, which is linked to antioxidant activity as indicated by SelP and GPx3 levels, may influence the tiredness domain of quality of life in thyroidectomized patients, even when thyroid hormone levels are within the reference range. Further research is necessary to clarify the role of selenium in the ongoing symptoms reported by some patients after thyroidectomy.

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P-36-009
The association of serum asprosin with metabolic parameters in Hashimoto's thyroiditis

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Thyroid hormones and adipokines share important physiological effects such as maintaining energy homeostasis and regulating glucose and lipid metabolism. Asprosin is a newly discovered adipokine that exhibits glucogenic effects. This study aimed to determine the possible relationship between autoimmune Hashimoto's thyroiditis, one of the most common thyroid disorders, and asprosin and related glucose and lipid metabolism parameters. The study included 26 newly diagnosed patients with adult Hashimoto's thyroiditis (18 female/8 male) and 26 healthy controls (17 female/9 male). Thyroid function tests, lipid panel, fasting glucose, and insulin were measured with routine autoanalyzers. Asprosin level in serum samples was analyzed by ELISA method. Significant differences were found between the groups regarding body mass index (BMI), thyroid-stimulating hormone (TSH), free thyroxine (fT4), anti-thyroid peroxidase antibodies (anti-TPO), anti-thyroglobulin (anti-Tg), and low-density lipoprotein cholesterol, but not asprosin and insulin resistance. In the hypothyroidism group, there was a positive correlation between asprosin and body mass index (BMI), triglycerides, insulin, and insulin resistance and a negative correlation with gender. After grouping according to gender, asprosin levels were significantly higher in both hypothyroidic and healthy women compared to men. Our results suggest that there is a potential relationship between Hashimoto's thyroiditis and asprosin levels and that insulin resistance may play a role in this connection. In addition, gender differences may have a significant effect on asprosin levels.

P-36-010
Adiponectin levels in type 2 diabetes: a meta-analysis

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Adiponectin, an insulin-sensitizing adipokine, plays a key role in glucose metabolism and insulin resistance. While its levels are consistently lower in individuals with type 2 diabetes mellitus (T2DM), an optimal reference range for clinical use remains undefined. This meta-analysis estimates pooled serum adiponectin levels in T2DM and explores its potential as a biomarker. A systematic search of PubMed, Google Scholar, and Cochrane Library identified cross-sectional or case-control studies reporting serum adiponectin levels in T2DM patients and controls. Only human studies using ELISA for total adiponectin were included. A random-effects model was applied, heterogeneity was assessed with I^2, and publication bias was evaluated via funnel plot asymmetry. Nine studies involving 2103 subjects (T2DM = 1131, controls = 972) were included. The pooled standardized mean difference (SMD) of serum adiponectin levels in T2DM patients was -1.18 (95% CI: -1.73 to -0.63, $p < 0.001$), indicating significantly lower adiponectin in T2DM. Study heterogeneity was high ($I^2 = 95.57\%$). No strong evidence of publication bias was detected. Adiponectin levels are significantly reduced in T2DM, reinforcing its potential as a biomarker. Further research is needed to establish clinically relevant thresholds for diabetes risk assessment and management.

P-36-011
Assessing total protein and albumin levels in children with type 1 diabetes and healthy controls

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In healthy children, total protein (TP) and albumin (ALB) levels reflect adequate nutritional status and liver function. Children with type 1 diabetes (T1D) may show variations in protein levels due to factors such as nutritional status, glycemic control and comorbidities like kidney disease. Poorly controlled diabetes can lead to complications that further impact protein levels. This study aims to assess and compare TP and ALB levels in children with T1D against healthy controls. This prospective study was conducted at the Clinical center University of Sarajevo and included 90 healthy children and 110 children with T1D, aged 2-17 years, matched for age and gender. TP (g/L), ALB (g/L), and hemoglobin A1c (%) were measured using standard laboratory procedures. Nonparametric tests (Mann-Whitney and Kruskal-Wallis) were applied, with $p < 0.05$ considered statistically significant. Median (IQR) TP values in children with T1D 69 (67-72 g/L) was significantly lower than those of healthy controls 75 (73-78 g/L), $p < 0.001$. Median (IQR) ALB values in children with T1D 39 (38-41 g/L) was also significantly lower than those of healthy controls 75 43 (42-44 g/L), $p < 0.001$. Hemoglobin A1c showed significant negative correlations with TP (rho = -0.561, $p < 0.001$) and ALB (rho = -0.682, $p < 0.001$). Furthermore, we divided the group with T1D in three groups according to level of disease control (good: HbA1c < 7%, intermediate: HbA1c 7.1-8.4% and poor: HbA1C > 8.5%). Between these three groups there is a significant difference in the values of TP ($p = 0.021$) and ALB ($p = 0.011$). This study reveals that children with T1D have significantly lower TP and ALB levels compared to healthy controls, highlighting potential nutritional deficiencies and impaired liver function. Poor glycemic control adversely affects protein status. These findings stress the importance of regular monitoring of protein levels in T1D children and the need for strategies to improve glycemic control and nutritional health.

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LB-R-36-01
Assessment of 17-hydroxyprogesterone (17-OHP) and testosterone measurement in congenital adrenal hyperplasia (CAH) in newborns – a case report

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The term congenital adrenal hyperplasia (CAH) describes a family of autosomal recessive disorders in which there is impaired cortisol synthesis due to a deficiency in one of the enzymatic steps required for cortisol production. 21-Hydroxylase deficiency, in which there are mutations in *CYP21A2*, the gene encoding the adrenal 21-hydroxylase enzyme, is the most common form of CAH. The diagnostic hormone is17-hydroxyprogesterone (17-OHP), which is markedly elevated in 21-hydroxylase deficiency. The objective of this study was to monitor the concentration of 17-OHP and testosterone in the treatment of CAH in newborns. 17-OHP and testosterone analysis was performed from the newborn's serum due to suspicion of CAH on a Dynex DSX® analyzer using the ELISA technique and Vitros 5600 analyzer at the Department of Clinical Biochemistry and Laboratory Medicine Clinical Center University of Sarajevo. The concentration of 17-OHP, testosterone, progesterone, Na, K, at the age of one month were determined. The results are: Na 134 mmol/L, K 6.6 mmol/L, progesterone 152.0 nmol/L, testosterone 17.9 nmol/L. The result of 17-OHP is 1148.66 ng/ml. After corticosteroid therapy, the concentration of 17-OHP decreases and results are: after 1 month 3.20 ng/ml; after 3 months 2.34 ng/ml; after 5 months 0.52 ng/ml. The results of testosterone after one month 0.2 nmol/L, 10 months < 0.45 nmol/L and progesterone values after one month 28.40 nmol/L, and 10 months < 0.6 nmol/L. Laboratory evaluation of adrenal androgens is useful in evaluating the effectiveness of treatment and should be consistently timed; 17-OHP, androstendione, and testosterone are considered the best markers when assessing the appropriateness of current glucocorticoid therapy and low normal levels may be an indication of over-treatment. After Cortef tbl (hydrocortisone) and Astonin tbl (valganciclovir chloride) therapy was included, 17-OHP, testosterone and progesterone were within reference values.

LB-R-36-02
Abstract withdrawn.

LB-R-36-03
Skeletal muscle hypertrophy in the non-obese diabetic Goto-Kakizaki rat

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Type 2 diabetes mellitus (T2DM) is highly prevalent in obese patients and leads to skeletal muscle loss. The Goto-Kakizaki (GK) rat is a spontaneous non-obese model of T2DM. Skeletal muscles of type 1 diabetic rats show overload-induced hypertrophy similar to controls. We investigated whether the same occurs in the extensor digitorum longus (EDL) muscle of GK rats. Glucose and insulin tolerance tests confirmed the diabetic condition in GK rats. EDL hypertrophy was induced by synergistic ablation of the anterior tibialis muscle. After seven days, hypertrophied and contralateral muscles were collected for analysis of weight, fiber cross-sectional area, protein synthesis (AKT, mTOR, rpS6, 4E-BP1), degradation markers (atrogin-1, MuRF-1) by western blot, and gene expression of *AKT2*, *IGF-1*, *TGF-β*, *VEGF*, and *collagens type 1 and 3* (RT-qPCR). This study was approved by the Ethics Committee of Cruzeiro do Sul University (14/2018). GK rats had lower basal EDL muscle mass but showed similar hypertrophic gain compared to controls. At baseline, GK muscles showed higher total mTOR and MuRF-1, and lower p-AKT and p-rpS6. After hypertrophy, GK rats showed increased p-mTOR and p-rpS6, and decreased total mTOR. Basal gene expression of *IGF-1*, *TGF-β*, *VEGF*, and *collagen 1* was elevated in GK, while *AKT2*, *IGF-1*, *TGF-β*, and *VEGF* decreased after hypertrophy. Both groups increased *collagen 3* expression post-hypertrophy. We conclude that muscle mass loss in GK rats is associated with enhanced protein degradation, as indicated by elevated MuRF-1 levels at baseline. This effect appears partially compensated by increased total and phosphorylated mTOR, both at baseline and after hypertrophy. This study was supported by FAPESP (2018/09868-7; 2019/02175-9; 2019/19097-0), CAPES, CNPq, and Cruzeiro do Sul University.

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LB-R-36-04
Determinatıon of reference ranges of serum TSH, fT4, and fT3 hormones by direct method in pregnant women

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This study aimed to determine the reference ranges of serum TSH, fT4, and fT3 hormones in healthy pregnant women and healthy non-pregnant women of similar age using the direct method. Participants were selected among individuals who applied to the outpatient clinics of Izmir Tepecik Training and Research Hospital, based on the CLSI EP28-A3c guidelines. Individuals with a positive anti-TPO test were excluded. A total of 512 subjects' thyroid hormone levels were analyzed. No statistically significant difference was observed in TSH levels between the pregnant and non-pregnant groups. However, significant differences were found in fT4 and fT3 levels. When evaluated by trimester, the lower limit of TSH was found to be lower in the first trimester compared to the other trimesters, and no significant differences were observed between the other groups except between the 1st and 3rd trimesters. fT4 and fT3 levels were lower in the pregnant group compared to the non-pregnant group, and these levels decreased progressively across the trimesters. The reference ranges determined in this study differed from those recommended by international guidelines and assay manufacturers. *The authors marked with an asterisk equally contributed to the work.

Immunology

P-37-001
Human B cells are protected by ursodeoxycholic acid via the inhibition of SARS-CoV-2 spike protein binding to ACE2

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B cells are essential for the defense against various infectious agents including severe acute respiratory syndrome coronavirus 2 (SARS- CoV-2) causing coronavirus disease 2019 (COVID-19). COVID19 is caused by interaction of the spike protein (SP) with the receptor-binding domain (RBD) and its receptor, angiotensin converting enzyme 2 (ACE2). Bisphenol A (BPA), a plasticizer and endocrine-disrupting chemical, can enter the human body through several exposures. Previously, we reported human B cell death by BPA treatment via autophagy induction. We still need aid in dealing with unknown or long-term effects of COVID19 in ecotoxicologically affected B cells. We observed an increase in ACE2 gene expression in human B cells by BPA treatment. Then, we investigated the role of COVID19 on human B cells using spike protein and whether ursodeoxycholic acid (UDCA) contributes to restore SP to ACE2 under our experimental conditions. UDCA also inhibits B cell damage aggravated by co-treatment of BPA and SP. These results suggest that UDCA could be a preventive agent to SP-exposed B cells under BPA exposure. This research was supported by the Basic Research Program through the National Research Foundation of Korea (NRF) funded by the Ministry of Science and ICT (grant number RS-2023-00244570), Republic of Korea.

P-37-002
Determination of non-HLA antibodies and detection of their complement-binding properties in patients with end-stage renal disease

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Alloimmunization is a major obstacle to successful kidney transplantation, mainly due to the formation of anti-HLA antibodies (anti-HLA-Abs). In addition to sensitization against HLA, non-HLA antibodies (non-HLA-Abs) could also have an important impact on graft survival. The aim of this study was to determine the specificities of complement activation of non-HLA-Abs and anti-HLA-Abs and the correlation between non-HLA-Abs and complement-binding non-HLA-Abs in the serum of patients with end-stage renal disease. Serum samples from 74 patients on the waiting list for kidney transplantation were analyzed using complement-dependent cytotoxicity and solid phase assays. The 91.9% of the subjects developed non-HLA IgG Abs, of which 45.9% were complement binding. Anti-HLA

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Abs were found in 43.2% of the sera tested. The most common anti-HLA-Abs specificities were HLA-A2 (14.9%), broad B15 (67.6%), broad Cw3 (12.2%), DR15 (10.8%) and DQ6/DQ8 (10.8% each). Among the complement-binding non-HLA IgG Abs, the highest frequencies were for anti-PRKCZ (12.2%), anti-DEXI (8.1%) and anti-STAT6 (5.4%)[I]. Complement-binding non-HLA-Abs, but not anti-HLA-Abs, were found in 28.4% of subjects, while both were detected in 17.6%. No correlation was found between non-HLA IgG and complement-binding non-HLA Abs based on the number of antibody specificities and the number of different non-HLA IgG Abs and previous immunization events. The results suggest that non-HLA Abs, although often overlooked, may play a critical role in transplant outcomes. Routine testing for non-HLA Abs, in addition to mandatory anti-HLA Abs screening and identification, could improve immunologic risk assessment in transplant patients and optimize post-transplant care. Reference: 1 Mujić Franić A et al. (2024) Int J Mol Sci. 25(22):12103.

P-37-003
Peanut sprout extract enhances antitumor activity of NK cells and mitigates benign prostatic hyperplasia

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Natural killer (NK) cells are innate immune cells that are crucial for anticancer activity and have been developed as an immune cell therapy for cancers. However, their limited effectiveness has prompted research into methods to enhance NK cell activity through combination therapies. Health supplements capable of boosting immune surveillance against tumor cells are gaining attention owing to their potential benefits. Resveratrol, a stilbenoid produced by several plants including peanuts and grapes, reportedly exerts anticancer effects and can activate immune cells. The peanut sprout extract cultivated with fermented sawdust medium (PSEFS) is rich in resveratrol, leveraging its health benefits in terms of the dry weight of herbal products, thus maximizing the utilization of resveratrol's beneficial properties. Our study compared the efficacy of resveratrol and PSEFS and revealed that PSEFS significantly enhanced NK cell activation compared with an equivalent dose of resveratrol. We investigated the ability of PSEFS to potentiate NK cell anticancer activity, focusing on NK cell survival, tumor cell lysis, and NK cell activation in PSEFS-administered mice. Additionally, we found that PSEFS showed a greater potency than saw palmetto extract in mitigating benign prostatic hyperplasia in a rat model. Our findings suggest that PSEFS could be a potential NK cell booster for cancer immunotherapy.

P-37-004
Inflammatory immune response and immune cell activation by adhesion protein of *Porphyromonas gingivalis*

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Various components of gram-negative bacteria are responsible for causing diseases and inflammation. In our previous study, we found that lipopolysaccharide from *Escherichia coli* and *Porphyromonas gingivalis* showed significant differences in their immune activation abilities [1]. In addition, an adhesion protein of *E. coli*, FimH, has well defined to induce inflammation with immune cell activation [2]. However, research on Mfa1, one of the adhesion proteins of *P. gingivalis*, is lacking. We performed an *in silico* study to confirm the inflammation-inducing potential of Mfa1 and found that Mfa1 has strong binding affinity to toll-like receptor 2 in mice and humans. In addition, when Mfa1 protein was treated to bone marrow derived dendritic cells (DCs), the expression levels of co-stimulatory molecules and major histocompatibility complex were increased, and the production of pro-inflammatory cytokines IL-6, IL-12, and TNF-α was also elevated. In addition, intraperitoneal administration of Mfa1 promoted activation of splenic DCs as well as upregulation of IL-6, IL-12, and TNF-α in the serum. In addition, repeated administration of Mfa1 induced differentiation of splenic T cells into IFN-γ-expressing T helper 1 cells. Since *P. gingivalis* is a bacterium that causes periodontitis, Mfa1 was administered to the gums and found that the size of the salivary lymph nodes (sLNs) increased and the DCs and T cells in sLNs were activated. Therefore, these data suggested that Mfa1, an adhesion protein of *P. gingivalis*, may be a major factor that can induce periodontitis. References [1] Wang, Yuhua, et al. (2016) Molecules 21.8, 1086. [2] Zhang, Wei, et al. (2020) Nature Communications 11.1, 1187.

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P-37-005
Myeloid HIF-2α deficiency drives neutrophil hyperactivation and renal fibrosis development

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The transcription factor HIF-2α plays a crucial role in immune regulation and tissue homeostasis. In our study, we found that myeloid-specific ablation of HIF-2α (*Epas1*$^{mye-/-}$) leads to spontaneous glomerulosclerosis in male mice, highlighting a potential link between hypoxia signaling and renal fibrosis. Our results show early and progressive kidney injury, with elevated expression of *Kim1* and *Ngal* as acute kidney injury (AKI) markers, detected as early as 3 months of age ($p = 0.03946$) and exacerbated by 320 days ($p = 5.16E-06$ and $p = 0.00089$, respectively). Additionally, these mice exhibit upregulation of pro-inflammatory and fibrotic genes, suggesting a dysregulated immune response. Proteomics analysis identifies neutrophil degranulation as an enriched pathway (Z-score = 4.243) in young basal *Epas1*$^{mye-/-}$ male mice. Functionally, bone marrow neutrophils show hyperactivation, characterized by increased expression of *Mpo* ($p = 0.04537$) and *Mmp9* ($p = 0.00043$). Furthermore, they display upregulated hypoxia response genes, including *Hif1a* ($p = 0.00701$), *Glut1* ($p = 0.05141$), and *Vegf* ($p = 0.03493$). In contrast, bone marrow-derived macrophages (BMDMs) exhibit a shift toward M2 polarization, indicating an impaired inflammatory resolution. These findings suggest that disrupted crosstalk between macrophages and neutrophils may serve as a key driver of renal fibrosis. The hypoxic environment of the bone marrow, essential for hematopoiesis and immune homeostasis, might be altered in these mice, leading to uncontrolled neutrophil activation and impaired macrophage function. This miscommunication could further contribute to excessive neutrophil recruitment and degranulation in the kidney, exacerbating fibrosis and disease progression. Overall, our study uncovers a previously unrecognized role of myeloid HIF-2α in neutrophil regulation and kidney pathology, providing new insights into hypoxia-driven inflammatory responses and potential therapeutic targets for renal fibrosis.

P-37-006
Thioredoxin-interacting protein-derived peptide (TN13) rescues mice under LPS-induced acute respiratory distress syndrome and sepsis

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Acute inflammatory responses are crucial for the host mortality and morbidity. The pathogenesis of acute respiratory distress syndrome (ARDS) or sepsis is accompanied by systemic inflammation. p38 MAPK is a crucial regulator of inflammatory responses and is a potential target for acute inflammatory diseases, including ARDS and sepsis. Here, we report that TAT-TN13 peptide (TN13), a p38α MAPK inhibitor, inhibits severe inflammatory diseases, including ARDS and sepsis, *in vivo*. TN13 exerted protective effects on lung injuries of mice against lipopolysaccharide (LPS)-induced ARDS by inhibiting inflammatory cell recruitment and cytokine production. TN13 treatment rescued mice survival from severe sepsis. It inhibited inflammatory responses in a mouse model of LPS-induced sepsis. TN13 regulated inflammatory responses by inhibiting the p38 MAPK/NF-κB signaling pathway. These results collectively suggested that TN13 could be an effective treatment option for severe inflammatory diseases to save lives.

P-37-007
Necessity of optimized post-transplant panel reactive antibody monitoring in kidney transplant recipients: risk of false-negative results due to localized antibody phenomena

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Panel reactive antibody (PRA) measurements assess pre-existing alloimmune sensitization by detecting anti-HLA Class I and Class II IgG in transplant recipients. Post-transplant PRA dynamics, including timing of early and delayed immune responses and false-negative results likely due to localized antibody sequestration, remain poorly understood. We evaluated anti-HLA Class I and II IgG PRA levels in frozen serum samples from 30 kidney transplant recipients using a microbead-based flow cytometry assay. Assessments were conducted on pre-transplant (day 0) and post-transplant (days 3 and 7) samples. PRA level ≥ 1% for HLA Class I and/or II was considered positive. Patients' history was retrospectively evaluated case-by-case. On day 0, 13.33% of patients (n = 4) exhibited HLA Class I IgG PRA positivity. By day 3, 3.33% (n = 1) remained positive, while on day 7, 6.67% (n = 2) demonstrated positivity. For HLA Class II IgG PRA, 6.67% (n = 2) were positive on day 0. By day 3, 3.33% (n = 1) remained positive, and on day 7,

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6.67% (n = 2) exhibited positivity. Notably, one patient who tested negative for anti-HLA antibodies on days 0, 3, and 7 was retrospectively found to have PRA and donor-specific antibody positivity on post-transplant day 17. All PRA-positive patients had at least one sensitization event before transplantation, including blood transfusions, surgical interventions, pregnancy, or prior transplantation. Post-transplant PRA may exhibit a transient or persistent positive pattern or show false negativity likely due to antibody sequestration in graft. Additionally, delayed PRA positivity can occur and may not be evident in early assessments. Further studies are needed to optimize the evaluation of post-transplant PRA monitoring. This study was supported by the Scientific and Technological Research Council of Türkiye (TÜBITAK) under Grant No. 222S882 and by the Scientific Research Projects Coordination Unit of Istanbul University, Project No. TDK-2023-39901.

P-37-008
Increased sensivity to infection due to memantine-induced blocking of inlammatory cell trafficking

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Memantine is a selective N-methyl-d-aspartate receptor antagonist, used clinically for treating Alzheimer's disease. The proposed mechanism of action is the blocking of inflammatory cells infiltration into the brain and spinal cord. Previously, we uncovered the potential of memantine to ameliorate symptoms of experimental autoimmune encephalomyelitis (EAE) in old rats [previously published in: Bufan B et al (2024), Biomedicines 12 (4):717]. Here, we have treated female dark agouti rats (3 and 22 months old) with memantine (60 mg/kg body weight) for 7 days (1st–7th day post-immunization for EAE). On the 7th day, we assayed peritoneal and blood leukocytes for their phenotype and functionality. Memantine treatment reduced the expression of the surface major histocompatibility complex class II (MHCII) on peritoneal macrophages. Peritoneal macrophages were weakly responsive to *in vitro* LPS stimulation. Macrophages from old rats produced more nitric oxide (NO) *ex vivo* compared to macrophages from young rats. Cellularity of peritoneal cavity was inversely correlated with NO production, however memantine treatment seemed to reverse that correlation in old rats. Peritoneal cells from memantine-treated rats responded differently to phorbol 12-myristate 13-acetate (PMA) – there was an increased dihidrorhodamine fluorescence in all leucokytes, which was abrogated with memantine treatment, and in the old rat group there was a stronger PMA stimulation but reduced inhibitory effect of memantine. Among blood lymphocytes, there was a decreased expression of CD49d ($CD43^+CD62L^+CD49d^+$), as well as an increased percentage of $CD62L^+$ cells. Finally, while our results show a more pronounced effect of memantine in young rats, we have noticed that old rats under memantine treatment showed signs of non-EAE distress (rough fur, lethargy, respiratory issues). These results show the fast effect of memantine on a systemic level, as well as the potential downsides of its mechanism of action. *The authors marked with an asterisk equally contributed to the work.

P-37-009
The effect of *Candida parapsilosis* on immune regulation in health and oral carcinoma

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Trained immunity is part of the innate immune system that relies on epigenetic modifications and metabolic reprogramming to establish immune memory. Unlike adaptive immunity, it does not involve antibodies, and the secondary immune response can be activated by other pathogens whose antigens are recognised by the same pattern recognition receptors. This phenomenon can be found in professional immune cells as well as in epithelial cells. To study this biological event, we made a model system where we used healthy and oral cancer cell lines as the host. Oral cancer is among the most prevalent malignancies worldwide, with particularly high incidence rates. The most frequent form of oral cancer is squamous cell carcinoma (SCC), primarily caused by tobacco use and excessive alcohol consumption. Oral fungal infection may be a consequence of antitumor therapy, but recent studies suggest that fungal overgrowth itself may contribute to carcinogenesis by producing carcinogenic metabolites or altering immune responses. *Candida albicans*, the most common pathogenic fungus in the cancer microbiome, is frequently detected in these malignancies. We stimulated both healthy and oral squamous cell carcinoma (OSCC) cell lines with *Candida parapsilosis* GA1 and CLIB214 strains for 24 hours. After a five-day resting period, we re-exposed the host cells to *Candida albicans* SC5314 and WO-1 strains to assess the impact of prior fungal stimulation. We examined the effect of pre-treatment on cell damage, cytokine production, fungal adhesion capacity and gene expression changes in host cells. Our results revealed that pre-treatment with *C. parapsilosis* reduced cytotoxicity, increased the production of pro-inflammatory cytokines IL-6 and IL-8, enhanced the adhesion ability of *C. albicans* strains to the host. These findings suggest that prior exposure to *C. parapsilosis* may induce a trained immunity-like response in epithelial cells, influencing host-pathogen interactions and immune modulation in oral cancer.

P-37-010
Modulation of macrophage polarization by endothelial and vascular smooth muscle cells in an *in vitro* co-culture model of atherosclerosis

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Atherosclerosis is a chronic inflammatory vascular disease marked by monocyte activation, endothelial dysfunction, and VSMC phenotypic changes. Macrophages polarize into pro-

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inflammatory M1 or anti-inflammatory M2 phenotypes, interacting with ECs and VSMCs through proteins, cytokines, and exosomes. This study utilizes *in vitro* co-culture models to investigate these interactions via soluble mediators and exosomes, providing insights into the atherosclerosis mechanisms. THP-1 monocytes were differentiated into macrophages with PMA and treated with TNF-α or LPS as a control experiment. Additionally, macrophages co-cultured with VSMCs (V-MAC) or HUVECs (EC-MAC) using inserts under same conditions to assess the effects of VSMCs and HUVECs on macrophages polarization. Flow cytometry was used to assess macrophage polarization, while qPCR analyzed gene expression. Macrophages exosomes were characterized by NTA and western blotting. In macrophages, flow cytometry results showed that LPS stimulation induced M1 polarization. M1-associated genes (IL1β, IL6, and MCP1) were upregulated, while M2 markers were downregulated. In EC-MAC co-culture, the inflammatory response was further enhanced, promoting M1 polarization. In contrast, V-MAC co-culture resulted in increased IL1β and IL6, whereas MCP1 and NOS2 expression remained unchanged. This suggests that the macrophages exhibited a partial M1-like phenotype rather than fully adopting the classical M1 state, while the inflammatory response was still elevated. The downregulation of M2 markers in both co-culture conditions indicates that the transition toward the M2 phenotype was suppressed. These findings highlight that ECs and VSMCs differentially modulate macrophage polarization by altering the inflammatory environment, with exosome-mediated communication playing a crucial role. Understanding these interactions provides insights into the potential role of exosome-mediated pathways in modulating inflammation and vascular remodeling. *The authors marked with an asterisk equally contributed to the work.

P-37-011
Modulation of BCG-induced immune response using β-glucan: boosting immune regulation and reducing severe inflammatory reactions

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BCG (Bacillus Calmette-Guérin) vaccination is well-known for its ability to stimulate immune responses, which lead to trained immunity and various inflammatory responses. The addition of β-glucan, a recognized immunomodulator, may help redirect this response toward a more balanced immune response. The interaction between BCG and β-glucan in modulating monocyte and macrophage populations remains an unexplored topic. Aim of this study was to investigate the effects of BCG and β-glucan on immune cell populations in mice. Sixteen animals were divided into four test groups, with three groups receiving intraperitoneal treatment with BCG, β-glucan, or a combination of both. Flow cytometry analysis of peritoneal cells, spleen, bone marrow, and PBMC showed varying immune responses among the groups. BCG alone triggered a robust inflammatory reaction, increasing the population of M1 macrophages and dendritic cells, along with the expression of MHCII and CD11b. β-glucan also expanded these populations, indicating independent immune activation. However, in the BCG + β-glucan group, a decrease in inflammatory monocytes was observed, suggesting that β-glucan tempers excessive inflammation when combined with BCG. The reduction of $MHCII^+CD11b^{low}F4/80^+CD11c^-$ macrophages in BCG + β-glucan group compared to the β-glucan group alone suggests that β-glucan modulates BCG-induced inflammation by favoring immune regulation over prolonged activation. Furthermore, $MHCII^+CD19^+$ B cells were progressively reduced in the BCG and β-glucan group, most significantly in the BCG + β-glucan group, indicating a shift in B cell dynamics toward regulatory or antibody-secreting phenotypes. These findings indicate that β-glucan does not merely enhance BCG-induced activation but modulates it, balancing inflammatory and regulatory immune pathways. The combination of BCG and β-glucan leads to controlled immune activation, limiting excessive inflammation while supporting immune adaptation. *The authors marked with an asterisk equally contributed to the work.

P-37-012
Fraction of classical monocyte subpopulation in relapsing-remitting multiple sclerosis (RRMS) patients before and after glucocorticoid treatment: a pilot study

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Monocytes are involved in the immune response in multiple sclerosis (MS). Relapse symptoms of MS are often treated with glucocorticoid drugs which possess anti-inflammatory and immunosuppressive effects. In general, classical monocytes show pro-inflammatory properties. The aim of this pilot study was to determine the percentage of classical monocytes in the relapse and, on average, 5 weeks after glucocorticoid treatment. Four patients (two male and two female) with relapsing-remitting multiple sclerosis (RRMS) were recruited in the relapse phase. The mean age of the patients was 44.8 ± 6.61 years. Their blood was sampled and analysed by flow cytometry. The classical monocyte subpopulation was determined based on the CD14 and CD16 expression (CD14+ +CD16−). Before glucocorticoid treatment, the mean expanded disability status scale (EDSS) score was 3.4, while after the treatment, the mean EDSS score was 1.9. The mean fraction of classical monocytes before the treatment was 78.9 ± 7.3%, whilst after the treatment, it was 77.6 ± 6.8% ($p = 0.69$). These results so far suggest that there is no effect of glucocorticoid treatment on the fraction of classical monocyte subpopulation in studied patients.

P-37-013
Could T-regulatory cells cells permit senescent cell accumulation with age?

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Senescent cells accumulate in the body, which contributes to the development of age-associated diseases and general senility. The elimination of senescent cells slows down with age, which is attributed to the aging of the immune system itself. We hypothesize that this process can be mediated by the formation of peripheral tolerance to age-associated autoantigens through the involvement of T-regulatory cells. Given that tumor and senescent cells share common features, we assume the presence of similar mechanisms of tolerance for senescent cells. Our aim was to detect the increase in the content of T-regulatory cells

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depending on the accumulation of cells with a senescent phenotype. We measured the percentage of senescent cells in the tissues of young and old mice, focusing on cells with increased β-galactosidase activity and lipofuscin accumulation, factors known to be associated with the aging process. The senescent cell content assessed by three independent methods (Sudan Black B staining, X-gal, or the Spider-βGal reaction kits) was increased in tissues of old animals. These methods have labile specificity regarding distinct cellular processes. We also revealed the age-dependent differences in T-lymphocyte subpopulations associated with accumulation of senescent cells. This approach allows us to compare the efficiency of protocols for estimating the severity of tissue aging and the potential role of immune system in this process. Acknowledgments: This work was supported by Russian Scientific Foundation, project 24-15-20003.

P-37-014
Omega-3 fatty acids modulate lymphocyte metabolism, reduce inflammation, and improve glycemic control in nonobese diabetic Goto-Kakizaki rats

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The Goto-Kakizaki (GK) rat is a model for studying insulin resistance and type 2 diabetes mellitus (T2DM) without obesity. Fish oil (FO), rich in omega-3 fatty acids, may modulate immune imbalance in non-obese individuals with T2DM. This study evaluated FO supplementation on T cell polarization and metabolism in GK rats. Eight-week-old GK and Wistar rats were divided into control (CT) and FO-supplemented groups (2 g/kg, 3 times weekly for 8 weeks): GK-CT (n = 14), GK-FO (n = 15), Wistar-CT (WT-CT, n = 15), and Wistar-FO (WT-FO, n = 11). Glucose and insulin tolerance tests (GTT, ITT) were performed. After euthanasia, mesenteric lymph node lymphocytes were analyzed for glucose consumption, glucose transporter 1 (GLUT-1) expression, cell proliferation, T cell subsets, and cytokine levels using flow cytometry. Gene expression related to T cell profiles and metabolism was assessed via PCR. Enzymatic kinetics of hexokinase (HK), phosphofructokinase (PFK), citrate synthase (CS), pyruvate kinase, and lactate dehydrogenase were evaluated using a colorimetric assay. FO improved glucose tolerance and insulin sensitivity, increased regulatory T (Treg) cell polarization, and suppressed T helper 1 (Th1) and Th17 profiles. Metabolically, FO reduced glucose consumption, downregulated GLUT-1, and decreased CS and PFK activity. CS gene expression was higher in GK-CT than in GK-FO and WT-FO. Hypoxia-inducible factor 1-alpha (HIF-1α) expression was higher in GK-CT lymphocytes than in GK-FO and WT-CT, while acyl-coenzyme A dehydrogenase was lower in GK-CT. FO supplementation decreased PFK expression in GK. In conclusion, FO improved glucose tolerance and insulin resistance in GK rats, modulating immune responses by enhancing Treg cells and reducing pro-inflammatory Th1/Th17 profiles. These effects are linked to metabolic modulation, with reduced aerobic glycolysis. Some data previously published in: Lobato TB et al. (2024). Nutrients 16(23), 4106. Funding: FAPESP: 2018/098687; CNPq and CAPES.

P-37-015
Modulation of the immune response by N-glycans in an H1sD2 glycoprotein model for allergen-specific immunotherapy

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Allergen-specific immunotherapy (AIT) is the only treatment capable of providing long-term relief from allergies by modifying the immune response. However, its limitations necessitate further research and novel strategies, including the use of adjuvants to enhance innate immune activation. Among posttranslational modifications, N-glycosylation plays a critical role in antigen interactions with pattern recognition receptors, influencing immune responses. Hemagglutinin (HA), the surface glycoprotein of the influenza virus, is a potential adjuvant in AIT due to its immunomodulatory properties. This study focuses on H1sD2 glycoforms, which combine the receptor-binding domain of HA (H1s) with the major house dust mite (HDM) allergen Der p 2 (D2). H1sD2 glycoforms were designed *in silico*, expressed in *Pichia pastoris*, purified, and characterized by mass spectrometry, which confirmed their protein sequences, glycopeptides, and intact masses. The immunoreactivity of the glycoforms was confirmed by immunoblot analysis with the HDM allergic human sera. To assess their immunomodulatory potential, H1sD2 glycoforms were tested on differentiated THP-1 macrophages, measuring cytokine gene expression (IL-4, IL-10, and IFN-γ). Furthermore, peripheral blood mononuclear cells (PBMCs) from HDM-allergic individuals were treated, and cytokine levels were analyzed by ELISA. Polarization of THP-1-derived macrophages into M0 and M2 phenotypes was verified through the expression of surface markers CD206, CD163, and CD54. Additionally, flow cytometry and confocal microscopy were used to investigate the interactions of H1sD2 glycoforms with the appropriate receptors on M0 and M2 macrophages. The results of this study demonstrate the influence of H1sD2 N-glycan decoration on the expression pattern of different cytokines as well as the binding profile to mannose-binding receptors, highlighting its potential role in improving AIT efficacy.

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P-37-016
Lymphocyte metabolism in diabetic Goto-Kakizaki and obese Wistar rats with Walker 256 tumor

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Lymphocytes undergo metabolic reprogramming in response to inflammatory conditions caused by chronic diseases like obesity and type 2 diabetes (T2DM), impairing immune function. In the tumor microenvironment, cancer cells compete with lymphocytes for nutrients, weakening T cell responses. This study aimed to evaluate lymphocyte metabolism in Walker 256 tumor progression in obese Wistar rats vs. non-obese diabetic Goto-Kakizaki (GK) rats. Wistar and GK rats (6 weeks old) were divided into 6 groups: 2 GK groups with a balanced diet, and 4 Wistar groups, including 2 with a high-fat, high-sugar (HLHS) diet to induce obesity. Tumor cells were inoculated in GK and Wistar groups. After 8 days, animals were euthanized, and tumor tissue, plasma, and mesenteric lymph nodes were collected. Leptin levels in plasma were measured by ELISA, cytokines in tumor tissue and lymphocytes by flow cytometry, lymphocyte lactate production and enzyme activity related to metabolism by spectrophotometry. GK rats consumed more food but had less adipose tissue than Wistar rats on the HLHS diet, which led to higher fat accumulation and plasma leptin levels. GK and HLHS Wistar rats showed spontaneous insulin resistance, confirmed by glucose tolerance test and insulin tolerance test. Lymphocyte analysis revealed lower Th1 responses in GK rats with tumors. Increased lactate production and enzyme activity (hexokinase and pyruvate kinase) in GK lymphocytes. Control GK rat lymphocytes had higher IL-10, IL-5, GM-CSF, and IL-9 secretion, and elevated IL-4 and IL-13 in the tumor microenvironment, promoting cancer survival. Obese Wistar rats with tumors had reduced IL-17A, indicating a weaker immune response. The results show that insulin resistance and lymphocyte metabolic reprogramming in GK and obese Wistar rats impair effective immune responses against tumors. These findings suggest that managing metabolic conditions may improve immune response to cancer. Funding: Fapesp (23/12318-7), CNPq and CAPES.

Inflammation

P-38-001
Effect of p-cresol on macrophage plasticity

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Tissue damage and homeostasis maintenance are both aided by macrophages, which are cells of the immune system. Depending on the environmental conditions, these cells can differentiate into M1 inflammatory and M2 anti-inflammatory phenotypes, which synthesize and release different cytokines. The accumulation of endogenous p-cresol, which is produced by intestinal bacteria and metabolized in the liver, can occur in the circulation and organs. p-cresol levels in the blood of certain pathological conditions are 5 to 25 times higher than those of healthy individuals. Our aim in this study was to investigate how p-cresol influences macrophage polarization. Raw264.7 macrophage cell cultures were used to achieve this goal. It has been found in a recent study that p-cresol alters macrophage polarization. Cresol exposure at various concentrations increased the expression of the pro-inflammatory HBGB1 protein, pro-apoptotic caspase-3 and pro-apoptotic Bad protein in macrophages. At the same moment, there was a decrease in the expression of anti-apoptotic Bcl-2 protein. Based on the results, it can be concluded that cresol with a high concentration activates inflammatory processes in macrophages and triggers apoptosis.

P-38-002
Short-chain fatty acids and indole derivatives act as agonists of muscarinic receptors in the intestine

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Inflammatory bowel disease (IBD) involves chronic gut inflammation driven by immune and neural dysregulation, including the cholinergic system. Microbial metabolites like short-chain fatty acids (SCFAs) and indole derivatives help regulate inflammation and support intestinal homeostasis. This study aims to evaluate the effects of SCFAs and indole derivatives on the cholinergic system in an *in vitro* IBD model using the Caco-2 cell line. IBD was modeled by treating Caco-2 cells with lipopolysaccharide (LPS, 10 μg/mL), followed by administration of SCFAs (acetate, butyrate, propionate) and indole derivatives (indole-3-acetic acid, indole-3-propionic acid) at 500 μM. Gene expression of cholinergic markers (CHAT, mAChR1/3, α7nAChR, ACHE) and intestinal barrier proteins (ZO-1, OCL) was analyzed via qPCR. Inflammatory markers (TNF-α, IL-6) were assessed using qPCR and ELISA. Cholinesterase activity was measured using the Ellman method, and mucus production was evaluated using Alcian Blue/PAS staining. To investigate the role of mAChR3, experiments were repeated with darifenacin, a selective mAChR3 antagonist. Data were analyzed using GraphPad Prism and reported as mean ± SEM. Results: Acetate, butyrate, indole-3-acetic acid, and indole-3-propionic acid exert anti-inflammatory effects through mAChR3, while propionate primarily acts via mAChR1. Blocking mAChR3 abolished butyrate's anti-

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inflammatory effect. LPS induction increased α7nAChR expression. Mucin production was elevated under inflammatory conditions; however, acetate and butyrate suppressed mucin expression, whereas propionate, indole-3-acetic acid, and indole-3-propionic acid did not exhibit this effect. Gut microbiota-derived metabolites, such as SCFAs and indole derivatives, may suppress inflammation via cholinergic signaling in the intestine. Understanding these mechanisms could provide valuable insights into IBD pathophysiology and inform the development of targeted therapies.

P-38-003
Miglustat treatment reverses altered secondary lipids and inflammatory markers in lysosomal sialidase Neu1 deficient fibroblasts

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Lysosomal sialidase, encoded by the *NEU1* gene, is responsible for the removing of terminal sialic acids from sialoglycoproteins. Mutations in *NEU1* cause sialidosis, which is an ultra-rare lysosomal storage disorder. Early onset, dysmorphic type of sialidosis can result in stillbirths, hydrops fetalis, mental retardation, and visceromegaly. Previously, accumulation of sialyloligosaccharides, sialoglycoproteins and glycolipids (GM3, GD3, GM4, and LM1) in fibroblasts of sialidosis patients, brain and visceral organs has been reported. In this study, we aimed to clarify whether there is a link between secondary lipids and chronic inflammation in fibroblasts from sialidosis patients and mice models and carried out inflammation-related gene expression and shotgun lipidome analysis. First, we showed elevated levels of hexosylceramides (HexCer) and altered expression of proinflammatory cytokines (Ccl2, Ccl3, Ccl5, and Cxcl10) in both human and mouse fibroblasts. Later, we inhibited glucosylceramide (GlcCer) synthase with miglustat to reduce the accumulated HexCer which also causes alteration of the cytokine and interleukin gene expression. After miglustat treatment, PA, PC, PG, PI PS, DAG, TAG and SM lipid levels were increased in both sialidosis fibroblasts. Distinctly, it was shown that CE levels were significantly decreased only in mice fibroblasts; however, CE levels elevated in human fibroblasts. Additionally, we demonstrated that inflammatory gene expressions such as Ccl3 were reduced after treatment in both mouse and human fibroblasts. Our data shows that miglustat treatment may be a potential treatment strategy to reduce inflammation and regulate altered secondary lipids in sialidosis patients. *The authors marked with an asterisk equally contributed to the work.

P-38-004
Exploring alternative DNA-sensing pathways in vascular smooth muscle cells during sterile inflammation

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Atherosclerosis, the leading cause of cardiovascular disease, is a chronic inflammatory condition marked by fatty deposits in arterial walls forming plaques. These plaques are covered by a fibrous cap formed by vascular smooth muscle cells (SMCs), which can transition between contractile and proliferative phenotypes. Immune cell accumulation destabilizes the fibrous cap, increasing the risk of rupture and prothrombotic events. Atherosclerosis involves sterile inflammation triggered by damage-associated molecular patterns (DAMPs), such as self-DNA, which activate pattern recognition receptors and drive inflammation. We have previously shown that proliferative human coronary artery SMCs (hCASMCs) respond to DAMPs, while their contractile phenotype suppresses this proinflammatory response [previously published in: Liu L et al. (2021) Front Physiol 12, 710968]. Additionally, we explored the role of the cGAS-STING pathway, a key innate immune sensor of double-stranded DNA (dsDNA), in hCASMCs, demonstrating its role in inducing proinflammatory cytokines [previously published in: Bankell E et al. (2024) Sci Rep 14, 1-19]. However, cGAS-STING inhibition did not fully suppress the dsDNA-induced inflammatory response, suggesting involvement of alternative DNA-sensing mechanisms. This raises the hypothesis that SMCs, beyond their structural role in fibrous cap formation, may act as immune-like cells in atherosclerosis. Their ability to respond to diverse DAMPs suggests an active role in modulating immune responses within plaques. Our current research investigates alternative DNA-sensing pathways driving type I interferon and proinflammatory cytokine responses in hCASMCs. By uncovering how these pathways contribute to sterile inflammation, we aim to identify new mechanisms driving atherosclerosis progression and explore novel therapeutic strategies for chronic vascular diseases.

P-38-005
Interchangeability of citrullinated adenosine deaminase from different sources

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It is known that rheumatoid arthritis (RA) is a chronic inflammatory autoimmune joint disorder characterized by autoimmune features. The discovery of new citrullinated proteins as novel autoantigens/detection of autoantibodies against them enhances the efficiency of RA diagnosis/treatment. Adenosine deaminase (ADA) is a key enzyme in purine metabolism, essential for lymphoid node formation. In this work, the interchangeability of *in vitro* and *in vivo* citrullinated ADAs from different sources was investigated. *In vivo* citrullinated ADA (ADA_{SF}) from synovial fluids of RA patients was isolated. Human donor erythrocytes and bovine lung were purified and citrullinated in vitro, as $ADA_{Ercit.}$ and $ADA_{Lcit.}$, respectively. Antibodies towards *in vitro* citrullinated preparations were produced, as $IgG_{Ercit.}$ (0.53 mg/ml) and $IgG_{Lcit.}$ (0.43 mg/ml), respectively. The cross-interactions of produced $IgG_{Lcit.}$ (1.84 μg/ml), depending on its concentration, with 50 ng/well ADALcit., ADA_{SF} and $ADA_{Ercit.}$ were presented as absorbance values of o-phenylenediamine: 0.98 ± 0.002; 0.54 ± 0.01 and 0.43 ± 0.01, respectively. In comparison, for $IgG_{Ercit.}$ (3.5 μg/ml) under the same conditions, there were: 0.91 ± 0.02, 0.63 ± 0.01 and 1.04 ± 0.01, respectively. Notably, the produced antibodies exhibited similar interaction strength with ADA_{SF}. Using a competitive ELISA assay, it was observed that the produced IgGs (300 ng/

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well) detected ADA only in SFs (50 µl/well) of RA patients, unlike in different types of arthritis. The obtained results suggest the produced IgGs, particularly $IgG_{Lcit.}$ (as a more affordable/acceptable option), could be used for detecting citrullinated ADA in biological fluids of RA patients. *The authors marked with an asterisk equally contributed to the work.

P-38-006
Protectin D1, an omega-3–derived lipid mediator, resolves mast cell-driven allergic inflammation via FcεRI signaling

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Protectin D1 (PD1) derived from docosahexaenoic acid (DHA) has shown promise in resolving inflammation. Mast cells are critical drivers of allergic inflammation, releasing inflammatory mediators such as histamine and pro-inflammatory cytokines. This study assesses the effectiveness of PD1 in counteracting mast cell-mediated allergic inflammation. *In vivo*, two well-established mouse models were employed: IgE-mediated passive cutaneous anaphylaxis (PCA) and ovalbumin-induced active systemic anaphylaxis (ASA). The oral administration of PD1 markedly suppressed PCA reactions, including ear swelling, plasma extravasation of Evans blue and mast cell degranulation. In the ASA model, oral PD1 administration dose-dependently alleviated hypothermia and reduced elevated serum levels of IgE, histamine, and IL-4. Mechanistic insights were gained through studies in the RBL-2H3 and primary mast cells derived from mouse bone marrow, where PD1 inhibited IgE-mediated degranulation and decreased intracellular calcium influx by blocking FcεRI signaling pathways involving Lyn, Fyn, and Syk kinases. Additionally, PD1 suppressed pro-inflammatory cytokine production by inhibiting the activity of critical transcription factor; nuclear factor-κB. These findings suggest that PD1, a bioactive lipid derived from DHA, is a very promising therapeutic candidate for mast cell-derived allergic inflammation.

P-38-007
Amygdalin alleviates atopic dermatitis-like skin inflammation via inhibition of Th2 immune responses

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Atopic dermatitis (AD) is a chronic skin disease marked by immune dysregulation such as upregulated T helper (Th) 2 responses. While Th2-targeted therapies for AD are under development, their application is limited by side effects such as hypereosinophilia and arthritis. Amygdalin is a glucoside known for its anti-inflammatory and antioxidant effects. It is an essential component of bitter apricot kernel, traditionally utilized to alleviate inflammatory skin diseases such as boils and acne. This study focused on investigating the therapeutic effects of amygdalin on AD. Its effectiveness was evaluated both *in vivo*, using the AD mouse model induced by 2,4-dinitrochlorobenzene (DNCB) and *Dermatophagoides farinae* extract (DFE), and *in vitro*, using activated leukemia T lymphoblasts and keratinocytes. Amygdalin was shown to reduce the infiltration of immune cells in lesions and both total and DFE-specific immunoglobulin E (IgE) levels in mouse serum. Of note, it explicitly suppressed the expression of Th2 cytokines including interleukin (IL)-4, -5, and -13, as well as tumor necrosis factor (TNF)-α in ear tissues with AD induced by DNCB/DFE. These phenomena were corroborated by observations in CCRF-CEM cells, where amygdalin notably reduced the levels of IL-4 and TNF-α by inhibiting nuclear translocation of nuclear factor of activated T cells 1 and nuclear factor-κB. These findings suggest that amygdalin effectively alleviates allergic skin inflammation by suppressing the Th2 and inflammatory responses, making it a promising candidate for AD treatment.

P-38-008
Tolerogenic dendritic cell-inducible hybrid nanoparticles alleviate asthma in mice

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Asthma is a chronic allergic inflammatory disease of the airways that can have fatal effects by impairing the oxygen transport function of the lungs. In this study, asthma is treated using a drug delivery system that has recently garnered increased attention. To suppress the activity of immune cells involved in asthma, dexamethasone (Dex) is loaded on a poly lactic-co-glycolic acid polymer and wrapped in lipid-containing monophosphoryl lipid A (MPLA), a toll-like receptor 4 ligand. The hybrid nanoparticles containing Dex and MPLA (DM-HNPs) effectively target dendritic cells (DCs) and macrophages and inhibit the activation of DCs in mice. Moreover, DM-HNP increases the expression of programmed cell death-ligand 1 and the production of interleukin-10 in the Alum-stimulated DCs, indicating its promotion of tolerogenic DC differentiation. DM-HNPs further induce the generation of regulatory T cells. DM-HNP has been shown to be effective not only in suppressing the onset of asthma but also in treating it by regulatory T cell generation. Overall, DM-HNPs alleviate asthma induced by immune regulatory cells and can serve as a potential treatment for patients with asthma.

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P-38-009
ROCK2 inhibition regulates inflammation and airway hyperreactivity in an experimental model of allergic asthma

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Bronchial asthma is a chronic inflammatory disease characterized by airway hyperreactivity, inflammation, and lung remodeling. Rho-associated coiled-coil-containing protein kinase (ROCK) signaling is implicated in these pathological processes, yet the distinct roles of ROCK1 and ROCK2 remain unclear. While non-selective ROCK inhibitors modulate all key asthma features, data on selective ROCK2 inhibition are limited. We investigated the effects of intraperitoneal administration of a more selective ROCK2 inhibitor in an ovalbumin-induced guinea pig model of allergic asthma, comparing results with saline-treated, and untreated sensitized animals. Asthma characteristics were assessed as follows: 1. Airway hyperreactivity – measured by specific airway resistance (sRaw) after histamine inhalation and cough frequency during citric acid inhalation; 2. Inflammation Evaluated by cytokine levels (IL-2, IL-4, IL-5) in lung homogenates; 3. Lung remodeling – analyzed via smooth muscle actin, collagen III/V, and TGF-β1 in lung tissue. Increased selective ROCK2 inhibition significantly reduced airway hyperreactivity by decreasing cough and sRaw and suppressed inflammation by reducing IL-2, IL-4, and IL-5 levels. However, it did not significantly affect lung remodeling markers, suggesting pathway-specific effects. Our findings highlight the distinct biochemical role of ROCK2 in airway inflammation and hyperreactivity and suggest that targeted ROCK2 inhibition could be a promising approach for modulating specific molecular pathways in asthma. However, non-selective ROCK inhibitors may have broader therapeutic potential. This work was supported by grants: APVV-23-0261, APVV 19-033, VEGA 1/0042/24, VEGA 1/0060/25.

P-38-010
Significant influence of body mass index (BMI) and pain/function towards knee osteoarthritis Kellgren–Lawrence grading

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Knee osteoarthritis (KOA) is a chronic degenerative joint disease causing pain, functional limitations, and reduced quality of life. KOA severity is commonly classified using the Kellgren–Lawrence (KL) grading system based on radiographic changes. However, structural severity on X-rays does not always align with clinical symptoms. As a result, the relationship between KL grading and key clinical parameters influencing disease progression remains unclear. Therefore, this study aims to examine the association between KL grading and clinical parameters, including body mass index (BMI), pain severity, functional limitations, and inflammatory markers in KOA patients. This analytical cross-sectional study assessed KOA severity using the KL grading system based on manually evaluated digital anteroposterior knee X-rays. BMI was determined by height-to-weight ratio. Pain severity and functional limitations were evaluated using the Visual Analog Scale (VAS) and the Western Ontario and McMaster Universities Arthritis Index (WOMAC). Blood investigations measured plasma levels of erythrocyte sedimentation rate (ESR) and C-reactive protein (CRP). A total of 172 KOA patients were included, with 73% female and 27% male with ages ranging from 40 to 65 years. Grade 2 KOA was the most prevalent comprising, 53% (92 patients), followed by Grade 3 at 47% (80 patients). Independent t-test analysis showed a significant association between increasing KL grading and BMI ($p < 0.05$), suggesting body weight influences KOA severity. Higher KL grading was also associated with higher VAS and WOMAC scores ($p < 0.05$), indicating increased pain and functional limitation. No significant association was found between KL grading and inflammatory markers ESR and CRP. In conclusion, statistical analysis demonstrated proportional associations between KOA KL grading and BMI, VAS and WOMAC score but not with chronic or acute phase of the disease. *The authors marked with an asterisk equally contributed to the work.

P-38-011
Effect of L-theanine on isthmin-1 in heart tissue of doxorubicin-treated rats

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L-theanine (LTE) is the main amino acid in *Camellia sinensis* tea plants. It not only contributes to the flavour of tea but also has various health benefits. Besides its sedative and calming activities, LTE also has immunomodulatory effects. Doxorubicin (DOX) is a chemotherapy drug used to treat cancer. However, it damages the heart by activating inflammatory pathways, leading to cardiotoxicity. Isthmin-1 (ISM-1) is a novel adipokine that promotes glucose uptake and plays essential roles in inflammation and fibrosis. ISM-1 plays indispensable roles in the regulation of glycolipid metabolism and cell survival. Tumour necrosis factor alpha (TNF-α) is an intercellular signalling protein, a cytokine that can be released by various types of immune cells in response to cellular damage, stress or inflammation. In this study, the effect of LTE on ISM-1 levels in heart tissue of doxorubicin-treated rats was investigated. For this purpose, 3 groups ($n = 6$) were created with 18 Wistar male rats as control, DOX and DOX + LTE. DOX was administered i.p. at a dose of 6 mg/kg once a week for 3 weeks. LTE was given by oral gavage at a dose of 200 mg/kg for 21 days. ISM-1 and TNF-α levels as inflammation indicators in the heart tissues of rats were measured with commercial ELISA kit. While TNF-α level increased statistically significantly in the DOX group

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compared with control group, both ISM-1 and TNF-α levels increased significantly in the DOX + LTE group compared with control group. In conclusion, in addition to DOX administration increasing inflammation in rats, LTE showed a further enhancing effect on inflammation in DOX-treated rats.

P-38-012
The role of the of ER stress in the proinflammatory reaction of macrophages

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Recent evidence highlights the role of endoplasmic reticulum (ER) stress in proinflammatory responses, particularly through the key genes *IRE1a*, *PERK* and *ATF6*. This study investigates how these genes influence the inflammatory state of macrophages, focusing on their interactions with the NF-κß signaling pathway. We utilized THP-1 cells with targeted knockouts of *IRE1a*, *PERK* and *ATF6* (THP-1 IRE1KO, THP-1 PERKKO and THP-1 ATF6KO), with non-knockout THP-1 cells serving as controls. All cells were differentiated into macrophages using phorbol 12-myristate 13-acetate (PMA) at 50 pg/ml and subsequently treated with lipopolysaccharide (LPS) at 100 ng/ml for 24 hours to induce a proinflammatory response. Cytokine expression levels (TNF and IL-1ß) were assessed via quantitative PCR (qPCR), while secreted cytokine concentrations were measured using ELISA. Results from qPCR showed that LPS treatment significantly increased IL-1ß and TNF gene expression in control THP-1 cells ($p < 0.05$). In the THP-1 IRE1KO group, IL-1ß and TNF expression levels decreased by 1.3-1.4-fold and 1.6-fold, respectively, compared to controls. Upon LPS treatment, the expression levels of IL-1ß and TNF in the THP-1 IRE1KO group decreased relative to the control group. The THP-1 ATF6KO group also exhibited reduced IL-1ß and TNF expression, with no increase following LPS treatment. The THP-1 PERKKO group showed unchanged IL-1ß and TNF levels relative to controls but had a slight 0.8-fold increase upon LPS treatment. ELISA results confirmed that cytokine secretion of IL-1ß and TNF was lower in THP-1 IRE1KO, THP-1 PERKKO and THP-1 ATF6KO compared to controls. These findings suggest that ER stress-related genes IRE1a, PERK and ATF6, may play a significant role in activating the NF-κß signaling pathway, promoting the expression of proinflammatory cytokines like TNF and IL-1ß. This work was supported by Russian Scientific Grant #25-15-00483.

P-38-013
The effects of quercetin, curcumin, and their combination on inflammation and blood-brain barrier proteins in a sepsis model

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The aim of the study is to investigate the protective or therapeutic effects of curcumin (C), quercetin (Q), and their combination (C + Q) in a sepsis animal model by inflammatory pathways, inflammasome activation, and tight junction protein levels in brain tissue. To evaluate the efficacy of polyphenols, a total of 30 experimental animals were divided into 5 groups (n = 6). Sepsis was induced by administering 5 mg/kg lipopolysaccharide (LPS) (i.p.) and for control, the rats were treated with dimethyl sulfoxide (DMSO) (i.p). The polyphenol groups were administered 50 mg/kg C, 100 mg/kg Q, and 25 mg/kg C + 50 mg/kg Q respectively. Six hours after LPS administration, the rats were sacrificed, and their hippocampus and prefrontal cortex were isolated and stored at -80°C. The measurements of TNF-α, IL-1β, NF-κB, NLRP-3, ZO-1, and claudin-5 were performed using the ELISA method in tissue homogenates. ZO-1 and Claudin-5 levels were found significantly lower in the LPS group compared to the control. However, for C, Q, and combination treatment groups were found significantly higher than in the LPS group. No significant differences were observed between the C, Q, and combination groups in terms of ZO-1 and Claudin-5 levels. TNF-α, IL-1β, NF-κB, and NLRP-3 levels in the LPS group were significantly higher compared to the control group. In contrast, TNF-α, IL-1β, NF-κB, and NLRP-3 levels in the C, Q, and combination treatment groups were significantly lower than those in the LPS group. In addition, no significant differences were observed between the C, Q, and combination groups regarding TNF-α, IL-1β, NF-κB, and NLRP-3 levels. This study demonstrates that C, Q, and the curcumin-quercetin combination suppress brain inflammation and preserve tight junction proteins in sepsis. However, no significant difference was found in the effectiveness between the polyphenol treatments. We suggest that future studies explore various combination doses and administration schedules, which may yield different outcomes. *The authors marked with an asterisk equally contributed to the work.

P-38-014
Role of inflammatory indices and CRP in diagnosing mesenteric lymphadenopathy in pediatric abdominal pain

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Mesenteric lymphadenitis (ML) is an inflammation of the mesenteric lymph nodes frequently detected by ultrasonography in the pediatric population. The aim of the study was to determine the levels of CRP, neutrophil to lymphocyte ratio

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(NLR), platelet to lymphocyte ratio (PLR), systemic inflammatory index (SII), systemic inflammatory response index (SIRI) and to evaluate the correlation of CRP, WBC, NLR, PLR, SII, SIRI with lymph node size and number in ML and control group individuals. The study group consisted of patients aged 0-18 years who underwent abdominal US imaging at Istanbul Atlas University Hospital for any reason between 01.09.2024 and 31.02.2024 and were found to have ML. This retrospective study included 260 children with ML and 200 healthy children. The main symptom of primary and secondary ML was abdominal pain. CRP, WBC, NLR, PLR, SII and SIRI were higher in ML compared to healthy children ($p < 0.05$). There was a positive correlation between CRP and NLR in the patient groups. Lymph node size and number were positively correlated with NLR and SII. CRP, WBC, NLR and SII values were found to be higher in secondary ML compared to primary ML. WBC and NLR were found to be statistically higher in patients with appendicitis in the secondary ML group compared to primary ML ($p < 0.05$). When the ROC curve was analyzed, when the threshold value of NLR was 4.4, ML could be predicted with 82% sensitivity and 80% specificity. The increase in NLR and SII correlated with CRP, which will help early diagnosis and treatment, are practical, cheap and easily accessible, and have effects on the number and size of lymph nodes, was emphasized. NLR>4.4 at presentation can predict ML with good sensitivity and specificity. It may be due to the fact that primary etiologic causes found in secondary ML significantly increase acute phase reactants.

P-38-015
Serum glial fibrillary acidic protein (GFAP), SERPINA3, IL-33 levels in patients with schizophrenia and their relationship with treatment resistance

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Recent studies have highlighted the inflammation theory of schizophrenia, suggesting a potential association between the disorder and elevated inflammatory markers. However, the relationship between inflammatory markers and treatment-resistant schizophrenia (TRS) is unclear. Neuroinflammation is characterized by activation of glial cells and GFAP is a marker of reactive astrocytes. Reactive astrocytes, the primary source of SERPINA3, also contain abundant IL-33, which mediates CNS inflammation and may play a role in schizophrenia pathophysiology. This study aims to investigate the relationship between the treatment response status of schizophrenia patients and the underlying inflammatory condition. Our study includes TRS patients, schizophrenia patients in the remission phase, and healthy control groups. Serum GFAP, SERPINA3, IL-33 levels were analyzed using the ELISA method, and NLR, MLR, PLR values obtained from the complete blood count were calculated. Correlations between PANSS total/subscale scores and these markers were analyzed. In our study, GFAP concentrations were significantly higher in the TRS group than in the control group. SERPINA3 concentrations, neutrophil levels and NLR were significantly higher in both TRS and remission groups compared to controls. Platelet levels were significantly higher in the TRS group compared to the control group and remission group. In the TRS group, MLR showed a positive correlation with PANSS positive score, while NLR correlated negatively with PANSS negative score. ROC analysis was performed to evaluate GFAP's ability to discriminate between TRS and remission schizophrenia patients, yielding an AUC of 0.676 ($p < 0.05$). Our study suggests that an increased inflammatory response may play a role in the pathogenesis of schizophrenia. The higher levels of inflammatory markers observed in treatment-resistant patients suggest a more severe inflammatory response, indicating that the degree of inflammation may be associated with treatment response status.

P-38-016
Investigation of inflammatory effect of pyrroquinoline quinone on HepG2 cell line

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Hepatic carcinoma is a cancerous growth that develops when cells in the liver multiply uncontrollably and abnormally. Currently, there is no definitive treatment known to offer a complete and lasting cure for patients with advanced or metastatic liver cancer. This type of cancer demonstrates resistance to chemotherapy drugs. The primary objective of most cancer treatments is to alleviate symptoms and slow the progression of the disease. Pyrroloquinoline quinone (PQQ) is an antioxidant molecule that safeguards cells from damage caused by free radicals. Also referred to as metoksatin, PQQ is a water-soluble ionic compound and an essential nutrient vital for growth and development. Recent studies suggest that PQQ can induce apoptosis in various types of cancer cells. Our research focused on examining the pro-inflammatory responses induced by PQQ in HepG2 cells, a human hepatocellular carcinoma cell line. The ECO5 and EC10 concentrations of PQQ (5.566 μM and 13.84 μM) were first established and then applied to HepG2 cells. Following this, we conducted qRT-PCR analysis on the total RNA extracted from all samples to examine variations in mRNA expression for specific genes (*CCL2*, *CCL5*, *CCL7*, *CXCL9*, *CXCL10*, *CXCL11*, *IL-1β*, *IL-6*, *NF-κB* and *TNF*). The administration of the EC05 dose resulted in enhanced mRNA expression of inflammation-linked cytokines. The genes *CCL2*, *CCL7*, *CXCL10*, *IL-6* and *TNF* exhibited fold increases of 8.50, 2.65, 5.82, 2.63, and 2.66, respectively. As with EC10 treatment, we found that the expression levels of *CCL2*, *CCL7*, *CXCL9*, *CXCL10*, *IL-1β*, *IL-6* and *TNF* genes increased 13.21, 9.27, 3.70, 9.33, 4.33, 2.48, and 7.93 times, respectively. Our findings suggest that the PQQ molecule has a multi-target pro-inflammatory effect; more research using animals is necessary to obtain more precise findings.

P-38-017
Quercetin reduces inflammation and kidney tissue damage in mice with endotoxemia

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Systemic inflammation induced by Gram-negative bacterial toxin is one of the common causes of acute kidney injury. The search for agents that can reduce inflammation and prevent renal

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dysfunction is relevant. Recently, much attention has been given to the potential health-beneficial properties of natural polyphenolic compounds, in particular quercetin, the most commonly occurring flavonoid. This work aimed to investigate the effect of quercetin on the renal expression levels of proinflammatory cytokine genes as well as histostructural changes in kidney tissue during experimental endotoxemia, induced by lipopolysaccharide (LPS). LPS (*E. coli* O111:B4, 3 mg/kg) was intraperitoneally injected into the mice for 24 h. Quercetin (4.5 mg/kg) was injected in 15 h before and 1 h after LPS. The expression levels of genes IL-1β, IL-6, TNF-α were detected by a TaqMan Thermo Fisher Scientific Assays by real-time PCR method. The standard histological technique was applied for histological evaluation. Administration of LPS caused an increase in the gene expression of proinflammatory cytokines in kidney tissue: IL-6, TNFα and IL-1β (2 -fold, 2.2-fold and 1.8-fold, respectively; $p < 0.05$). Significant morphological alterations in kidney tissue were observed, including circulatory disorders with severe damage to the histostructure of the vascular glomeruli and the epithelium of the proximal and distal convoluted tubules. Quercetin treatment prevented the increase in the expression of proinflammatory cytokine genes and significantly reduced destructive processes in Bowman's capsules, as well as dystrophic and necrotic changes in the capillary network of the kidneys. Thus, quercetin treatment had a protective effect on kidney tissue. Further studies are needed to establish the signaling pathways whose activation by quercetin helps reduce inflammation in kidney tissue and preserve its histostructure.

P-38-018
Boron-supplemented fecal microbiota transplantation alleviates LPS-induced liver and kidney damage via the semaphorin signaling pathway

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This study aimed to investigate the protective effects of fecal microbiota transplantation (FMT) on lipopolysaccaride (LPS)-induced liver and kidney damage. The effects of boric acid (BA) and boron nitride nanoparticles (hBN NPs), which we think may increase the effectiveness of FMT application, on liver and kidney damage were investigated through oxidative stress, inflammation, apoptosis and semaphorin signaling pathway. 70 Wistar albino rats were used in the study and divided into 7 groups (n = 10). Control, LPS (100 μg/kg), LPS + FMT (2 ml), LPS + hBN NPs (100 μg/kg), LPS + BA, LPS + FMT + hBNNPs, LPS FMT + BA. Fecal samples of the control group were collected in a sterile tube 7 days before transplantation. Briefly, a fecal pellet was collected from each donor (control) in the cage, dissolved in 3 mL of sterile PBS, and centrifuged at 500 g for 30 seconds, then the soluble fraction was administered by oral gavage (2 mL/rat). After 7 days, rats were sacrificed under anesthesia. Biochemical and pathological analyses were performed on liver and kidney tissues. Liver and kidney function tests (ALT, AST, BUN and creatinine levels), which increased compared to the control group as a result of LPS injury, were treated with FMT ($p < 0.05$). The oxidant-antioxidant status was evaluated, TAS, TOS, ROS, SOD, MDA levels showed significant results when treated with FMT compared to the injury group. Inflammation increased by LPS in liver and kidney tissues, IL-6 and TNF-a levels decreased in boron-added FMT treatment ($p < 0.05$). SEMA3A levels were found to be suppressed by fecal microbiota transfer ($p < 0.05$). Our current findings indicate that, in addition to the applied FMT treatment, boron and hBN therapy are effective in regulating inflammation and oxidative stress processes and contribute to the improvement of liver and kidney damage. Furthermore, microbiome analysis suggests that the identified strains may exert these effects through the semaphorin signaling pathway. *The authors marked with an asterisk equally contributed to the work.

P-38-019
Influence of IL-6 polymorphism rs1800795 on coagulation markers in COVID-19 patients and correlation of IL-6 serum levels with disease severity

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Interleukin-6 (IL-6) plays a crucial role in the inflammatory response and coagulation dysregulation observed in COVID-19. Genetic polymorphisms within the IL-6 gene, such as rs1800795, may influence coagulation parameters and disease severity. This study aims to evaluate the impact of IL-6 rs1800795 on key coagulation markers (prothrombin time, international normalized ratio, activated partial thromboplastin time and D-dimer levels) and to assess IL-6 serum levels in patients with varying COVID-19 severity. A total of 750 COVID-19 patients from the Bosnian population, categorized into three groups based on disease severity, were genotyped for the IL-6 rs1800795 (G>C) polymorphism using commercial genotyping kits. Genotyping was conducted on the Applied Biosystems QuantStudio5 RT-PCR System. Coagulation parameters – including PT, INR, aPTT, and D-dimer levels – were measured according to standard IFCC protocols and analyzed using linear regression. IL-6 serum levels were quantified with the automated immunochemical analyzer (Snibe Maglumi 600) and compared across disease severity groups. Linear regression analysis, adjusted for age and sex, revealed that homozygous carriers of the minor rs1800795 C allele had significantly lower aPTT levels ($p = 0.021$) compared to G allele carriers. However, no significant associations were found for PT, INR, or D-dimer levels. ANOVA analysis showed no significant differences in IL-6 serum levels between mild (50.71 ± 71.1 pg/mL), moderate (45.99 ± 64.2 pg/mL), and severe COVID-19 cases (58.21 ± 56.3 pg/mL), although a trend toward higher levels in severe cases was observed ($p = 0.079$). The IL-6 gene polymorphism rs1800795 may influence coagulation in COVID-19 patients, specifically aPTT, while IL-6 serum levels showed no significant correlation with disease severity. Further studies are needed to clarify its clinical relevance.

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P-38-020
Investigating the correlation between heparin-binding protein, pentraxin-3 and C-reactive protein in sickle cell anemia disease

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Sickle cell anemia (SCA) is a genetic hematological disorder resulting from a homozygous mutation in the beta-globin gene, leading to a formation of hemoglobin S. SCA is characterized by chronic inflammation and frequent vaso-occlusive crises. The aim of this study is to determine the potential role of C-reactive protein (CRP), pentraxin-3 (PTX-3), and heparin-binding protein (HBP) in reflecting disease's severity and inflammatory activity levels. This study includes 21 individuals diagnosed with SCA and 29 healthy individuals. Serum samples were obtained from the participants and analyzed for CRP, PTX-3, and HBP levels using the ELISA method with the DSX™ Four-Plate Automated ELISA processing system microELISA device. No significant difference was found between the patient and control groups in terms of CRP and HBP parameters ($p = 0.661$; $p = 0.935$, respectively), while a significant difference was observed in pentraxin-3 levels ($p = 0.020$). Correlation analysis between parameters showed a significant correlation between CRP and HBP, as well as pentraxin-3 and HBP ($r = 0.802$; $p < 0.001$, $r = 0.718$; $p = 0.001$, respectively), and moderate correlation was also observed between CRP and pentraxin-3 ($r = 0.618$; $p = 0.005$). Pentraxin-3 may have greater potential than CRP and HBP parameters in the diagnosis and the follow-up of SCA patients, while the latter two may have a potential role in the diagnosis and monitoring of acute crises.

P-38-021
Anti-inflammatory effects of *Acorus calamus* ethanolic extract in LPS-stimulated RAW 264.7 macrophages

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Inflammation is a critical biological response necessary for host defense but can lead to diseases like rheumatoid arthritis, inflammatory bowel disease, neurodegenerative disorders, and septic shock when uncontrolled. Macrophages play a key role in initiating and resolving inflammation by releasing pro-inflammatory cytokines. *Acorus calamus* (Sweet flag) is a perennial wetland plant traditionally used to treat ailments such as fever, asthma, bronchitis, digestive issues, and inflammation. This study investigated the anti-inflammatory effects of *Acorus calamus* ethanolic extract on lipopolysaccharide (LPS)-stimulated RAW 264.7 macrophages. The cells were treated with different extract concentrations (50, 100, and 200 μg/mL) after LPS stimulation. The levels of interleukin-6 (IL-6), tumor necrosis factor-alpha (TNF-α), and interleukin-1 beta (IL-1β) were measured using enzyme-linked immunosorbent assay (ELISA). The results showed that the extract significantly reduced cytokine production in a dose-dependent manner. Significant inhibition was observed at 100 μg/mL and 200 μg/mL ($p < 0.05$) compared to the LPS-only group, with the most substantial reduction at 200 μg/mL. These findings suggest that the extract effectively modulates inflammatory pathways in macrophages. In conclusion, Acorus calamus ethanolic extract exhibits strong anti-inflammatory activity, particularly at concentrations of 100 μg/mL and above. It holds promise as a natural therapeutic candidate for managing inflammation-related conditions. Further research is needed to clarify its mechanisms and assess its efficacy in *in vivo* models.

P-38-022
Effect of blocking of APJ receptor on liver pathology in TNBS-induced colitis in rats

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Inflammatory bowel disease (IBD) is a disease that can affect the liver in addition to the digestive system. The intestine and liver are anatomically connected by the portal vein, and damage to the liver can occur due to intestinal pathology or pharmacological agents. The apelinergic system, consisting of apelin and APJ receptor, plays important roles in inflammatory processes. Activation of the apelinergic system generally has protective effects in the intestine in IBD, but different effects are observed in liver. Therefore, the aim of this study was to investigate how the APJ receptor blockage affects the gut-liver axis and the liver pathology in trinitrobenzene sulfonic acid (TNBS)-mediated colitis. For this purpose, 32 Wistar male rats (8–10 weeks, 200–250 g) were randomly divided into 4 groups: control, Ala, colitis, and colitis + Ala. While saline was administered intrarectally to the control, TNBS (100 mg/kg) to the colitis group. The APJ receptor antagonist Ala13-apelin-13 (30 μg/kg/day, i.v) for 3 days after saline or TNBS administration to the Ala and colitis + Ala groups, respectively. In subjects with colitis, damage to the intestine ($p < 0.01$) and liver ($p < 0.01$), and increased TNF-α ($p < 0.01$) and LPS ($p < 0.05$) in portal blood and TNF-α ($p < 0.01$), MDA ($p < 0.05$) and MPO activity ($p < 0.001$) in liver were observed. APJ antagonist administration reduced the colon damage ($p < 0.01$), but did not affect the increase in TNF and LPS in portal blood. In the liver, injury and the TNF-α did not change in the colitis + Ala, MDA increased ($p < 0.05$), but the MPO activity decreased ($p < 0.05$). According to our results, although blocking the APJ receptor reduced colitis symptoms, it could not repair liver damage. APJ blockade, shows a protective effect in different pathological processes in the liver, may not be effective in liver damage due to TNBS colitis. This work was supported by Akdeniz University Scientific Research Projects Coordination Unit (Project code: TYL-2024-6584). *The authors marked with an asterisk equally contributed to the work.

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P-38-023
Identification of novel hematological biomarkers in axial spondyloarthritis: a case-control study

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Axial spondyloarthritis (axSpA) is a chronic inflammatory condition affecting the spine, with limited biomarkers for monitoring disease activity. This study explored hematological indices, such as neutrophil-, platelet-, and lymphocyte-derived ratios, as non-invasive tools for assessing inflammation and disease severity. It also compared radiographic (r-axSpA) and non-radiographic (nr-axSpA) forms. This retrospective analysis compared hematological data from a control group (n = 123, 49% female) and a patient cohort (n = 93, almost 49% female). ANOVA was used to compare ratios of%Neutrophils (%N/L), Platelets (PLT/%L) and%Monocytes to Lymphocytes (%M/L), %Neutrophils to Hemoglobin (%N/Hgb), white blood cell count to mean platelet volume (WBC/MPV), and white blood cell count to red blood cell count (WBC/RBC) between the control group and r-axSpA and nr-axSpA. When comparing disease groups to the control, the analysis revealed statistically significant differences in several hematological ratios. Specifically, the%N/L ratio was significantly different in r-axSpA compared to control ($p < 0.05$). Highly significant differences were observed for%N/Hgb and PLT/%L ratios in r-axSpA versus control (both $p < 0.005$). Very highly significant differences ($p < 0.0005$) were found for%M/L ratio in n r-axSpA versus control, and for WBC/MPV and WBC/RBC ratios in both n r-axSpA and r-axSpA compared to the control. Notably, the differences in WBC/MPV and WBC/RBC ratios for r-axSpA were exceptionally pronounced ($p < 0.0005$). Findings revealed significant alterations in specific hematological ratios in both nr-axSpA and r-axSpA compared to healthy individuals, with r-axSpA showing more pronounced disturbances. Ratios like%N/L,%N/Hgb, PLT/%L,%M/L, WBC/MPV, and WBC/RBC may help differentiate between healthy individuals and axSpA subtypes, offering potential for disease classification and monitoring. *The authors marked with an asterisk equally contributed to the work.

P-38-024
Pharmacological Bcl-2 inhibition with navitoclax as a therapeutic strategy against pancreatitis

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Acute pancreatitis (AP) is a severe inflammatory disease of the pancreas characterised by extensive tissue damage driven by necrotic cell death in pancreatic acinar cells (PACs). This necrosis intensifies inflammation and worsens disease progression, yet effective treatments remain unavailable. Chronic pancreatitis (CP), in contrast, is a progressive fibroinflammatory disorder marked by sustained inflammation and irreversible fibrosis, driven by activation of pancreatic stellate cells (PSCs). In this study, we evaluated the ability of Bcl-2 inhibitors, venetoclax and navitoclax, to modulate cell death pathways in pancreatic cells *in vitro* and *in vivo*, shifting the predominant mechanism from necrosis to apoptosis. *Ex vivo* models employing cerulein or ethanol/palmitoleic acid (EtOH/POA) to induce PAC injury demonstrated that both compounds significantly reduced necrosis while increasing apoptosis, ATP levels, and overall cell viability. These findings were further corroborated *in vivo* using mouse models of AP induced by cerulein or EtOH/POA. Venetoclax, in particular, showed superior efficacy in reducing necrosis and overall disease severity with minimal adverse effects. In contrast, navitoclax, while promoting apoptosis, also induced thrombocytopenia and a slight increase in apoptosis within healthy pancreatic tissue. While vvenetoclax provided no therapeutic benefit in CP, navitoclax exacerbated pancreatic fibrosis and atrophy, possibly by influencing PSC activity. Notably, both inhibitors affected calcium signalling, a critical factor in AP pathology, and upregulated the calcium-binding proteins S100A8 and S100A9, suggesting additional mechanisms of action beyond apoptosis regulation. These results highlight venetoclax as a promising candidate for AP treatment by mitigating necrosis and shifting the mode of acinar cell death, offering a potential new avenue for therapeutic intervention.

P-38-025
Phytocannabinoids as potential therapeutics for intestinal inflammation

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Phytocannabinoids, such as cannabidiol (CBD), have shown potential in the treatment of inflammatory diseases, including

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intestinal inflammation. However, the therapeutic effects of many phytocannabinoids are still not well understood. We investigate the effects of ten phytocannabinoids on intestinal inflammation using human *in vitro* models. Initially, impact of phytocannabinoids was assessed in THP-1 monocyte-derived macrophages stimulated with lipopolysaccharide. Changes in inflammatory cytokine production were analysed using enzyme-linked immunosorbent assay (ELISA), and luciferase reporter assay was used to measure their influence on the nuclear factor kappa B (NF-κB) pro-inflammatory signalling pathway. The most effective phytocannabinoids reduced TNF-α levels by 22 ± 5% and IL-6 by 59 ± 4%, while the strongest inhibitors of NF-κB activation achieved a reduction of 42 ± 2%. To further evaluate potential of phytocannabinoids, an advanced *in vitro* intestinal barrier model has been developed using a Transwell system with Caco-2 and HT-29 cells forming the epithelial layer, combined with THP-1 monocytes or peripheral blood mononuclear cells (PBMCs) to mimic the immune response. The effects of phytocannabinoids on transepithelial electrical resistance (TEER) and inflammatory cytokine production are currently being assessed. These preliminary findings highlight the potential of specific phytocannabinoids, particularly cannabichromene (CBC) and cannabidivarin (CBDV), as promising therapeutic candidates for intestinal inflammation. This work was funded by the Grant Agency of the Czech Republic (GACR), No. 22-20860S and by the grant of Specific university research – grant No. A2_FPBT_2025_022.

P-38-026
Novel plant-based dietary anti-inflammatory agents for the prevention of inflammatory bowel diseases: inhibition of nitric oxide and interleukin-1β production by thyme extracts and rosmarinic acid

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Among non-communicable intestinal diseases, inflammatory bowel diseases (IBD) are a major health concern, and their incidence and mortality have been increasing. As major triggers can be pointed genetic factors, environmental toxicants or dietary habits, and the onset is associated with oxidative and inflammatory events, leading to intestinal barrier damage. Recently we reported the potential of *Thymus* spp. aqueous extracts (TAE) as dietary antioxidants, which increased nuclear factor erythroid 2-related factor 2 (Nrf2) protein expression, reducing oxidative stress markers [1]. In this work, we studied the potential of TAE's and their main component rosmarinic acid (RA) as prophylactic anti-inflammatory agents. Safety profile was assessed in RAW 264.7 cells (macrophages) exposed for 24 h to TAE or RA, using Alamar Blue assay. To assess inflammatory mediator's production, RAW 264.7 cells were pre-exposed to TAE or RA for 24 h, after which test solutions were removed and replaced by culture medium (CM) or CM plus lipopolysaccharides (LPS; 1 μg/mL) for 24 h. Nitric oxide (NO) release was evaluated by Griess assay, protein expression and intracellular NO were evaluated by flow cytometry, and cytokines' production by ELISA. Mucosal irritant activity and angiogenesis modulation were evaluated in CAM (chorioallantoic membrane)-based assays. Concentrations ≤50 μg/mL (TAE) or 20 μM (RA) were non-cytotoxic, and pre-exposure of RAW 264 cells to these concentrations reduced NO and interleukin (IL)-1β production, while not reducing the levels of key proteins, tumor necrosis factor alpha or IL-6. No irritant properties or angiogenesis modulation were observed in the CAM assay. TAE have potential as anti-inflammatory agents, being RA the main contributor to this effect, which should be further explored for the development of improved IBD prevention solutions. [1] Martins-Gomes et al. (2024) Antioxidants, 2024;13(11):1287. Funds by FCT: UIDB/04033/2020(CITAB), SFRH/BD/145855/2019(CMG).

P-38-027
Molecular structure influences the reduction in the production of inflammatory markers in macrophages exposed to different flavonoids

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The incidence of chronic diseases, including cardiovascular diseases, cancer and arthritis, has been increasing worldwide. A common feature among these pathologies is inflammation, which has been the focus of countless studies. Despite the large number of anti-inflammatory drugs, some present undesirable side effects or the patients develop resistance. Thus, there is a need to develop novel and safer anti-inflammatory agents. The aim of this study was to understand the impact of differences in the molecular structure of flavonoids on the anti-inflammatory potential. Thus, we exposed a macrophage cell model (RAW 264.7) to the flavonoids (eriodictyol, kaempferol or quercetin; 0-100 μM) for 24 h, to select non-cytotoxic concentrations. Anti-inflammatory activity was evaluated in pre-exposure and co-exposure assays, using non-cytotoxic concentrations. In pre-exposure, cells were treated with flavonoids (5, 10, 20 μM) for 24 h, test solutions were removed and replaced by LPS (lipopolysaccharide, 1 μg/mL, 24 h). In co-exposure, cells were co-treated with flavonoids and LPS simultaneously (24 h). Inflammatory mediators (nitric oxide (NO), cytokines) and protein expression were assessed by colorimetric assays, flow cytometry and ELISA. We observed that at non-cytotoxic concentrations (≤20 μM), the 3 flavonoids reduced total NO production, with the co-exposure assay being more efficient, which is likely due to combined radical scavenging and decreased NO production. Flow cytometry revealed reduction in iNOS protein in cells exposed to the flavonoids. The number of hydroxyl groups and structural differences in C-ring between flavonoids was shown to differently modulate cytokine

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production and cyclooxygenase 2 expression. The flavonoids tested show potential as anti-inflammatory molecules, specifically targeting NO production. Future studies should focus on signaling pathways upstream to iNOS, to further explore the molecular targets of the flavonoids. Funded by FCT UIDB/04033/2020(CITAB).

P-38-028
Evaluation of a cardiovascular rehabilitation program on inflammasome formation in neutrophils of infarction patients with and without diabetes

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The risk of acute myocardial infarction is twice as high in diabetic patients. These conditions have an inflammatory origin and modulate neutrophil function, which plays a key role in inflammation, its resolution, and inflammasome formation, leading to increased inflammatory cytokine production. Anti-inflammatory drugs aid in diabetes management by preventing inflammasome activation. Moderate exercise exerts anti-inflammatory effects, enhances neutrophil function, and is a recommended non-pharmacological treatment for myocardial infarction. This study evaluated the effects of cardiovascular rehabilitation (CR) on inflammasome-related mRNA expressions in neutrophils from diabetic myocardial infarction patients. The study included 35 patients, aged between 40 and 70 years, who were followed at the outpatient clinic of the Dante Pazzanese Institute of Cardiology (IDPC). Patients were divided into four groups. (1) Myocardial infarcted (MI), (2) MI with T2DM (MI + T2DM), (3) MI and CR (MI + CR), and (4) MI with T2DM and CR (MI + T2DM + CR). The CR program consisted of aerobic and resistance exercises of moderate intensity, performed for 1 hour, totaling 37 sessions. Neutrophils were collected from the patients' peripheral blood at the beginning and end of the study period. The mRNA expression of genes involved in inflammasome activation (*IL-1β*, *TLR2*, *TLR4*, and *TLR9*) was assessed using RT-qPCR. Data were presented as mean ± standard deviation. The nonparametric Mann-Whitney test was used for comparisons between groups, and the Wilcoxon test was used for comparisons between phases (pre- and post-intervention). No changes were observed in the mRNA expression of genes involved in inflammasome activation in the neutrophils of patients from the four studied groups, either before or after participation in the CR program. We conclude that the CR program does not affect mRNA expression of genes involved in inflammasome activation in neutrophils. (Funding FAPESP 2016/22073-8, CAPES, CNPq).

P-38-029
Impact of shift work on neutrophil function and cell death in military police officers

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Military police work is a high-risk profession due to daily exposure to violence and human brutality. As a result, it is considered one of the most stressful occupations and is associated with several chronic and metabolic diseases. The immune system plays a crucial role in this context, as it is responsible for the body's defense mechanisms and is modulated by hormones and plasma mediators released during stress and the circadian cycle. Neutrophils, the most abundant immune cells, are essential for pathogen recognition, destruction, and the regulation of inflammatory processes. However, the impact of police work on neutrophil function and cell death remains unclear. This study aimed to investigate the effects of work shift and service time on neutrophil function and death in military police officers from the Radio Patrol 190 unit in downtown São Paulo. Blood samples were collected from 96 volunteer officers at rest, before their work routine. Participants were divided into four groups based on years of service: < 3 years, 3-10 years, and >10 years. The analyzed neutrophil functions included migration, phagocytosis, reactive oxygen species (ROS) production, and cytokine release. Additionally, cell death parameters such as membrane integrity, DNA fragmentation, and mitochondrial membrane potential were evaluated. Our findings demonstrated no significant differences in neutrophil function and death based on years of service. However, shift work significantly influenced these parameters. Neutrophils from officers working night shifts exhibited increased ROS production, higher DNA fragmentation, and reduced secretion of pro- and anti inflammatory cytokines (TNF-α, IL-1β, IL-6 and IL-10) following LPS stimulation. This cytokine reduction, combined with enhanced neutrophil apoptosis, may contribute to impaired inflammatory regulation, potentially increasing susceptibility to metabolic and autoimmune diseases (Funding FAPESP 22/05414-7, CNPQ and CAPES).

P-38-030
The relationship between anti-inflammatory pro-resolving lipid mediators and Behçet's disease

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Inflammation is a key biological process that protects against tissue damage and pathogens while maintaining homeostasis. Its

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resolution phase involves pro-resolving mediators such as lipoxins, resolvins, protectins, and maresins, synthesized from polyunsaturated fatty acids (PUFA) in macrophages. These mediators have anti-inflammatory effects and play a role in resolving inflammation. If acute inflammation is not controlled, chronic inflammation can develop, leading to pathological conditions and tissue damage. Behçet's disease (BD) is a recurrent, multisystemic inflammatory vasculitis affecting joints, eyes, blood vessels, the nervous system, and the gastrointestinal tract, along with oral and genital ulcers. Despite research on inflammatory molecules, no biomarker has been identified to predict disease progression or organ involvement. Moreover, no studies have investigated the relationship between BD and pro-resolving lipid mediators. Our study included 48 BD patients, 16 age- and gender-matched healthy controls, and 16 ankylosing spondylitis (AS) patients as a patient control group. Lipoxin, Maresin, Protectin, and Resolvin levels were assessed using immunoassays. Lipoxin levels were significantly lower in the BD group ($p = 0.015$) and AS group ($p < 0.001$) compared to healthy controls and lower in AS patients than BD patients ($p = 0.038$). Maresin levels were significantly lower in AS patients compared to BD ($p = 0.026$) and healthy controls ($p = 0.012$). Protectin levels were significantly reduced in BD ($p = 0.048$) and AS patients ($p < 0.001$) compared to healthy controls and were lower in AS patients than BD patients ($p = 0.032$). Lower PLM levels in patient groups suggest dysregulated inflammation resolution. These findings highlight the potential role of PLMs in BD and the anti-inflammatory effects of colchicine used in its treatment.

P-38-031
The association between l-homocitrulline levels and impotence in man

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Erectile impotence (EI) is a widespread issue that affects a considerable portion of the male population, with its underlying causes stemming from a multifaceted array of physiological (such as neurogenic and arteriogenic)mechanisms. Homocitrulline (Hcit) is one methylene longer than citrulline and it can be synthesised from lysine residues in the polypeptide chain nonenzymatically by the action of cyanate or by post-translational modification. In this study, we employed the advanced analytical technique of liquid chromatography-tandem mass spectrometry (LC-MS/MS) to examine the levels of homocitrulline in men and explore its potential relevance to erectile impotence. 50 patients with EI and 50 individuals without EI were included in the study. Serum levels of homocitrulline and lysine were quantified using a LC-MS/MS system from AB Sciex, specifically the API 3200 model (Applied Biosystems/MDS Sciex). Biochemical parameters of the patients were assessed with the Beckman Coulter AU 5800 autoanalyzers (Beckman Coulter, Brea, USA). Additionally, C-reactive protein (CRP) concentrations were determined using the IMMAGE 800. There was no significant difference between the median age range of the patients (46 yrs (35-60) for patients and 50 yrs (37-60) for controls, $p = 0.919$). Homocitrulline levels were quantified as 1385 ng/ml (111-5510) in the patient group and 293 ng/ml (45-4260) in the controls, ($p < 0.001$). Lysine levels were 40.750 ng/ml (10.000-104.000) in patients and 55400 ng/ml (124.00-128.000) in controls ($p < 0.001$). Hcit/Lys ratio were 0.333 (0.003-0.441) in the patient group and 0.005 (0.001-0.126) in controls. Also, Hcit/Lys index was found to be strongly correlated with EHS score and there was no correlation with IIEF-5 score. Our findings indicate that homocitrulline levels are significantly elevated in patients with erectile dysfunction, suggesting that homocitrulline may be a useful marker for the diagnosis, and could be a treatment marker of this condition.

P-38-032
Cabernet sauvignon wine phenolics as modulators of 12-HETE, PGE2 and TXA2 production in human platelets

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Inflammation underlies the pathophysiology of numerous diseases, including neurodegenerative disorders, cancer, and cardiovascular diseases. Metabolites of arachidonic acid play a key role in inflammation. These bioactive compounds, including prostaglandins and leukotrienes, act as potent inflammatory mediators. Thromboxane A_2 (TXA_2) and prostaglandin E_2 (PGE_2) are products of the cyclooxygenase pathway of arachidonic acid metabolism, while 12-hydroxyeicosatetraenoic acid (12-HETE) is a product of the lipoxygenase pathway. The aim of this study was to investigate whether polyphenol-rich wines, renowned for their cardioprotective properties due to the French Paradox, can act as modulators of arachidonic acid metabolism, specifically, production of 12-HETE, PGE_2, and TXA_2. The impact on arachidonic acid metabolism was investigated *ex vivo* using human platelets. Platelets were pretreated with cabernet sauvignon wine samples, followed by induction of the inflammatory response with calcimycin (Calcium Ionophore A23187) and $CaCl_2$. Quantification of 12-HETE, PGE_2 and TXA_2 in the cell lysate was performed using LC-MS/MS.[1] Significant differences between the samples are observed in the inhibition of 12-HETE (non-active (n.a.) – 49.4%), PGE_2 (n.a. – 67.6%), and TXA_2 (n.a. – 48.6%) production. Quantitative analysis of 21 phenolic compounds was conducted using HPLC-UV/VIS.[2] The most abundant phenolic compounds were gallic acid, malvidin-3-*O*-glucoside and catechin (up to 103, 80.0, 57.6 mg/L, respectively). A modest correlation between phenolic content and biological activity was observed. The obtained results further support the hypothesis that moderate wine consumption may exert beneficial health effects, potentially contributing to the modulation of pathological processes linked with inflammation and cardiovascular diseases. References: 1. Lesjak et al. *Lebenson. Wiss. Technol.* 53.2 (2013): 530-539. 2. Majkić, et al. *Int. Food Res.* 121 (2019): 151-160.

LB-R-38-01
Prevalence and clinical characteristics of patients with asthma–bronchiectasis overlap in Algeria: a retrospective study

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Asthma and bronchiectasis are both chronic airway diseases with symptomatic and clinical outcomes similarities. Overlap of these two conditions would imply more worsening of symptoms and life quality. However, data about asthma–bronchiectasis overlap

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(ABO) are lacking in Africa and specifically in Algeria. Our study aims to assess its characteristics and to estimate its prevalence in a cohort of an asthmatic population. Medical records of asthmatics attending the pulmonology department of the public health center of Azazga city (Algeria), were retrospectively analyzed. Data about clinical characteristics, exacerbation, ICU and hospital admission were extracted. Chi-squared test was used to assess the links between categorical variables, and Student-t test to compare means. Data analysis was performed using SPSS. Of the 203 included patients, 83 (40.9%) were males, and the mean age was 51.87 ± 16.56 years. ABO was found in 11 patients with a prevalence of 5.4%, however, no significant differences were found in sex ratio, smoking status, comorbidities or symptoms ($p > 0.05$). Interestingly, pulmonary fibrosis was more important (67%) in the ABO group ($p < 0.001$) but not allergy (3%, $p = 0.047$). The treatment was similar between the two groups, including inhaled corticosteroids, short- and long-acting beta agonists, or leukotriene receptor antagonists ($p > 0.05$). Moreover, respiratory infections were linked with more exacerbations in ABO group ($p = 0.036$). Bronchiectasis diagnosis in patients with asthma is a key for providing an adequate management to better control symptoms, exacerbations and/or infections.

LB-R-38-02
Tear pharmacokinetics and systemic exposure of dexamethasone canalicular inserts in rabbits

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A dexamethasone canalicular insert, Dextenza®, has been used to treat anterior inflammation of the eye, including keratoconjunctivitis sicca. This study aimed to investigate the tear pharmacokinetics and systemic exposure of dexamethasone in New Zealand White rabbits after inserting the preparation into the punctum and to correlate the data with the *in vitro* dissolution test. Dextenza was inserted into the puncum of rabbits. A paper strip serially collected tears, and dexamethasone concentrations in tears and plasma were measured using HPLC-MS/MS. The time courses of tear and plasma dexamethasone concentrations were characterized. The release of dexamethasone from the insert to tears in rabbits was completed in ~5 days, much faster than in humans and dogs (30 days). The time course of plasma dexamethasone concentration was fully characterized, in contrast to the fact that systemic exposure was merely observed in the other species. The present results might be attributed to the anatomical structure of the lacrimal sac beneath rabbits' canaliculi. The *in vitro* dissolution pattern represented an excellent correlation with the *in vivo* release in tears. We first examined the pharmacokinetic study of canalicular insert in rabbits, which could be applied to the other insert studies in the species. *The authors marked with an asterisk equally contributed to the work.

LB-R-38-03
The systemic immuno inflammation index (SII) as a biomarker for predicting the severity of acute appendicitis

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Acute appendicitis (AA) is the main cause of acute abdominal pain. Despite the classic symptoms, early identification can sometimes be difficult. Several scoring systems have been developed to improve the diagnosis of AA; however, they are unable to distinguish between simple and complicated appendicitis. The systemic inflammation index (SII) is a new biomarker based on platelet, neutrophil and lymphocyte counts. The aim of this study was to investigate the role of the SII in predicting the severity of acute appendicitis in adults. This was a retrospective study of 202 patients with AA. According to the Alvarado score, the study population was divided into two categories: uncomplicated (UAA, $n = 100$) and complicated (CAA, $n = 102$). Biological tests were performed, including complete blood count (CBC), SII (platelets × neutrophils/lymphocytes), platelet-lymphocyte ratio (PLR), neutrophil-lymphocyte ratio (NLR), biochemical and coagulation tests. SII rates in patients with CAA were significantly higher than in those with UAA. SII had the highest discriminatory capacity for determining CAA compared with other biological parameters ($AUC = 0.694$), Multivariate logistic regression analysis of factors affecting the diagnosis of CAA showed that the SII, NLR, PLR and CRP were statistically significant. Analysis of the correlation between the biomarkers SII and the ALVARADO score showed significantly positive correlations. A severity score was developed from the clinical and biological variables that were significant. The variables related to the factors possibly associated with the severity of acute appendicitis were obtained. Multivariate regression analysis was performed. Final score points were assigned according to the values of the regression coefficient. This model showed high discriminatory capacity to differentiate CAA from UAA with an AUC of 0.857. Our results highlight the value of SII as a promising and easily available biomarker that can be used to assess the severity of AA.

LB-R-38-04
Therapeutic potential of isolated mitochondrial transplantation derived from adipose stem cell spheres for immunomodulation and tissue repair

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Mitochondria, essential for regenerative capabilities, provide the necessary energy for cell function. Previously, we observed that chitosan-induced adipose-derived stem cell (ASC) spheres exhibit a unique and compact mitochondrial morphology characterized by densely packed cristae, enhanced mitochondrial activity, and increased ATP production. Epigenetic modification of H3K27me3 and the involvement of PPARγ were identified and

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validated using the EZH2 inhibitor GSK126 and the PPARγ agonist rosiglitazone. Mitochondria isolated from these spheres (Sph exMito) exhibited superior structural stability and delivery efficiency, suppressed pro-inflammatory cytokine expression in LPS- and TNFα-induced inflamed cells, and rescued damaged neural cells [previously published in: Chang MM et al. (2025) *Stem Cell Res Ther* 16, 129]. Moreover, endothelial dysfunction is a hallmark of various life-threatening vascular diseases. Compared to mitochondria isolated from ASCs, Sph exMito retained better structural integrity and membrane potential. These mitochondria were efficiently internalized by inflamed human umbilical vein endothelial cells (HUVECs), fused with endogenous mitochondria, and subsequently restored mitochondrial morphology and energy metabolism. Treatment with Sph exMito also promoted vascular-like tube formation in HUVECs and reduced the adhesion of pro-inflammatory M1 macrophages. Additionally, extracellular vesicles (EVs) secreted by inflamed HUVECs induced M1 polarization in macrophages. In contrast, EVs derived from Sph exMito-treated HUVECs reprogrammed macrophages toward an anti-inflammatory M2 phenotype. This study demonstrates that mitochondrial transfer from ASC spheres exerts dual therapeutic effects: restoring endothelial function and modulating immune responses. The EZH2–H3K27me3–PPARγ axis plays a critical role in enhancing mitochondrial function and delivery efficiency, offering a promising cell-free therapeutic strategy for tissue regeneration.

LB-R-38-05
Transbuccal films in veterinary medicine: the clinical effects of dosage forms on hematological and biochemical parameters of blood

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In modern veterinary pharmacology and therapy, the ability to control dosage, delivery, release of a drug, as well as the absence of post-injection reactions is extremely important. There is a need to improve technological solutions in the creation and development of new dosage forms – a convenient transbuccal drug delivery system with controlled release, taken without water, without the use of auxiliary instruments, with minimal time and effort on the part of animal owners and medical personnel. The purpose of a comparative study of two dosage forms is to identify a possible reaction of the body and changes in the clinical and biochemical parameters of the blood of experimental animals with a course of non-steroidal anti-inflammatory drugs (NSAIDs) with a single active ingredient, Carprofen. Blood parameters were monitored in 24 dogs from two experimental groups and 10 control animals. When examining the blood of animals in the 2nd experimental group (12 animals) that received the medicinal product Norocarp in the dosage form of tablets for oral administration, a reliable increase in the level of leukocytes by 4.54%, an increase in the level of glucose by 1.5 times, a decrease in the level of total protein by 4.9% in relation to the control were revealed, which indicates the development of stress hyperglycemia in dogs. The level of erythrocytes decreased in both experimental groups: with the drug Carprofen in the dosage form of transbuccal films by 11%; with the drug Norocarp by 15%; and the hematocrit level decreased by 22.5% in animals of the experimental group with the drug. This study has been supported by the RUDN University Strategic Academic Leadership Program.

Metabolism and metabolomics

P-39-001
Exploring Sirtuin 3 and sex-dependent differences in adipocyte differentiation and metabolic stress induced by western diet

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Obesity is a major global health concern, with its prevalence rapidly increasing worldwide. A Western diet (WD), combined with other factors, contributes significantly to the onset of obesity, a key risk factor for numerous health-compromising conditions. Excessive nutrient overload can lead to metabolic dysregulation in adipose tissue, including mitochondrial dysfunction. Sirtuin 3 (Sirt3) is a mitochondrial deacetylase with an important role in maintaining energy homeostasis and ensuring efficient mitochondrial activity. Additionally, sex differences in susceptibility to obesity-associated metabolic disorders are well known yet often overlooked in biomedical research study designs. The effects of Sirt3 in the context of sex-specific metabolic imbalance in adipose tissue have not been thoroughly investigated. Therefore, we aimed to analyze the interplay between Sirt3 and sex in mitigating metabolic stress induced by a WD in adipocytes. For this purpose, we utilized previously established cell lines of mouse embryonic fibroblasts (MEFs) derived from Sirt3+/+ and Sirt3−/− mouse models. We differentiated MEFs of both sexes into adipocytes using a differentiation medium containing 3-isobutyl-1-methylxanthine, dexamethasone, insulin, and rosiglitazone. After seven days, the differentiated cell population was exposed to palmitic and oleic acids in high-glucose conditions for six hours to mimic the conditions of a WD *in vitro*. Furthermore, the metabolic status of the differentiated adipocytes was assessed using a variety of molecular techniques, including qPCR, western blot, immunofluorescence, and metabolomic analysis. Our results revealed Sirt3- and sex-dependent differences in the adipogenic capacity of MEFs, with Sirt3 deficiency and the female sex significantly enhancing adipogenesis. These findings provide valuable new insights into the role of Sirt3 in sex-specific metabolic regulation and its contribution to the development of metabolic disorders associated with obesity.

P-39-002
Early dynamics of pancreatic β-cell adaptation to reduced mass and diet-induced stress

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Diabetes, affecting millions globally, is characterised by β-cell dysfunction and death, making it crucial to understand how remaining β-cells adapt and compensate under metabolic stress

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to maintain insulin homeostasis. We aim to explore how reduced β-cell mass affects early metabolic and molecular adaptation during the first weeks of diet-induced stress. To achieve this, we used the NSG RIP-DTR mouse model, that expresses the human diphtheria toxin receptor (DTR) driven by the rat insulin 2 promoter (RIP) in an immunosuppressed background. We ablated ~50% of β-cells through DT administration and exposed the mice to a high-fat diet (60% calories from fat, HFD) plus high glucose water (20%, HGW) for 4 weeks. Different metabolic parameters were evaluated at 2 and 4 weeks and transcriptomic analysis of isolated islets was performed. While serum cholesterol increased by 2 weeks, triglyceride elevation was observed only at 4 weeks in mice with intact β-cell mass, but not in DT-treated animals. Initially, DT-treated mice showed impaired glucose tolerance when challenged with HFD + HGW; however, by 4 weeks, they demonstrated metabolic adaptation. Transcriptional profiling of pancreatic islets revealed distinct temporal patterns of gene expression, with early increased β-cell differentiation and stress responses pathways transitioning to adaptive mechanisms by 4 weeks, particularly in pathways related to protein folding and glucose homeostasis. These findings suggest a remarkable adaptive mechanism of the remaining pancreatic β-cells under metabolic stress conditions that could be therapeutically targeted for maintaining β-cell function in diabetes treatment. This research was funded by the Romanian Academy and by the Romanian Ministry of Research, Innovation, and Digitization, PNRR program, CF 197-2022/PNRR-III-C9-2022-I8 (760059/23.05.2023) and from the grant RO-NO-2019-0544; 21/2020 BETAUPREG/the NO Grants 2014-2021.

P-39-003
Gut-cochlea axis and age-related hearing loss: exploring potential biomarkers

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Age-related hearing loss (ARHL) affects one-third of individuals over 65 years old and is linked to the onset and evolution of cognitive decline, possibly driven by inflammatory processes involving alterations in gut microbiota. Accordingly, ARHL, cognitive impairment, and alterations in gut metabolites may be interconnected within a context of an inflammatory response, suggesting the potential existence of an "auditory-gut-brain" axis. The molecular basis underlying this axis are being studied here by using the *Dusp1* knockout mouse, an experimental model of premature ARHL. The dual phosphatase DUSP1 (MKP1) is a key regulator of the stress-response kinases and its absence results in accelerated hearing loss, primarily driven by heightened levels of oxidative stress, inflammation and cochlear cell apoptosis. A total of 40 plasma and faecal samples were collected from mice of varying age (3 and 12 months), sex (male and female) and genotype (*Dusp1* knockout and wild type) and were subjected to comprehensive multi-omics analysis, including proteomics and metabolomics. Plasma samples were evaluated for the presence of 48 inflammatory cytokines using the Olink® Proximity Extension Assay technology. Faecal samples underwent gut metabolome profiling via LC-ESI(+/-)-MS. Results analyzed using an integrated multi-omics approach revealed altered levels of short-chain fatty acids, such as butyric, acetic, and propionic acids, in the ARHL model. These intestinal metabolites are key regulators of inflammatory response and immune activation. Consistently, Olink analyses showed elevated levels of inflammatory markers, including IL-10, MIP-1β and TNF-α, in *Dusp1* knockout mice at both ages studied. These findings suggest that gut metabolites along with inflammatory cytokines in plasma may contribute to cochlear inflammation and influence ARHL, serving as potential biomarkers for this condition. Funding: THEARPY PID2020115274RBI00 and PROHEAR-PID2023-147347OB-I00 (MICIU/AEI/FEDER, UE).

P-39-004
Sweet habits and their effects on pancreatic β-cells' adaptive mechanisms

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Elevated glucose consumption disrupts metabolic balance which challenges pancreatic insulin secretion mechanisms. Pancreatic β-cells can dynamically modulate their functional states to counterbalance increased insulin demand. However, this compensatory mechanism can become overwhelmed in diabetic conditions. We aimed to characterize the effects of sustained high-glucose exposure on adaptive mechanisms to stress of β-cells in the context of increased inflammation and high-glucose metabolic challenge. Female non-obese diabetic (NOD) mice, aged 4 weeks, were administered either high-glucose water (HGW) or standard water (NW) across variable time intervals. Pancreatic tissues and isolated islet underwent comprehensive fluorescence microscopic and transcriptomic evaluations, respectively, after acute exposure (3 and 4 weeks of HGW). We found that prolonged HGW exposure (24 weeks) of NOD mice significantly reduced diabetes progression. Both at 3 and 4 weeks of HGW exposure, mice presented a small, but unsignificant delay in glucose control, suggesting a compensatory metabolic adaptation to increased insulin demand. Using immunofluorescence, we found that β-cells presented dynamic expression of proinsulin and insulin at the different experimental times, pointing to a modification of secretion capacity. Transcriptomic profiling of islets from 3 and 4 weeks-HGW exposed mice unveiled substantial metabolic pathway alteration, as compared to NW. Pancreatic β-cells demonstrate adaptive metabolic strategies to sustain insulin production under chronic glucose-induced stress, potentially mitigating diabetic progression. Acknowledgements. This research was funded by

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P-39-005
Targeting mitochondrial dysfunction in obesity and diabetes: therapeutic insights into *Hypericum perforatum* and hypericin

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Mitochondrial dysfunction is a link between obesity and diabetes, contributing to metabolic disorders through oxidative stress and inflammation. Herein, we studied the therapeutic potential of *Hypericum perforatum* extracts and one of its active compounds, hypericin, in modulating mitochondrial stress and related pathways in models of obesity and diabetes. We used 3T3-L1 adipocytes treated with pro-diabetic stimuli in order to simulate conditions of mitochondrial dysfunction. Cells were treated with extracts of *Hypericum perforatum* and hypericin to assess their effects on mitochondrial function, oxidative stress markers, and inflammation. Techniques such as qPCR for gene expression analysis, ELISA for cytokine measurement, and mitochondrial assays were used. Additionally, the interactions of hypericin with proteins associated with mitochondrial stress were assessed by molecular docking studies. They modulated the expression of genes related to mitochondrial biogenesis and stress response, such as PGC-1α and CEBPα, while inhibiting the expression of pro inflammatory cytokines like TNF-α and IL-6. In this way, the molecular docking analysis revealed that hypericin has a strong binding affinity with the key mitochondrial stress regulators, pointing toward its role in mitigating mitochondrial dysfunction within metabolic disorders. These results therefore place *Hypericum perforatum* and hypericin as potential therapeutic agents in the mitigation of mitochondrial stress and its downstream effects in obesity and diabetes, thus meriting further investigation into their modes of action and applications. This project was supported by the Scientific and Technological Research Council of Türkiye (TÜBITAK) under Grant No. 124Z443.

P-39-006
Investigation of the effect of triglyceride and fenofibrate treatment in volume-loaded rat heart model

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Cardiovascular diseases lead the death statistics, both domestically and internationally. During heart failure, hypertrophic changes of the heart can be observed. The condition can also develop as a result of chronic volume overload. In heart failure, the energetic homeostasis of the cells is also damaged due to the altered substrate preference of cardiomyocytes. AMP-activated protein kinase (AMPK), which is regarded as the central regulator of cell metabolism, stimulates the processes associated with ATP generation by phosphorylating the proteins of the catabolic pathways depending on the intracellular energy level. The metabolic state of the cells can be further modified if the condition is aggravated by hypertriglyceridemia. To understand these processes, we examined the left ventricular tissues of the hearts of untreated healthy and surgically created rats with chronic volume overload. An additional group of chronically volume-stressed animals received triglyceride feeding and, at the same time, fenofibrate, which is used in human therapy. In addition to the operated animals, we also created sham-operated controls for each group of animals. We examined AMPK at the mRNA level using RT-qPCR, and the expression of several key enzymes involved in carbohydrate and lipid metabolism under the control of AMPK in the left ventricular tissues of the animals, as well as tissue lipid levels were determined. Our results indicate that although the presence of heart failure did not significantly affect the expression of the isoforms and target genes of AMPK subunits expressed in the heart, triglyceride feeding and fenofibrate used in addition to triglyceride markedly increased the expression of the catalytic subunit of AMPK. Shifting the metabolism in the glycolytic direction can have a protective effect, and the use of fenofibrate can be beneficial in the therapy of heart failure aggravated by hypertriglyceridemia.

P-39-007
The application of 3D fluorescence spectroscopy in tandem with machine learning in IVF process: a pilot study

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There is an increasing trend of infertility as well as increases in the number of performed *in vitro* fertilization (IVF) processes. The key processes that determine IVF success are the window of implantation, endometrial receptivity and the selection of a competent embryo. The 5-day embryo quality is assessed by its key morphologic features: blastocyst stage of expansion, inner cell mass quality and trophectoderm epithelium quality. The quality assessment made by an embryologist may be considered subjective but on the other hand, preimplantation genetic analysis is an invasive method and can be quite costly. Research now focuses on non-invasive methods for embryo selection. One of the promising non-invasive approaches is the analysis of spent culture medium metabolites. The aim of this pilot study is to explore the possible application of metabolomic fluorescent analysis of spent embryo culture medium in the prediction of implantation outcome. We analyzed synchronous fluorescence spectra of diluted and non-diluted spent culture media of 5-day embryos. Using biostatistical and machine learning methods we were able to find fluorescence spectral markers, which could potentially differentiate between successful and unsuccessful embryo transfer. We observed significant differences in the fluorescence ratios of 405/479 nm and 419/464 nm in the average

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spectra between the culture media of embryos with successful and unsuccessful transfers. Given the small amount of material (10 µl) and the complexity of data mining, 3D fluorescence analysis poses a challenge for further research, both in terms of analyzing a larger number of samples as well as optimizing the method itself.

P-39-008
Nuclear magnetic resonance-based metabolomics in children with growth retardation

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Growth retardation is characterized by a child's height or weight being below the normative community standards for their age. This condition may not only affect physical growth but is accompanied by an increased risk of health issues such as type 2 diabetes and cardiovascular diseases. Metabolomics, which involves the comprehensive analysis of small-molecule metabolites that serve as intermediates or end products of metabolic pathways, provides a valuable approach for understanding the biochemical alterations in such conditions. This study aimed to investigate the metabolomic alterations in children with growth retardation in comparison to age-matched, normally growing peers. Nuclear magnetic resonance (NMR)-based metabolomics analyses were performed on urine and serum samples obtained from 25 healthy controls and 30 children with growth retardation aged between 6 months and 18 years. Participants with secondary causes of growth retardation were excluded, and all participants had normal routine biochemical parameters. Data analysis with MetaboAnalyst 6.0 revealed significant metabolite differences between the groups. In serum samples, 4-ethylbenzoic acid and N-acetylgalactosamine levels were significantly altered in children with growth retardation. In urine samples, higher levels of creatine and alanine were observed in the patient group relative to the control group. Pathway analysis revealed disruptions in starch, sucrose, and galactose metabolism. Notably, the lower levels of alanine and creatinine observed in the urine of children with growth retardation compared to controls may reflect nutritional deficiencies. Although these findings are not yet incorporated into routine laboratory assessments for growth retardation, they highlight distinct metabolic alterations that warrant further investigation. Longitudinal studies are essential to elucidate the long-term metabolic consequences of growth retardation and their implications for clinical management.

P-39-009
Sirtuin 3 deficiency and sex differences modulate adipogenic potential in mouse embryonic fibroblast (MEFs)

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The increasing prevalence of obesity has become a major public health problem. It affects more than 670 million people worldwide and has reached epidemic proportions. The combination of a genetic predisposition with the consumption of a Western diet (WD) and a sedentary lifestyle leads to the development of obesity. Common complications associated with obesity include the risk of developing type 2 diabetes, non-alcoholic fatty liver disease and cardiovascular disease. Sex differences in obesity-related diseases show females often have a more favourable metabolic profile than males, due to differences in the distribution and function of adipose tissue. Adipose tissue is now recognised as an important regulator of energy homeostasis, with its dysfunction during metabolic stress being associated with many obesity-related diseases. Sirtuin 3 (Sirt3), a highly conserved NAD^+-dependent protein deacetylase, deacetylates and activates mitochondrial enzymes involved in fatty acid oxidation, oxidative phosphorylation and the antioxidant system in response to metabolic stress. However, its role in sex-specific susceptibility to metabolic stress is unclear. We used previously established mouse embryonic fibroblasts (MEFs) derived from Sirt3 WT and Sirt3 KO mouse models (1). MEFs of both sexes were differentiated into adipocytes for seven days using a special differentiation medium. The metabolic status of the differentiated adipocytes was analysed using qPCR, western blotting, mitochondrial respirometry and metabolomic profiling. The results showed that differences in the ability of MEFs to differentiate into the adipocytic lineage are Sirt3 and sex dependent, with Sirt3 deficiency and female sex significantly increasing the adipogenic potential of MEFs. These results provide new and valuable information for gaining insights into adipogenic potential of each sex across different genetic backgrounds. 1. Previously published in: Šimunić et al. (2024) Mech Aging Dev 222: 111996. *The authors marked with an asterisk equally contributed to the work.

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P-39-010
The relationships between fatty acids, fatty acids desaturase-2, and attention deficit hyperactivity disorder

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Fatty acids are associated with attention deficit hyperactivity disorder (ADHD); however, the mechanisms underlying this link remain unclear. This research investigated the potential roles of fatty acid desaturase-2 (FADS2) and fatty acids in ADHD, involving 170 children aged 6-12, including those with ADHD (n = 85) and healthy controls (n = 85). ADHD symptoms were assessed using Conners' Parent Rating Scale. 24-hour dietary intake record (three-day) was analyzed for fatty acid consumption. Plasma fatty acids were measured using gas chromatography-mass spectrometry (GC-MS). FADS2 protein levels were analyzed using an ELISA kit and FADS2 gene expression was analyzed using real-time PCR. A structural equation model (SEM) was used to determine the relationship between fatty acids, FADS2, and ADHD. Higher dietary intakes of omega-6, polyunsaturated fatty acids, plasma linoleic acid, and the omega-6/omega-3 ratio were found in the ADHD group, correlating positively with ADHD symptoms. Plasma levels of monounsaturated fatty acids (MUFAs: vaccenic, nervonic, oleic), gamma-linolenic acid, and docosanoic acid were lower in the ADHD group and were negatively correlated with symptoms. FADS2 gene expression was higher in ADHD patients and was positively correlated with dietary omega-6 intake. SEM analysis revealed that higher levels of linoleic acid, omega-6 fatty acids, and FADS2, along with lower levels of MUFAs, gamma-linolenic acid, and docosanoic acid were associated with ADHD symptoms. In conclusion, altered fatty acid metabolism, particularly increased omega-6 fatty acids and FADS2 expression, alongside decreased MUFAs and specific fatty acids, may contribute to ADHD symptoms.

P-39-011
Effects of psycho-emotional stress on oxidative stress, energy metabolism, and epigenetic modifications in the rat hippocampus and prefrontal cortex

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Stress, a non-specific response to external changes, significantly impacts biochemical and physiological mechanisms in the brain. Prolonged psycho-emotional stress due to social isolation alters metabolic pathways, affecting cognitive function and emotional stability. This study aims to assess the impact of stress on oxidative stress markers, energy metabolism, and epigenetic modifications in the prefrontal cortex and hippocampus. Experimental animals were subjected to 30-day social isolation. Quantitative changes in corticosterone, serotonin, and nitric oxide (NO) levels were analysed, along with markers of oxidative stress, antioxidant enzyme activity (SOD, catalase, glutathione reductase, glutathione peroxidase), and glycolytic and mitochondrial enzyme activities (hexokinase, aldolase, aconitase, succinate dehydrogenase, fumarase). DNA methylation/demethylation changes were evaluated by analysing S-adenosylmethionine (SAM) levels and enzyme activities of DNMT and TET demethylases. Social isolation induced significant hormonal changes, including a 45% increase in serotonin and a 72% decrease in corticosterone. NO levels increased by 62%, correlating with elevated oxidative stress and reduced antioxidant enzyme activity. Energy metabolism was disrupted, with increased glycolysis (76% increase in hexokinase activity) and decreased mitochondrial function (60.5% reduction in aconitase activity). SAM levels and DNA methylation enzyme activity were significantly reduced, suggesting epigenetic alterations. In conclusion, prolonged psycho-emotional stress triggers oxidative stress, alters energy metabolism, and impairs DNA methylation, potentially leading to neurotoxic effects and cognitive deficits. These findings highlight the necessity for further research into protective interventions against stress-induced neurodegeneration. *The authors marked with an asterisk equally contributed to the work.

P-39-012
Pasteur effect in leukocyte energy metabolism of patients with mild, moderate, and severe COVID-19

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The Pasteur effect in cellular energy metabolism is defined as the switch to anaerobic glycolysis due to alterations in ambient oxygen concentration or inhibition of ETC and oxidative phosphorylation. This study aimed to determine the effect of SARS-CoV-2 infection on the energy metabolism of leukocytes among COVID-19 patients at various stages. A total of 90 cases were analyzed in asymptomatic, mild, moderate, and severe COVID-19 patients. Levels of aerobic glycolysis intermediates and products of anaerobic glycolysis were measured in leukocytes with LC-MS/MS analysis. A decrease in the rate of aerobic glycolysis was found as the disease progressed (p-values

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< 0.001), while anaerobic glycolysis rate was unchanged in all study groups because lactate levels had no difference between asymptomatic, mild, moderate, and severe groups (p = 0.754). Moreover, the ratios of lactate to TCA cycle parameters demonstrated a significant reduction in the rate of aerobic glycolysis of COVID-19 patients with different stages (p-values < 0.001). Furthermore, ROC curve analysis for citrate, a-ketoglutarate, fumarate, lactate, lactate/citrate, lactate/a-ketoglutarate and lactate/fumarate had significant cut-off values in Moderate and Severe patients (p < 0.001). Our results suggest that the Pasteur effect from aerobic to anaerobic glycolysis was shown to be induced as the severity of COVID-19 progresses. *The authors marked with an asterisk equally contributed to the work.

P-39-013
The role of N-alpha-acytelarginine in blood pressure regulation in the spontaneously hypertensive rat

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The spontaneously hypertensive rat (SHR) is salt-sensitive, and treatment with L-NAME, a non-selective nitric oxide synthase (NOS) inhibitor, exacerbates its hypertensive phenotype by further reducing nitric oxide (NO) levels. This reduction leads to increased vascular resistance and elevated blood pressure due to diminished NO-mediated vasodilation. NOS is the enzyme responsible for producing NO from L-arginine. In HXB/BXH recombinant inbred (RI) strains, derived from SHR and Brown Norway (BN) progenitors, a quantitative trait locus (QTL) associated with blood pressure responses to L-NAME was identified on chromosome 4, with the peak linkage at 118 Mbp. Correlation analysis of the hepatic metabolome revealed the strongest association between L-NAME-induced blood pressure changes and N-alpha-acetylarginine (r = 0.56, p = 0.001). The abundance of N-alpha-acetylarginine in the liver is regulated by a metabolomic quantitative trait loci (mQTL) on chromosome 4, which overlaps with the L-NAME blood pressure QTL at 115.6 Mbp. Further correlation analysis of hepatic N-alpha-acetylarginine concentrations with liver mRNA expression identified a cis-regulated expression QTL (eQTL) for Nat8f1 (N-acetyltransferase 8, GCN5-related family member 1; 118.3 Mbp, r = -0.68, p = 2.72×10) and Nat8f4 (118.3 Mbp, r = 0.60, p = 3.82×10). These genes colocalized with the mQTL and blood pressure QTL, making them strong candidate genes. The availability of L-arginine is a key determinant of NO production by NOS. Increased acetylation of arginine may reduce its availability for NOS, thereby decreasing NO synthesis. Higher arginine acetylation leads to reduced NO bioavailability, impaired vasodilation, and increased blood pressure. These findings suggest that genetically determined arginine acetylation contributes to NO depletion and predisposes SHR to hypertension.

P-39-014
Cd36 regulates hepatic concentrations of ceramides and very long chain fatty acids: correlations with blood pressure and lipolysis

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The spontaneously hypertensive rat (SHR) harbors a deletion variant of the Cd36 (fatty acid translocase) gene, which has been identified as a genetic determinant regulating glucose metabolism, lipid metabolism, and blood pressure. However, the mechanisms through which Cd36 influences these metabolic and hemodynamic traits remain unclear. In this study, we analyzed the liver metabolome to identify metabolites directly regulated by Cd36. Hepatic Cd36 mRNA expression showed significant correlations with several metabolites, including PC 37:1 (phosphatidylcholine) (r = 0.57, p = 0.00085), CL 70:7 (cardiolipin) (r = 0.42, p = 0.018), PE 36:1 (phosphatidylethanolamine) (r = 0.61, p = 0.00026), Cer 41:2;O2 (ceramide) (r = 0.60, p = 0.00036), Cer 41:1;O2 (r = 0.59, p = 0.00048), Cer 42:1;O2 (r = 0.59, p = 0.00048), PI 36:1 (phosphatidylinositol) (r = 0.62, p = 0.00020), FA 26:1 (fatty acid) (r = 0.42, p = 0.018), FA 32:1 (r = 0.66, p = 0.00006), and FA 34:1 (r = 0.68, p = 0.000023). These metabolites are closely linked as metabolomic quantitative trait loci (mQTL) near the Cd36 gene locus. Our findings demonstrate that the Cd36 gene directly regulates the concentrations of these metabolites, which are significantly associated with basal and stimulated lipolysis in epididymal adipose tissue and in isolated adipocytes. Additionally, ceramides and ultra-long-chain fatty acids were found to correlate with blood pressure regulation. While ceramides are well-documented in their roles in lipolysis and blood pressure regulation, the involvement of ultra-long-chain fatty acids in blood pressure control remains largely unexplored. These results provide novel insights into how Cd36 influences lipid metabolism and blood pressure regulation. *The authors marked with an asterisk equally contributed to the work.

P-39-015
Unveiling the hidden dangers: micro/nanoplastic-induced disruptions in lipid metabolism and developmental toxicity in zebrafish embryos

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The effects of microplastics on endocrine disruption, neurotoxicity, and the reproductive system have been demonstrated in numerous studies. Additionally, micro and nanoplastics are known to impact lipid, carbohydrate, and protein metabolism. Microplastics/nanoplastics are substances that we are exposed to in many sectors and fields in today's world, where plastic production and consumption are continuously increasing. They are environmental pollutants resulting from the degradation and wear of plastic products. The

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effects of microplastic (MP) exposure, including oxidative damage, cytotoxicity, neurotoxicity, energy loss, apoptosis mechanisms, and impacts on the reproductive system, have been demonstrated in various model organisms. MPs accumulate in various regions of the body and, by penetrating biological membranes depending on their size, can interfere with biochemical pathways and affect cellular function. The zebrafish (*Danio rerio*) is one of the important model organisms used in development and toxicology studies. It is known that the accumulation of micro/nanoplastics in the organism over short, medium, and long periods leads to adverse effects on lipid metabolism. In our study, the effects of 100-nanometer polystyrene particle exposure on growth and development in zebrafish embryos were morphologically examined and visualized. Additionally, lipid accumulation in the exposure groups was investigated using oil-red-o staining compared to the control. This study is significant for understanding that MP/NP exposure disrupts embryonic development and leads to negative effects on lipid metabolism due to bioaccumulation, as well as toxicity related to accumulation in different tissues. Key words: zebrafish, nanoplastic, lipid, oil-red-o.

P-39-016
Metabolomic insights into infertility: distinct biomarkers in PCOS, DOR, and NOR

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Infertility affects approximately 15% of reproductive-age couples, with polycystic ovary syndrome (PCOS) and diminished ovarian reserve (DOR) being major contributors. Metabolomic analysis of follicular fluid provides insights into the underlying metabolic disturbances associated with these conditions. This study aimed to identify metabolic biomarkers distinguishing PCOS, DOR, and normal ovarian reserve (NOR) using an untargeted metabolomics approach. Follicular fluid samples from 119 participants (PCOS: $n = 39$, DOR: $n = 40$, NOR: $n = 40$) were analyzed using liquid chromatography–high-resolution mass spectrometry. Metabolites were identified through HMDB, MassBank, and MoNA databases, with pathway analysis conducted via KEGG. Statistical analysis was performed using R and Python, incorporating one-way ANOVA, t-tests, and Mann-Whitney U tests with False Discovery Rate correction. Significant metabolic alterations were observed across groups, with taurocholate, mycalemide, and N,N-dimethylglycine exhibiting strong discriminatory potential. PCOS patients showed decreased levels of taurocholate and trehalose-6-phosphate, whereas NOR patients had elevated N,N-dimethylglycine and argininosuccinate levels. The DOR group exhibited increased levels of 1-methyl-2-pyrrolidone and haplopine. These findings highlight distinct metabolic signatures in PCOS and DOR, suggesting potential biomarkers that could enhance diagnostic and therapeutic strategies. This study underscores the utility of metabolomics in precision medicine approaches for reproductive health.

P-39-017
Investigation of the effects of potassium chloride and sodium bicarbonate salts on metabolic activities and cellular protein concentrations in *Saccharomyces cerevisiae* cells

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High concentrations of salts are known to cause over accumulation of ions to levels that may cause the production of excessive reactive oxygen species and metabolic toxicity. Thus, high salt intake eventually leads to specific autoimmune diseases and cardiovascular diseases in humans. In this study, it was aimed to study the influences of common salts used in agriculture as fertilizers or natural pesticides which can affect human health upon exposure. Model organisms are essential when it comes to toxicological exposure and factors affecting human health. *Saccharomyces cerevisiae* is the model organism which has a considerable number of gene orthologs responsible for human diseases so it can provide similar cellular environment. The high concentrations of ionic solutes are known to reduce water activity and limit the cellular growth. However, there isn't any study showing the effects of high concentrations of potassium chloride (KCl) and sodium bicarbonate ($NaHCO_3$) salts on metabolic activity and cellular protein concentrations in *S. cerevisiae* cells. In this study, the effects of 0, 19, 44, 69 and 100 mM KCl and $NaHCO_3$ salts on metabolic activity and cellular protein concentrations were investigated by using MTT [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide] and Bradford assays. According to the MTT assay results, none of the KCl concentrations caused a significant decrease in metabolic activity compared to negative control ($p > 0.05$), while 69 and 100 mM of $NaHCO_3$ caused significant decreases ($p < 0.05$). On the other hand, the cellular total protein contents were unchanged by the increased concentrations of the KCl or $NaHCO_3$ ($p > 0.05$). To conclude, it can be said that high concentrations of $NaHCO_3$ were responsible for the decreases in metabolic activities while they didn't affect the cellular protein content. The findings obtained in this study provided evidence for the evaluation of the effects of environmental toxicity on biological systems by comparing the toxic effects of different salts on yeast cells.

P-39-018
Regulation of non-shivering thermogenesis though AKT pathway by piperine attenuates white adipose tissue loss in colon cancer-induced cachexia

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Increased non-shivering thermogenesis (NST) in white adipose tissue (WAT) is one of the significant phenomena of cancer cachexia. We observed changes in NST of WAT with piperine, a natural compound, as a novel therapeutic approach for cancer cachexia. We designed cancer cachexia *in vivo* and *in vitro* models using CT26 colon cancer cells. Piperine increased body weights and inguinal white adipose tissue in CT26-induced cachexia mice. Piperine also suppressed the expression of increased NST by

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uncoupling protein 1 and peroxisome proliferator-activated receptor gamma coactivator 1-alpha. AKT phosphorylation, known to inhibit NST, was decreased in WAT of cancer cachexia, whereas it was increased by piperine. Ly294002, AKT inhibitor, treatment increased NST and piperine did not affect the NST factors increased by LY294002. Hence, inhibition of NST by increasing AKT phosphorylation is suggested as a new approach for cancer cachexia, and piperine is proposed as a new therapeutic agent.

P-39-019
Effects of prenatal maternal smoking exposure on the newborn metabolomic profile: *in vitro* diagnostic NMR spectroscopy of umbilical cord blood plasma

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Maternal smoking continues to be a significant issue for newborns, leading to various negative outcomes both in the short term and long term, including throughout childhood and into adulthood. Maternal smoking during pregnancy disrupts placental morphology, which, in turn, restricts fetal-maternal circulation and fetal nutrition. We aimed to elucidate the effects of maternal smoking during pregnancy on newborn metabolism and metabolomic profiles. Accordingly, we conducted a targeted metabolomic analysis of umbilical cord blood (UCB) serum samples from newborns exposed to maternal smoking, utilizing a nuclear magnetic resonance (NMR) spectrometry device in the In-Vitro Diagnostic (IVDr) category developed specifically for clinical screening. The study included 37 newborn participants, 12 with maternal smoking exposure (MSE), and 25 healthy controls. A total of 144 metabolites, including lipoprotein-lipid derivatives, were quantitatively analyzed in UCB plasma samples by IVDr NMR spectrometry. Moreover, alterations in clinical and demographic data between the MSE and control groups and their correlation with the metabolites showing variation were also examined. In the comparison of metabolomics data between groups, glutamic acid ($p < 0.01$) and citric acid ($p < 0.05$) levels were found to be significantly higher in the MSE group compared to the control group. Conversely, levels of histidine ($p < 0.05$), glucose ($p < 0.05$), and VLDL-1 phospholipid ($p < 0.01$) were significantly lower in the MSE group. In clinical data group comparisons, the birth weight SDS ($p < 0.01$), and birth length SDS ($p < 0.05$) values were significantly lower in the MSE group compared to the control group. Weak positive correlations were observed between birth weight SDS with levels of glucose ($r = 0.29$) and histidine ($r = 0.24$). IVDr NMR spectrometry technology can be used functionally in advanced studies to investigate various chemical/toxic agents of both maternal and environmental origin on newborn metabolism.

P-39-020
Is xanthurenic acid 8-O sulfate an early marker of metabolic syndrome?

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A ketogenic diet (KD), described as high-fat, low-carbohydrate composition, causes metabolism to switch from glucose oxidation to lipid metabolism, increasing ketone body production as an alternative energy source. KD is studied for potential benefits in obesity, metabolic syndrome and neurodegeneration, but the effects of prolonged KD on skeletal muscle metabolism have not been fully explored. In this study, we investigated the changes of body mass and metabolic adaptations in skeletal muscle of 3 months-old C57BL/6 mice of both sexes – male (M) and female (F) – fed either a standard diet (SD) or a KD for 12 weeks. The KD included soybean oil, coconut oil and coconut fat and it contained 84% fat, 11% protein and 5% carbohydrate. The percentage change of body mass was calculated weekly. MALDI-TOF imaging mass spectrometry (IMS) was performed in negative mode and 300-700 m/z range on skeletal muscle tissue sections of both sexes and diets. Partial least squares discriminant analysis (PLS-DA), human metabolome database (HMDB) and METASPACE database were used for tentative metabolite identification. Results showed that KD M and F had a higher percentage change of body mass than SD M and F. Additional, KD M significantly gained mass earlier, after the 4th week of experiment, and KD F later, after 7th week. We detected separation of four groups (KD M and F, SD M and F) in metabolomics data. Xanthurenic acid 8-O-sulfate was one of the 15 putatively identified signals at m/z 307,97 and it was significantly higher in KD M compared to SD M. Xanthurenic acid 8-O-sulfate is considered to serve as a natriuretic hormone that enhances glycosuria. Since KD M showed both highest body mass percentage change and xanthurenic acid 8-O-sulfate levels compared to SD M, a potential correlation between the two is suggested what could point to xanthurenic acid-8-O-sulfate as an early marker for metabolic syndrome.

P-39-021
Rehabilitomics: metabolic effect of cardiac rehabilitation after acute myocardial infarction

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Ischemic heart disease is the leading cause of death from cardiovascular diseases worldwide. The beneficial effects of cardiac rehabilitation through physical exercise on disease progression in patients after acute myocardial infarction (AMI)

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are well known, while the metabolic effects remain unexplored. This study evaluated the metabolic responses to cardiac rehabilitation using MS-based metabolomics and lipidomics on dried blood spots (DBS). Seventeen patients with AMI underwent cardiac rehabilitation for three weeks. DBS samples were collected before and after the first week training, before and after the second week training, and the before and after the last week training. The comparison of pre- and post-training samples showed a positive long-term metabolic effect on energy metabolism, probably due to increased patients' performance after the entire cardiac rehabilitation. By contrast, lipidomics captured a short-term effect during the first week of training, highlighting a protective role of phosphatidylserines (PSs), which immediately increased after the first training. In animal models, it has been suggested that PSs decrease after AMI[1], and that PS supplementation has cardio-protection effect[2]. Thus, it seemed that a protective response after AMI and following cardiac rehabilitation has been established in our patients, probably because of PSS1 enzyme upregulation. Moreover, we found that the basal blood levels of N-acetyl-L-tyrosine (NAT) increased during rehabilitation. NAT has been shown to induce mitohormesis, a mitochondrial rescue response triggered by stress stimuli[3]. Take together, our results shed light on short- and long-term metabolic effects induced by cardiac rehabilitation in AMI patients and indicate NAT as potential biomarker of cardiac rehabilitation efficacy and as possible post-AMI therapy. References: 1 Wang J et al. (2023) Front Cardiovasc Med 10, 1122571. 2 Schumacher D et al. (2021) Int J Mol Sci 22, 4401. 3 Matsumura T et al. (2020) EMBO Rep 21, e49211.

P-39-022
Toward precision theranostics: deciphering the complexity of tumor microenvironment by quantitative and spatial metabolomics

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Senescence, an irreversible cell cycle arrest, can be readily induced in tumors by many chemo- and immunotherapies. The senescence-associated secretory phenotype (SASP) modifies the tumor microenvironment and is responsible for therapy resistance, while the metabolic role of SASP remains poorly understood. This study aims to quantitatively and spatially depict the senescent-cell metabolome to identify markers relevant for cancer diagnostics and therapy. Chemotherapeutic senescence induction in senescent and control two hepatocellular carcinoma cell lines, H-Ras and AMp19, were characterized by ^{1}H-NMR spectroscopy-based metabolomics. Mouse AMp19-cell subcutaneous xenograft tumors were subjected to validation of senescence induction with H&E, p21, p53 and Ki73. Tumor senescence radiotracer uptake area autoradiography and spatial metabolomics by timsTOF-fleX MALDI-2 imaging MS were analyzed with particular interest in low-molecular-weight metabolites and lipids. Senescent H-Ras-cell metabolomics highlighted changes in creatine, myo-inositol, and phospholipid pathways, emphasizing senescence's impact on cellular growth and extracellular matrix degradation[1]. Senescence radiotracer uptake *in vivo* co-localized with the spatial MALDI imaging-characterized metabolite distribution *ex vivo* indicated senescent region-specific feature distribution. Sphingolipid and phosphoethanolamine pathway alterations were characterized that are highly relevant for pre- and anti-inflammation regulatory omega-3 and -6 fatty acid balance in cell cycle arrest. Metabolomics characterization alongside *in vivo* imaging is an insightful approach to pinpoint the senescent tumor metabolic heterogeneity during cellular growth, energy, and repair pathways. As certain lipids modulate SASP, their metabolic interactions have a potential to guide therapeutic and diagnostic strategies targeting senescent tumor cells and mitigate their adverse effects. Reference: [1]Knopf P, et al. 2024 https://doi.org/10.1002/nbm.5157.

P-39-023
Glutamate and gamma-aminobutyric acid (GABA) dysregulation in adolescent and adult patients with major depressive disorder

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Gamma-aminobutyric acid (GABA) and glutamate are the primary inhibitory and excitatory neurotransmitters in the central nervous system, respectively, and their balance is crucial for normal brain function. Disruptions in this balance are implicated in neurological and psychiatric disorders, including major depressive disorder (MDD). While the traditional monoaminergic hypothesis of depression is being reconsidered, emerging evidence highlights the role of neuroplasticity and glutamatergic dysfunction. Acute and chronic stress can dysregulate glutamate release, leading to synaptic depression in the prefrontal cortex and hippocampus. This study aimed to compare plasma levels of GABA and glutamate in depressed adolescents and adults with healthy controls and to assess the effects of short-term antidepressant therapy during hospitalization. A total of 65 depressed adults and 48 adolescents were compared with 66 healthy adult and 28 adolescent controls. GABA levels were significantly lower in both adult ($p = 0.0435$) and adolescent ($p = 0.0043$) MDD patients, with no significant changes after short-term antidepressant treatment. Glutamate levels were significantly higher in adolescent MDD patients ($p = 0.0220$) but not in adults. However, antidepressant therapy increased glutamate levels in adults ($p = 0.0045$) while having no effect in adolescents. These findings support the involvement of GABAergic and glutamatergic dysregulation in MDD pathophysiology. However, interpreting peripheral

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neurotransmitter levels as direct indicators of brain function requires caution. Further research into these alterations could contribute to novel treatment strategies targeting glutamatergic signaling in depression. This project was supported by the EU NextGenerationEU through the Recovery and Resilience Plan for Slovakia under the project No. 09I03-03-V04-00207.

P-39-024
Lysine p-nitroanilidine triggers the unfolded protein response in rhabdomyosarcoma cells

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This study investigates whether lysine p-nitroanilidine (LPN) modulates the cytotoxicity of rhabdomyosarcoma (RD) muscle cells exposed to atorvastatin. We focused on muscle cells because statins, which inhibit cholesterol synthesis, can have unintended consequences on skeletal muscle tissue. Specifically, statin use has been linked to muscle damage, leading to weakness, pain, and in severe cases, rhabdomyolysis. Our approach employs an *in vitro* model of statin-induced muscle damage. Using this cellular model and untargeted metabolomics (GC/MS), we identified decreased levels of aminoadipic acid, a metabolite derived from lysine catabolism. Our objective is to investigate whether LPN, an *in vitro* inhibitor of lysine α-ketoglutarate reductase (first step in lysine catabolism), modulates the cytotoxic effects of atorvastatin. Notably, LPN in combination with atorvastatin decreased cell viability (crystal violet – MTT), increased apoptosis (PARP cleavage), and elevated levels of LC3B, GRP78, and CHOP proteins, indicative of unfolded protein response activation. Furthermore, we detected increased levels of pThr172-AMPK, a marker of disrupted cellular energetics. Given these findings, we assessed mitochondrial function and revealed that this lysine derivative alone impairs mitochondrial oxygen consumption rates. Finally, incubation of this lysine analog with HepG2 cells did not induce increased apoptosis or altered markers by WB, suggesting that the effects observed in RD cells may not be related to altered protein synthesis. Although cytotoxicity was not reduced by this inhibitor, these findings could be reinterpreted as a potential therapeutic strategy for rhabdomyosarcoma, a type of cancer with poor prognosis. In conclusion, LPN (alone or in combination with atorvastatin) enhanced the cytotoxicity of our statin-induced muscle damage model, and this effect was accompanied by or preceded the activation of the unfolded protein response and mitochondrial disruption.

P-39-025
Neonatal urine metabolic signature reflects systemic adaptations linked to preterm birth

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Preterm birth is linked to several long-term health complications. Characterizing the early metabolic changes involved would facilitate a better understanding of this complex phenotype. With this aim, the urinary metabolome of premature infants (n = 45) was compared with that of term neonates (n = 96), both at one month of age. Liquid chromatography coupled to mass spectrometry (LC-MS) and nuclear magnetic resonance (NMR) techniques were applied, leading to the identification of 521 and 74 compounds, respectively. Partial least square discriminant analysis (PLS-DA) revealed a clear and robust distinction between the two groups. In both approaches, models built were significant (pR2Y and pQ2 < 0.05) and with an extremely high performance (R2Y and Q2Y close to 0.9). Univariate analysis also revealed notorious differences in the urine composition between both cohorts, as almost half of the metabolites identified presented significant differences between groups. Some of the top discriminant metabolites included vitamin derivates (4-pyridoxate, 1-methylnicotinamide), amino acid metabolites (4-hydroxyproline, L-lysine) or compounds related to renal function (guanidinosuccinate, guanidinoacetate, urea, creatinine). Finally, multiblock analysis was performed to organize metabolites by biological function and ease interpretation. A distinctive preterm metabolic fingerprint emerged, involving nitrogen metabolism, growth, cell defense, nervous system regulations and both metabolic and microbiota alterations. These results suggest that the metabolic signature in neonatal urine could reflect the increased risk of preterm newborns for suboptimal development including impaired growth, increased adiposity, intestinal dysbiosis or poor neurological development. In conclusion, neonatal urine mirrors the impactful metabolic adaptations occurring in prematurity, which could hypothetically explain health complications linked to this phenotype.

P-39-026
Metabolomic profiling of a yeast model of Niemann-Pick type C1

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Niemann-Pick type C (NPC) disease is an autosomal recessive neurodegenerative disorder caused by loss-of-function point mutations in either NPC1 (95% of the cases) or NPC2, which are involved in lipid transport through the endocytic pathway. NPC is characterized by an accumulation of high amounts of lipids (cholesterol and sphingolipids) in the late endosomal/lysosomal network. We have previously shown that yeast lacking Ncr1p (NPC1 homolog) recapitulate features of NPC cells, including the accumulation of sphingolipids, trafficking defects, iron overload, and mitochondrial dysfunction, leading to a shortened lifespan [Previously published in: Vilaça R et al. (2018) Biochim Biophys Acta – Mol Basis Dis 1864, 79–88; Martins T et al. (2023) Int J Mol Sci 24, 6221]. In this study, we used mass

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spectrometry-based untargeted metabolomics to obtain the metabolomic profile of *ncr1*Δ cells. KEGG analysis revealed ten significantly enriched pathways in the mutant, including those related to the metabolism of amino acids, C5-branched dibasic acid, nicotinate and nicotinamide, 2-oxocarboxylic acid, thiamine, and carbon. Notably, several metabolites altered in *ncr1*Δ cells have been implicated in epigenetic regulation of aging, and this mutant exhibits changes in histone H3 that have been associated with cell death during chronological aging. These findings identify potential metabolite biomarkers that may contribute to the shortened lifespan of the yeast model of Niemann-Pick type C1. This work was funded by national funds through FCT – Fundação para a Ciência e a Tecnologia, I.P., under the project UIDB/04293/2020.

P-39-027
Analysis of mitochondrial respiratory function of blood cells in women with gestational diabetes: comparison with normoglycemic pregnant women

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Gestational diabetes (GD) is defined as a state of hyperglycemia registered for the first time in pregnancy. Reduced mitochondrial function has been observed in skeletal muscle and blood cells of individuals with type 2 diabetes, as well as placental tissue and skeletal muscle of women with gestational diabetes. The aim of this study was to explore the mitochondrial function of peripheral blood mononuclear cells (PBMCs) of healthy pregnant women and women with gestational diabetes mellitus. The study included 15 patients diagnosed with gestational diabetes, as well as 21 healthy pregnant women between 24 and 28 weeks of gestation. At the visit, body weight and height measurements, a 2-hour oral glucose tolerance test (OGTT), and venous blood sampling were conducted. Blood samples were collected in EDTA tubes, and PBMCs were isolated using LymphoPrep density gradient medium. Mitochondrial function of PBMCs was measured by high-resolution respirometry. Mean age (30.71 ± 4.97 years in control and 35.41 ± 5.31 in GD group) and body mass index (25.66 ± 4.8 kg/m^2 in control and 28.22 ± 4.48 in GD group) of participants were higher in GD group compared to healthy controls ($p < 0.05$). Mitochondrial respiration was lower in PBMCs of individuals with gestational diabetes, compared to healthy controls in ROUTINE respiration of intact cells (5.35 ± 0.26 in control and 4.5 ± 0.33 in GD group, $p < 0.05$) and complex I-linked oxidative phosphorylation capacity (7.58 ± 0.46 in control and 5.85 ± 0.71 in GD group, $p < 0.05$). In conclusion, mitochondrial function of peripheral blood cells of women with GD was impaired compared to normoglycemic pregnant women. As this is an easily measurable, minimally invasive parameter, it is a potential candidate for a biomarker that could be used clinically for screening, prognosis, or monitoring the effectiveness of therapy in gestational diabetes.

P-39-028
Potential serum biomarkers in bladder cancer by nuclear magnetic resonance (NMR) spectroscopy

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Bladder cancer (BC) is a widespread and vital disease, with 573 278 new diagnoses worldwide. The gold standard methods for diagnosing BC are urine cytology and cystoscopy. The cytopathology of urine specimens is a widely used non-invasive test for detection. Since cytology has low sensitivity, limiting its use in low-grade BC, new molecular-based tests are needed for the early diagnosis of recurrence in patients. In the literature, there are limited studies focused on non-muscle invasive bladder cancer (NMIBC) that investigate biomarker discovery and enhance our understanding of tumor progression. This study examined serum metabolites in NMIBC patients and controls to differentiate cancer progression and reveal the underlying mechanisms of bladder cancer. 100 serum samples ($n = 50$ cancer, $n = 50$ control) were analyzed using a 600 MHz IVDr nuclear magnetic resonance (NMR) spectrometry (Bruker BioSpin GmbH, Germany). Statistical analysis of the metabolites was performed using MetaboAnalyst 6.0. An orthogonal partial least squares discriminant analysis (OPLS-DA) model was employed to distinguish between NMIBC patients and controls. For pathway analysis, the KEGG metabolic pathway database was used. To investigate potential changes in the serum metabolic profile associated with cancer, NMIBC patients were compared to controls. In the serum samples from NMIBC patients, the levels of choline, alanine, histidine, leucine, tyrosine, glutamine, glycine, glutamate, and threonine metabolites were altered with cancer progression. The identified metabolites were especially associated with amino acid and fatty acid metabolism. The results indicate that specific serum metabolites can function as early-stage-based biomarkers in BC and provide new insights into the mechanisms underlying NMIBC. However, further studies are needed to explore their usability as potential biomarkers for the early diagnosis of patients with NMIBC.

P-39-029
AKR1B10 promotes metabolic reprogramming in hepatocellular carcinoma (HCC)

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Aldo-keto reductases (AKRs) are a superfamily of oxidoreductases that can utilize electrons from NADPH to reduce numerous substrates. AKR1B10 is one of the members of this family, which participates in the detoxification of carbonyl compounds, retinoic acid homeostasis and lipid metabolism. The

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role of AKR1B10 in gastrointestinal cancers differs according to the tissue origin. Thus, while we and others have shown a tumor suppressive role of AKR1B10 in colorectal cancer, many studies have reported that high AKR1B10 expression in hepatocellular carcinoma (HCC) is associated with worse prognosis. To mechanistically address this, we aimed to examine whether the expression of AKR1B10 can affect the metabolic characteristics of HCC cells. Evaluation of publicly available transcriptome data revealed that glycolytic HCC tumors with high expression of AKR1B10 showed worse survival than their oxidative counterparts. A metabolomics and fluxomics analysis was carried out in glucose and glutamine-starved HCC cell lines ectopically expressing AKR1B10 with differing metabolic characteristics (glycolytic or oxidative). The AKR1B10 expressing oxidative cell line (HuH7) revealed a slower Krebs cycle, poor energetics and increased glycolysis intermediates, all hallmarks of the Warburg effect. The AKR1B10 expressing glycolytic cell line (SNU423) retained high glycolysis despite glucose and glutamine deprivation. Thus, we have shown for the first time AKR1B10 expression may lead to metabolic reprogramming in HCC, particularly in relation to central carbon metabolism. The preferential activation of the Warburg effect with AKR1B10 expression in oxidative cells may explain the worse prognosis observed with the expression of this protein in HCC. This newly identified metabolic vulnerability can be therapeutically exploited in future studies.

P-39-030
High resolution lipidomic profiling of human adipose tissues from different anatomical locations

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Adipose tissue, the largest endocrine organ in the human body, plays a crucial role in energy storage, metabolic regulation, and thermogenesis. It also serves as a reservoir for environmental pollutants, drug metabolites, and other apolar lipophilic molecules. However, adipose tissue dysfunction is implicated in various pathological conditions, including obesity, metabolic syndrome, chronic diseases, and cancer. Adipose tissue is categorized into white, brown, and beige types, with a recently identified fourth type involved in hematopoiesis: the bone marrow adipose tissue (BMAT), whose characteristics remain to be fully elucidated. The present study aims to provide a comprehensive lipidomic analysis of adipose tissues from different abdominal locations (subcutaneous, mesenteric, omental, and perirenal) and from bone marrow (BM) obtained from healthy renal transplant donors ($n = 10$) and BM transplant donors ($n = 7$). Histopathological confirmation of the samples was performed using hematoxylin and eosin staining. Lipid profiles were analyzed using liquid chromatography-quadrupole time-of-flight mass spectrometry (LC-qTOF-MS) and visualized through principal component analysis (PCA). The findings revealed distinct lipid profiles among the different adipose tissues, highlighting their phenotypic diversity and providing a foundation for future research. These results may have significant implications for understanding pathological conditions such as hematological disorders, metabolic diseases, osteoporosis, and cancer.

P-39-031
The association between lipid levels and pulmonary function tests

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Lipid metabolism is one of the factors determining the progression of various lung diseases. This study explored the association between pulmonary function tests (PFTs) and biochemical parameters, including lipids, monocyte HDL ratio (MHR), glucose, and HbA1c in individuals undergoing routine health checkups. Methods: A total of 153 subjects' (51% male) cholesterol, LDL-C, HDL-C, triglyceride and glucose were analyzed using Abbott Architect c8000® auto-analyzer. HbA1c was run on Lifotronic® H9 HPLC. Monocyte count was calculated using the Abbott CELL-DYN Ruby® fully automated blood counter. PFTs were conducted using a spirometer, and data were analyzed via SPSS 27.0. The glucose, cholesterol, LDL-C, HDL-C, triglyceride, and HbA1c levels were higher in the obese and overweight group than controls ($p < 0.05$). Similarly, the glucose, cholesterol, triglyceride, and HbA1c levels were higher in diabetic and prediabetic groups than controls ($p < 0.05$). High lipid groups exhibited elevated levels of monocytes and MHR, with HDL being lower. Remarkably, smokers had significantly higher MHR (0.012) than non-smokers (0.010), and males (0.013) had higher MHR than females (0.008). PFT differences were significant for smokers, who showed lower FVC (3.70 ± 0.99L) and FEV1 (3.05 ± 0.77L) compared to non-smokers (FVC: 4.23 ± 1.16 and FEV1:3.44 ± 0.84L). Similarly, females had lower FVC (3.14 ± 0.59L), FEV1 (3.05 ± 0.77L), and PEF (5.1 ± 1.16L) than males (FVC: 4.23 ± 1.16L, FEV1: 3.44 ± 0.84L, PEF: 7.3 ± 1.89L). However, no significant differences in PFTs were observed across BMI, HbA1c, and lipid subgroups. In conclusion, body mass index, HbA1c, lipid levels, and smoking status significantly influence several biochemical and metabolic markers. Smoking and gender affect PFTs, while BMI and lipid levels do not significantly impact respiratory measures.

P-39-032
Changes in the profile of fatty acids and fatty acid derivatives in the liver of C57BL/6 mice subjected to long-term caloric restriction

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For decades, researchers have been searching for the most effective way to combat obesity, which is now a global health issue. Among the proposed dietary strategies, caloric restriction

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(CR) involves reducing energy intake by 10–30% relative to the body's energy requirements. This dietary intervention profoundly influences whole-body metabolism, leading not only to weight reduction through fat mass loss but also to the slowing of aging-related diseases, including neurodegenerative disorders, autoimmune diseases, and cardiovascular diseases. CR induces metabolic adaptations in response to fluctuations in the availability of essential nutrients. However, the effects of prolonged CR on hepatic fatty acid composition remain insufficiently characterized. The present study aimed to investigate the impact of sustained CR on hepatic levels of medium- and long-chain fatty acids, as well as fatty acid derivatives. The research was conducted on C57BL/6 mice (n = 24; 12 females, 12 males) subjected to lifelong 30% caloric restriction. Fatty acid composition was analyzed using gas chromatography, while fatty acid derivatives were quantified via liquid chromatography. The dynamic alterations in the hepatic lipid profile of labeled fatty acids suggest that lipolysis is actively engaged in the liver during fasting states. Furthermore, the data indicate the potential activation of de novo lipogenesis, likely serving as a compensatory mechanism to maintain energy homeostasis. Notably, a significant increase was observed in the hepatic concentrations of fatty acid derivatives with anti-inflammatory properties, including resolvin and lipoxin A4, whereas no elevation was detected in pro-inflammatory eicosanoids. These findings underscore the necessity of considering targeted supplementation strategies in the context of prolonged CR to optimize metabolic and inflammatory homeostasis. Previously published in: Palma J et al. (2023) Prostaglandins Other Lipid Mediat 169:106764.

P-39-033
Exploring the relationship between blood iron concentrations and the clinical course of multiple sclerosis

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Iron is the transition metal most abundant in nature, life, and also an essential cofactor of many cellular enzymes. Iron is essential for the general vital functions of every cell, as well as for certain neuronal activities. Excessive iron concentrations promote oxidative stress and mitochondrial damage, leading to cell death, including neuronal death. Neurodegeneration can therefore be defined by changes in ionic homeostasis and/or pro-oxidant-antioxidant balance, two states that differ significantly for different types of brain cells and also change with ageing. The findings clearly indicate that abnormal iron levels may contribute to the development of chronic diseases such as multiple sclerosis (MS). The pathogenesis of MS is complex and based on the interaction between genetic and environmental factors. The aim of this study was to look for an association between blood iron values and the health history and functional status of patients with MS. The study group consisted of 143 patients with a clinical diagnosis of MS. Determination of the presence of the test element in serum was performed using an ELAN ICP-MS mass spectrometer. Analysis of the concentration of the test element in terms of gender showed that men had significantly higher iron concentrations compared to women. It was noted that people with a family history of other autoimmune diseases have significantly higher iron concentrations. Also, significantly higher levels of iron concentrations are found in people who do not have de novo disease. Significantly lower levels of iron concentrations are also found in people with a family history of MS. It was also investigated that there is a statistical trend towards a negative weak correlation between iron concentration and age of onset of first symptoms. The blood iron concentration of the MS patients studied was not a differentiating factor between their functional status and disease course.

P-39-034
Loss of adenosine A3 receptor protects mice against high fat diet induced obesity

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During obesity, excess lipid accumulation leads to adipocyte hypertrophy and apoptosis in gonadal white adipose tissue (gWAT). Fatty acids and distress signals from dying adipocytes recruit monocytes and promote an M1 inflammatory shift in adipose tissue macrophages (ATMs). These M1-like ATMs engulf apoptotic adipocytes, release pro-inflammatory cytokines and resistin, triggering insulin resistance, while lipids from dying cells accumulate in the liver. Macrophages generate adenosine from ATP released during efferocytosis, and in brown adipose tissue (BAT), both neurons and adipocytes release adenosine. Adenosine regulates biological processes via adenosine receptors (ARs) in adipocytes and ATMs. A2Rs activate, while A3Rs inhibit adenylate cyclase, whose product, cAMP, promotes anti-inflammatory and thermogenic effects. A2AR activation protects mice from high-fat diet (HFD)-induced obesity. Here, we show that A3R loss partially protects mice from HFD-induced obesity and related pathologies despite similar food intake. Bone marrow transplantation experiments confirmed that hematopoietic cells were not involved. In wild-type mice, HFD reduced gWAT lipolytic enzyme expression, but this remained unchanged in A3KO mice. The absence of A3Rs did not alter thermogenic activity in gWAT or BAT, though BAT thermogenesis increased in wild-type mice. However, in inguinal WAT, thermogenic gene expression was significantly elevated in A3KO mice, suggesting enhanced browning. These findings indicate that A3R antagonists may offer a therapeutic strategy for obesity. *The authors marked with an asterisk equally contributed to the work.

P-39-035
The influence of copper concentration on selected clinical parameters in patients with multiple sclerosis

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Copper (Cu) plays a key role in the biosynthesis of neurotransmitters, which determines the proper conduction of nerve impulses. It protects cell membranes from damage and affects the myelination process of nerve fibers. The highest concentrations of copper are observed in organs characterized by high metabolic activity, such as the brain. It is essential for maintaining homeostasis, but excess copper can be toxic, promoting the initiation of inflammatory processes. Due to its properties, copper is a potential target for research on new diagnostic and therapeutic methods in the treatment of multiple sclerosis (MS). It has been shown so far that disorders of copper homeostasis often coexist with neurodegenerative processes. It

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has been studied that demyelination in the corpus callosum correlates with a significant reduction in the level of the soluble copper fraction, while the concentration of insoluble copper remains unchanged. It is hypothesized that the accumulation of insoluble forms of copper in the structures of the brain and spinal cord may contribute to the neuronal damage observed in MS, while the presence of more bioavailable forms of this element may exert a neuroprotective effect. The aim of the study is to assess the copper content in MS patients depending on selected clinical variables. The study group consisted of 143 patients with clinical diagnosis of MS, under the care of the Provincial Center for Demyelinating Diseases in Szczecin. Serum copper content was determined using the ELAN DRC-e ICP mass spectrometer. Statistical analysis did not show any statistical significance between the assessment of copper content in MS patients and the number of disease foci, the number of affected systems, the occurrence of de novo disease, the severity of relapses, kinship in the direct and collateral line, and the occurrence of autoimmune diseases. In men, compared to women, a statistical tendency towards increased copper content was observed.

P-39-036
Muscle hypertrophy and glucose homeostasis in Goto-Kakizaki rats: adaptive responses to mechanical overload despite insulin resistance

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Type 2 diabetes mellitus (T2DM) typically occurs in obese individuals, leading to muscle loss and impaired quality of life. The Goto-Kakizaki (GK) rat is a spontaneous T2DM model without obesity. Our group showed that despite muscle loss, GK rats exhibit hypertrophy of the EDL muscle after 7 days at the same magnitude as controls. This loss is linked to increased protein degradation, evidenced by elevated Murf-1 levels in the basal state. However, degradation appears partially compensated by increased total and phosphorylated mTOR, both at baseline and post-hypertrophy. This study evaluated EDL hypertrophy in control and GK rats and identified sustaining factors. Body mass analysis confirmed lower weight in glucose and insulin tolerance tests, plasma glucose/insulin levels, and HOMA index confirmed insulin resistance. EDL hypertrophy was induced via tibialis anterior ablation for 3, 7, 14, and 30 days. Hypertrophied and contralateral muscles were collected for wet and dry mass evaluation. The Ethics Committee of Universidade Cruzeiro do Sul (004/2024) approved this study. Results showed a progressive blood glucose reduction in GK rats following hypertrophy. At all time-points, wet and dry mass of contralateral and hypertrophied EDL in GK rats remained lower than in Wistar rats. However, the hypertrophy-induced muscle mass increase ratio did not differ between groups. Despite insulin resistance and lower baseline muscle mass, GK rats maintain hypertrophic capacity. Additionally, glucose reduction post-overload supports the hypothesis that muscle glucose uptake occurs independently of insulin action, possibly linked to muscle regeneration. Ongoing experiments assess muscle cross-sectional area and satellite cell activity. Progressive muscle growth suggests a sustained adaptive response to mechanical stimulus, showing GK muscles respond to overload like controls despite diabetes.

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LB-R-39-01
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LB-R-39-02
Metabolomics-based potential plasma metabolite biomarkers for metabolic syndrome and its five components: a case-control study

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Metabolic syndrome (MetS) is a global health problem, with prevalence estimates ranging from 20 to 30% among adults worldwide. MetS is characterized by components of abdominal obesity, hyperglycemia, hypertriglyceridemia, low high-density lipoprotein cholesterol (HDL-C) and hypertension. It is necessary to understand the complex pathophysiology of metabolic syndrome, a task that remains to be fully elucidated. In this regard, metabolomics has emerged as a pivotal tool, finding extensive application in the diagnosis of metabolic diseases, the identification of potential biomarkers, and the exploration of underlying pathophysiological mechanisms. In the present study, the MetS group comprised ten adults over the age of 60 who met at least three of the five components, while the control group comprised five adults over the age of 60 who did not have any of the five components. The groups were matched by age and gender, and shared a common geographic background, residing in the Marmara region. Plasma untargeted metabolomics was performed by ultra-high performance liquid chromatography tandem mass spectrometry (UHPLC-MS/MS) using orbital ion trap analyzer and operating in data-dependent acquisition (DDA) mode. In comparison with the control group; 45, 27, 10, 6 and 14 metabolites, particularly those related to fatty acid synthesis and the beta oxidation of long-chain fatty acids, were identified as being different in the abdominal obesity, hyperglycemia, hypertriglyceridemia, low HDL-C and hypertension components, respectively ($p < 0.05$ for all). One metabolite, N2-Acetylornithine, was found to be significantly lower in all five components of the MetS compared to the control group ($p = 0.025$, $\log_2$ fold change: -2.75). These potential metabolites need to be confirmed and quantified by targeted metabolomics studies. Furthermore, in order to elucidate their roles in the pathophysiology of MetS, it is necessary to conduct more in-depth studies with larger populations.

LB-R-39-03
Untargeted metabolomics reveals altered nucleotide and methionine metabolism in leukemia cells under serine/glycine restriction

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Although biochemical pathways and their components are well defined, how these pathways are regulated in different physiological conditions is still a subject of research. The aim of this study was to investigate the metabolic response of leukaemia cells to the restriction of serine amino acid, which has a function in many metabolic pathways. Differences in cell proliferation in serine, glycine and serine/glycine-free medium in K562 and U-937 leukaemia cell lines were investigated by cell viability assay. Phosphoglycerate dehydrogenase (PHGDH) and serine hydroxymethyltransferase 1/2 (SHMT1/2) gene expression was analysed in K562 cell line. Untargeted metabolomics was applied using LC-MS/MS. In K562 and U-937 cell lines, cell viability was significantly decreased in serine amino acid-free medium. Interestingly, in serine/glycine free medium, cell viability of K562 cell line was detected higher compared to U937 cell line. PHGDH and SHMT2 expression was detected significantly higher in K562 cell line in serine/glycine-free medium. Since SHMT1/2 genes are also linked to both folate and methionine cycle, the levels of purine, pyrimidine bases and methionine/SAM ratio were evaluated in the untargeted metabolomic analysis. Significantly, bases were detected more decreased in serine/glycine free medium indicating an increase in DNA replication in accordance with the proliferation assay. Moreover, methionine/SAM ratio was found slightly decreased in serine/glycine free medium suggesting epigenetic alterations might support increased cell proliferation in the absence of the amino acids. To conclude, we found that K562 cells better survive in serine/glycine medium compared to serine or glycine free medium. To understand changes in metabolites level in detail, isotope tracing experiment should be done. By elucidating the pathways that are induced with epigenetic changes upon serine/glycine deprivation in cancer cells, novel therapeutic targets might be identified.

Microbiology

P-40-001
Environmental factors governing killer toxin expression and competitive dynamics in mixed *Saccharomyces cerevisiae* communities

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The production and secretion of toxins by *Saccharomyces cerevisiae* represents a crucial biochemical defense mechanism that shapes microbial community dynamics. Our research investigates the

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molecular basis of these killer toxins and their role in mediating competitive interactions between different yeast strains. By characterizing both isolated and mixed yeast populations, we examine how the biosynthesis and secretion of these antimicrobial compounds influence population dynamics. The study focuses particularly on the biochemical parameters that regulate toxin production, including the expression of toxin-encoding genes and the post-translational modifications essential for toxin activity. Using fluorescently labeled *S. cerevisiae* strains and PCR-based genetic analysis, we identified distinct toxin variants and their corresponding immunity factors. The molecular characterization of these toxins was complemented by functional assays on both solid media and in liquid culture, allowing us to assess the kinetics of toxin production and its effects on susceptible strains. Our preliminary findings reveal that environmental conditions significantly modulate both the biochemical pathways of toxin synthesis and the sensitivity of target cells, suggesting complex regulatory mechanisms in toxin-mediated cell-cell interactions. This research provides insights into how biochemical warfare through toxin production influences microbial community composition, with implications for both industrial fermentation processes and medical applications.

P-40-002
Streptomyces netropsis IMV Ac-5025: whole-genome analysis and biosynthesis of metabolites with antimicrobial and elicitor properties

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Streptomyces species are key producers of antimicrobials and remain valuable sources for discovering bioactive compounds. This study aimed to conduct whole-genome sequencing and analysis of secondary metabolites of *Streptomyces netropsis* IMV Ac-5025 with antimicrobial and elicitor properties. Whole-genome sequencing was carried out using the Illumina NovaSeq 6000 and assembled with BacFlux v1.1.8. The analysis of the ethanolic extract of the strain was performed using a Vanquish UHPLC attached to a Q-Exactive mass spectrometer. Seeds pretreated with streptomycete metabolic complexes were germinated with *Rhizoctonia solani* CS3 101.169 to assess changes in hydrogen peroxide (H_2O_2) levels in wheat plants. H_2O_2 was quantified using a ClarioStar multi-channel reader. Morphometric parameters of pretreated wheat plants were measured at early ontogenesis. The genome assembly of *S. netropsis* IMV Ac-5025 resulted in 122 contigs, totaling 7 568 873 base pairs. Using antiSMASH, the analysis of biosynthetic gene clusters (BGC) identified 42 putative clusters, highlighting the strain's high biosynthetic potential (BGC of tiancimycin, aureothin, and althiomycin antibiotics). Other bioactive compounds, such as RiPPs (lanthipeptide-class-III, pristinin A3, citrulassin B), osmoprotectants (ectoine), siderophores (bacillibactin and EDHA), etc. were encoded. The presence of aureothin, distamycin, and isoaurostatin, was detected in the ethanolic extracts by LC-MS. The H_2O_2 levels varied from 119.36 to 239.68 nmol/g FW in wheat plants under infection-free conditions. Metabolic complex resulted in the reduction of elevated H_2O_2 levels in infected plants and prevented oxidative stress. These findings uncover new aspects of the strain's biosynthetic activity and hold promise for biotechnology. We hypothesize that streptomycete secondary metabolites have elicitor properties under biotic stress.

P-40-003
Binding analysis of potential inhibitors of bacterial conjugation to the coupling protein TrwB using EMSA and proteinase K digestion assays

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Antibiotics are widely used in modern medicine but abuse and misuse has led to the emergence of resistant bacteria; antibiotic resistance (AR) is a global health challenge. Type IV coupling proteins (T4CPs) are essential for bacterial conjugation, the main mechanism driving AR spread. Therefore, to control AR spread targeting, T4CP emerges as a promising strategy. Here, by proteolysis and electrophoretic mobility shift assays (EMSAs) we investigate the interactions between previously identified potential inhibitors and TrwB, T4CP of R388 plasmid. The soluble version of this membrane protein, TrwBΔN70, was digested with proteinase K in the presence/absence of the compounds to analyse whether the ligands induce a conformation change, rendering different proteolytic patterns. Some compounds prevented or reduced protein degradation, indicating that they could be conjugation inhibitors. We investigated if the compounds prevent TrwBΔN70 from binding DNA using EMSA. TrwBΔN70 was incubated with the plasmid pUC18 in the presence the compounds, followed by electrophoresis to discern DNA-protein complexes from free DNA. Only one compound induced partial dissociation of the complexes. These experiments are part of work to control the dissemination of AR by inhibiting specifically bacterial conjugation.

P-40-004
Targeting *Mycobacterium abscessus* salicylate synthase to disrupt iron acquisition mechanisms employing FDA-approved drugs

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Mycobacterium abscessus (Mab) is a nontuberculous mycobacterium (NTM) and classified as a rapid-growing mycobacterium (RGM). It is an opportunistic pathogen capable of invading human hosts, leading to severe infections, particularly in cystic fibrosis (CF) patients. The growing antibiotic resistance of Mab makes it increasingly difficult to treat with existing therapies, highlighting the urgent need for new strategies to combat this bacterium effectively. Certain genes within the Mab genome are designated as virulence factors, which are expressed differently depending on external conditions and play a crucial role in pathogenesis. One such virulence factor is the iron acquisition mechanism, mediated by mycobacterial

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siderophores. The biosynthesis of these siderophores begins with the enzyme salicylate synthase (SaS) [previously published in: Mori M. et al. 2023, Pharmaceutics 15, 502]. Since the development of novel inhibitory compounds can be a lengthy process, to accelerate the discovery of effective treatments, we adopted a drug repurposing strategy, aiming to identify existing FDA-approved drugs capable of inhibiting iron uptake in Mab. This approach involved an *in silico* analysis of the SaS crystal structure, followed by virtual screening of three FDA-approved drug databases. From this screening, eleven potential ligands were identified and subsequently tested in *in vitro* assays on the recombinant enzyme. Among these, fostamatinib, hydroxystilbamidine, and esomeprazole emerged as potent competitive inhibitors. Notably, hydroxystilbamidine also demonstrated moderate antivirulence activity in a mycobacterial model, suggesting its potential for use in combination with antibiotic treatments to enhance therapeutic efficacy. These findings confirm the potential of drug repurposing as a viable approach to target Mab infections. Further experimental validation and optimization of these inhibitors could pave the way for more effective treatments against NTMs infections. *The authors marked with an asterisk equally contributed to the work.

P-40-005
Development and optimization of magnetic bead-based tools for the isolation of *Coxiella burnetii* cells from tissue samples

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Coxiella burnetii, the causative agent of Q fever, presents significant diagnostic challenges due to its nonspecific clinical symptoms and variable serological responses. This study introduces a novel diagnostic approach using magnetic beads covalently coupled with *C. burnetii*-specific IgGs for isolating bacterial cells from infected tissues. Four bead types – epoxy, tosyl, amino, and carboxyl – were tested for optimal bacterial immobilization across varying concentrations of *C. burnetii* cells. After thorough washing, binding efficiency was assessed using protein quantification assays and *C. burnetii*-specific qPCR. Homogenized tissue samples were incubated with the functionalized magnetic beads, followed by magnetic separation and acidic elution to recover bound *C. burnetii* cells.Culture in axenic media was used to confirm viability. This study successfully developed and optimized a robust tool for isolating *C. burnetii* cells, offering potential advancements in diagnostic methodologies. Acknowledgments This study was financially supported by the project Vega 2/0125/24 and APVV-19-0519.

P-40-006
The RNA chaperone Hfq affects physiology and virulence of a human bacterial pathogen

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The RNA chaperone Hfq is one of the most abundant small proteins found in bacteria. While in Gram-negative bacteria like *Escherichia coli*, Hfq is known to promote the annealing of small non-coding RNAs (sRNA) and their target mRNAs, its role in Gram-positive bacteria remains poorly understood. In this study, we reveal unprecedented roles of Hfq in bacterial stress responses and virulence in the human pathogen *Listeria monocytogenes*. We demonstrate that Hfq expression is growth-phase dependent, with higher levels during stationary phase, suggesting a critical role under nutrient-limiting conditions. RNA-seq analysis comparing wild-type and Δhfq mutant strains confirms that Hfq is a key regulator of gene expression during stationary phase but not exponential growth. Remarkably, Hfq plays a previously unknown role in central metabolism by regulating acetyl-CoA production. Specifically, Hfq is essential for the expression of the pyruvate dehydrogenase complex, a critical enzyme complex converting pyruvate into acetyl-CoA, linking Hfq to the TCA cycle. We further show that Hfq is indispensable for oxidative stress resistance. Inactivation of hfq renders *L. monocytogenes* hypersensitive to sub-inhibitory concentrations of hydrogen peroxide (H_2O_2), impairing growth and viability. Our findings show that Hfq regulates catalase, a key enzyme for H_2O_2 detoxification, through a dual mechanism: indirectly influencing catalase transcription and directly stabilizing catalase mRNA under oxidative stress. Importantly, we demonstrate that Hfq is critical for *L. monocytogenes* survival in human macrophages, linking its oxidative stress resistance to virulence. Altogether, this work uncovers novel roles of Hfq as a central regulator of stress adaptation, metabolism, and virulence in *Listeria monocytogenes*, highlighting its importance in listeriosis pathogenesis. 1. Previously published in: Seixas AF et al. (2025) Free Radic Biol Med 227, 103-116.

P-40-007
Study of photodynamic inactivation and differential protein expression in rickettsial infections

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Rickettsiae (Alphaproteobacteria; Rickettsiales, Rickettsiaceae) are small (0.3–0.5 by 0.–2.0 μm) Gram-negative obligate intracellular bacteria that target and grow within the cytoplasm of eukaryotic vascular endothelial cells. They are transmitted by *Ixodes ricinus*, *Dermacentor reticulatus*, and *D. marginatus* ticks in Slovakia (Sekeyova, Z., Acta Virol, 2013). Carbon quantum dots (CQDs) are a fluorescent nanomaterial currently studied for use in photodynamic therapy (PDT), as an alternative for bacterial infections treatment. During PDT, CQDs generate superoxide, killing bacteria while mammalian cells remain intact. The cytotoxicity evaluation reveals that CQDs are bio and blood-compatible in a wide therapeutic window (Rajendiran et al. Polymers 2019, 11, 1670). Using RT-PCR, it was possible to demonstrate that the addition of CQDs to the culture medium

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and a phototherapy regime successfully lowered the R. conorii replication rate inside the cells. Our data showed a 98% antimicrobial efficiency after 72 hours applying the therapeutic regime. Study of the protein differential expression of *R. conorii*-infected VERO cells, performed using a Waters Synapt system and the ProGenesis QI software, revealed bacterial proteins related to growth, replication and ATP production as the most dysregulated. Among them, an increased expression of proteins involved in the Rickettsial lipopolysaccharide biosynthesis, and a lower expression of proteins involved in ATP synthesis and DNA repair was documented. In VERO cells, detoxification and metabolism were among the most affected cell functions. To monitor the cellular responses and the proteomic changes could be helpful to understand the mechanisms by which intracellular bacteria contend with cellular damage from PDT and how the host cells can take advantage of a treatment to overcome an infection. This knowledge could help to develop improved antimicrobial treatments to fight antibiotic resistance. APVV 23-00325, Vega 0011/25.

P-40-008
Changes in the microbiota of mice with allergic asthma are partially reversed by IGF1R blockade

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Asthma is a prevalent chronic condition that inflames and narrows the airways due to aberrant immune responses against allergens. IGF activity modulates immune responses in asthma. Functional IGF1R deficiency in mice attenuates allergic asthma symptoms. Although there is evidence linking asthma to gut microbiota composition through the so-called "gut-lung axis" and that IGF activity regulates gut microbiota diversity in mice, the relationship between IGF signaling and gut microbiota in asthma is largely unknown. Here we aimed to investigate the composition of the gut bacterial microbiota in a murine model of asthma, and how it is affected by therapeutic targeting of IGF1R. C57BL/6J mice were challenged by house dust mite (HDM) extract or PBS (control) for four weeks and therapeutically treated with the IGF1R tyrosine kinase inhibitor NVP-ADW742 (NVP) for the last two weeks, once allergic phenotype was established[1], and their fecal bacterial 16S rDNA was ultrasequenced. HDM-challenged mice showed a decrease in the α-diversity of the intestinal bacterial microbiome that did not significantly recover after NVP treatment, although a trend towards restoration was observed. The β-diversity was significantly different between the three groups, HDM, NVP and controls at the genus taxonomic level ($p < 0.008$), with the NVP mice situated in between, indicating a partial reversal effect mediated by IGF1R blockade. HDM-challenged asthmatic mice showed changes in relative bacterial abundance at the phylum, family and genus level, which were partially overturned by NVP treatment. NVP treatment also revealed specific changes in gut microbiota. Our results show that changes in the gut microbiota of allergic asthmatic mice are partly reversed by IGFR-targeted treatment, in addition to eliciting changes by itself. This suggests that the curative effects of anti-IGF1R treatment in asthma may be due in part to changes in the gut bacterial microbiome. ([1]*Biomedicines* 2021, 9: 912).

P-40-009
Screening of lactic acid bacteria strains with strong antagonistic activity, isolated from the vaginal microbiome

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The vaginal microbiome is a rich reservoir of lactic acid bacteria (LAB) with potent antagonistic activity against a variety of pathogens. The aim of the study was to isolate LAB from vaginal samples of healthy women of reproductive age and to assess various biological activities, to reveal strains with the highest antagonistic activity. The antagonistic activity against different *Candida* species was performed with the full diffusion method, while the antibacterial activity against some gram-positive and gram-negative bacteria was conducted with well-diffusion method. The caseinolytic activity of strains was examined electrophoretically. The cytotoxicity of LAB was studied on *Artemia salina* model. The impact of some ions and vitamins on the growth and antibacterial activity of LAB was examined. From the assessed 220 strains, only 6 were revealed which had the highest anti-yeast activity against clinical *Candida* species and antibacterial activity against bacterial test organisms. Antibacterial activity was observed after 24 hours of cultivation and persisted up to 48 hours, while antifungal activity appeared after 48 hours. Some of the six strains inhibited each other's growth and the anti-yeast activity of bacterial associations was significantly decreased. One of the strains totally hydrolyzed caseins after 9 hours. None of the selected strains possessed cytotoxic activity. The impact of some metal ions and vitamins on the antibacterial activity is strain-specific, in some strains they enhance antibacterial activity, while in others, they may have an inhibitory effect. The six LAB strains selected show promising potential for supporting women's health by defending against various infections. The work was supported by the Science Committee of RA, in the frames of the research project 23AA-1F010. *The authors marked with an asterisk equally contributed to the work.

P-40-010
Assessing the functionality of different soil types using a metagenomic approach

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Soil microbial communities play a key role in biogeochemical cycles, influencing the cycling of carbon, phosphorus, and nitrogen. Their metabolic activity determines soil fertility and the ability of ecosystems to recover from anthropogenic impacts. This study compares the application of a targeted sequencing approach using the 16S rRNA gene on the PacBio platform with shotgun sequencing, which can identify functional genes related to carbon (*gdh*, *mcrA*), phosphorus (*phoD*, *ppx*), and nitrogen (*nifH*, *nosZ*) metabolic pathways, while 16S rRNA gene analysis provides high-resolution taxonomic profiling of microbial communities. The study utilized soil samples characterized by physicochemical parameters, collected from Western Siberia,

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Russia—Luvic Chernozem, which are among the most fertile soils in the region. Through taxonomic analysis using 16S rRNA gene sequencing, we identified a "core" microbiota composition characteristic of this soil type and region, including *Streptomyces*, *Capillimicrobium* and *Conexibacter*. The next step involved analyzing functional genes in the studied microbiomes based on shotgun sequencing data. Using a compiled catalog of homologs, we searched for genes encoding essential enzymes for carbon, nitrogen, and phosphorus metabolism. Our findings indicate that the most abundant genes encode acyl-coenzyme A synthetase, phosphate importer ATP-binding protein, and sulfate adenylyltransferase. In contrast, the least abundant genes encode formamidase, 4-hydroxy-2-oxovalerate aldolase, and 7-cyano-7-deazaguanine synthase. As a result, we identified differences in microbiome composition and functional potential across different soil types using advanced sequencing methods, highlighting a promising approach for assessing soil functionality and propose strategies for soil microbiome management to enhance agroecological characteristics. This publication has been supported by the RUDN University Scientific Projects Grant System, project 202760-2-000.

P-40-011
Characterization of the ICEEc1 coupling protein from *Escherichia coli* ECOR31

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Antibiotic resistance (AR) is one of the greatest threats to human health. Conjugative plasmids and integrative conjugative elements (ICEs) constitute the main drivers of AR spread. Although ICEs outnumber conjugative plasmids, they still have been largely overlooked as vectors of AR[1]. ICEs are typically found integrated in the host chromosome and encode the machinery for their conjugation, which consists of: (i) proteins that form the relaxosome; (ii) proteins that form the Type IV secretion system (T4SS); and (iii) the Type IV coupling protein (T4CP) that links the relaxosome with the T4SS. T4CPs are essential for conjugation, but only a few (mainly plasmid-encoded) have been exhaustively studied, such as $TrwB_{R388}$, encoded by the conjugative plasmid R388, or $MobB_{CloDF13}$, which is one of the few T4CP encoded by a mobilizable plasmid, CloDF13. A homologue of this latest T4CP, $MobB_{ICE}$, was identified within the ICE*Ec1* from *Escherichia coli* ECOR31, which carries a widely distributed high-pathogenicity island among *Enterobacteriaceae*. $MobB_{ICE}$ shows 31% of identity and 42% similarity with $MobB_{CloDF13}$ and is predicted to contain four transmembrane domains (TMDs). $MobB_{ICE}$ was shown to be involved in the transfer of a plasmid carrying the origin of transference (*oriT*) present on ICE*Ec1*[2]. Here, through genetic engineering and conjugation assays, we show that $mobB_{ICE}$ requires $mobC_{ICE}$ to mobilize its *oriT*. The deletion of the TMDs-encoding region abolished mobilization. Additionally, we investigated if $MobB_{ICE}$ can substitute other T4CPs belonging to different types of mobile genetic elements. Finally, we purified a soluble version of $MobB_{ICE}$ and analyzed its DNA-binding and ATPase properties. A thorough characterization is essential to accomplish our ultimate goal, finding ICE conjugation inhibitors to reduce the spread of AR. References: 1. Botelho J & Schulenburg H. (2021). Trends Microbiol 29(1): 8-18. 2. Schubert, S. et al. (2004) Mol Microbiol 51: 837-848.

P-40-012
Hidden risks: antibiotic resistance and virulence profiles of bacterial strains isolated from operating room water systems in Romanian hospitals

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Filtered water systems in operating rooms, intended to ensure sterile environments, can act as reservoirs for microbial contamination, posing potential risks to patients, especially for the immunocompromised ones. This study presents the results of microbial identification, ability to form biofilms on inert surfaces, antibiotic susceptibility testing, virulence factors and gene profiling in strains isolated from operating room filtered water. 30 bacterial strains were isolated from water used in the operating room and identified through MALDI-TOF. The antibiotic resistance phenotypes were established by disk diffusion. Multiplex PCR reactions were performed for the detection of resistance determinants. The strains were tested for the production of different cell-associated and soluble virulence factors. The isolated strains were identified as: *Ralstonia insidiosa*, *Herbaspirillum huttiense*, *Cupriavidus necator*, *Acidovorax delafieldii*, *Shewanella baltica*, *Kocuria rosea*, *Derma nishinomiyaem*, *Moraxella oloensis*, *Xanthomonas campestris*, *Pseudomonas aeruginosa*, *Micrococcus lylae*, *Acinetobacter johnsonii*, *Acinetobacter lwoffi*, *Enterococcus faecalis*, *Staphylococcus warneri*. Many of these bacterial strains exhibited different antibiotic resistance phenotypes to beta-lactam groups and harbored the *bla* genes, *tet K*, *erm*, *aac* and *qnr*. The strains presented a large arsenal of virulence factors, caseinase, lecithinase hemolysins esculinase, lipase, gelatinase, DNase, amylase. The results showed that all bacterial strains expressed the ability to develop biofilms. This study reveals diverse bacterial species, including opportunistic pathogens, with varying antibiotic resistance profiles and the presence of clinically significant virulence factors. These findings emphasize the need for enhanced monitoring and stringent water filtration protocols to mitigate risks associated with waterborne pathogens in critical healthcare settings.

P-40-013
Killer activity and volatile organic compound profiles of yeasts from rowanberry and rosehip: potential for biocontrol applications

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Killer toxins, volatile organic compounds (VOCs), and other secondary metabolites produced by yeasts have promising

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applications as biocontrol agents in medicine, agriculture, and the food industry. This study investigated killer systems and VOC production in yeasts isolated from rowanberry (*Sorbus aucuparia* L.) and rosehip (*Rosa canina* L.) to explore their biocontrol potential. Yeast strains were obtained through a culture-dependent method and identified using molecular techniques. To verify the viral origin of killer phenotypes, dsRNA was extracted. VOC profiles of selected strains were analyzed using GC-MS. Most isolates (70%) from spontaneous fermentations of berries belonged to *Hanseniaspora uvarum* and *Metschnikowia pulcherrima*. The remaining 30% comprised genera such as *Pichia*, *Saccharomyces*, *Torulaspora*, and *Candida*. Approximately 20% of the isolates exhibited killer activity, which was tested at different levels of acidity. *Saccharomyces cerevisiae* exhibited the strongest killing activity, while *Pichia anomala* and *M. pulcherrima* showed broad-spectrum antagonistic activity. DsRNA-encoded phenotype was identified in *S. cerevisiae*, *S. paradoxus* and *T. delbrueckii* yeasts. VOC analysis revealed species-specific profiles: *H. uvarum* and *M. pulcherrima* predominantly produced ethyl acetate, whereas *S. cerevisiae* and *S. paradoxus* emitted 2-methyl-1-butanol. Killer strains of *S. paradoxus* produced higher amounts of ethyl acetate, ethanol, ethyl propionate, propyl acetate, 2-methyl-1-propanol, 3-methyl-1-butanol, and 3-methylbutanoic acid, compared to non-killer strains. These findings could lead to the development of new biocontrol tools that promote more sustainable and safer food production practices, as well as novel pharmacological agents for combating human pathogens. This project has received funding from the Research Council of Lithuania (LMTLT), agreement No S-PD-22-85. Part of the results are published in: Vepštaite-Monstaviče I et al. (2025) Foods 14, 288.

P-40-014
Interaction of OmpA protein of *Leptospira interrogans* with human host components

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Leptospirosis is a highly disseminate zoonosis of global importance. The etiological agent of the disease is pathogenic spirochete of the genus *Leptospira*. Elucidating the mechanisms of pathogenesis is crucial for disease prevention. Due to their location, outer membrane proteins (OMPs), including those containing OmpA domains, are attractive targets since they may elicit several activities, including adhesion, antigenicity and host cell stimulation. The objective of this project is to characterize functionally and immunologically the LIC_13056 protein from *L. interrogans*, which contains the OmpA domain. BLAST and Clustal Omega were used to construct a phylogenetic tree to compare the conservation of the LIC13056 gene among different *Leptospira* species. The *LIC_13056* gene was cloned into the pET-28a vector and expressed in *E. coli* C43 for 16 hours at 18°C. Purification was carried out using metal chelating chromatography. The purified protein, adsorbed in aluminum hydroxide, was used to immunize BALB/c mice for immunogenicity evaluation. The interactions of the recombinant protein with host components were performed by ELISA. The results showed that the LIC_13056 gene is well conserved among pathogenic species, indicating its potential involvement in leptospiral virulence. Animals immunized with rLIC13056 produced high antibody titers. The interaction of rLIC_13056 with α8 integrin, plasminogen (PLG), and components of the complement system, C4b, C8, C9, and C4BP were dose-dependent and saturable. Most important, binding to C9 component inhibits its polymerization and therefore the formation of membrane attack complex (MAC), contributing to bacteria immune evasion. The rLIC_13056 protein was able to capture PLG from normal human serum and convert it into plasmin. In conclusion, rLIC_13056 exhibited a broad binding spectrum with different human components, suggesting a potential role in host infection processes. Financial supported by: CNPq, FAPESP and Fundaçao Butantan.

P-40-015
Nanoscale fractionation: a powerful tool for profiling bioactive microbial metabolites

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In modern clinical practice, the increasing spread of drug-resistant microorganisms poses a serious threat. Since natural products form the basis of many existing therapies, developing novel antimicrobial agents with unique mechanisms of action is urgently needed. Traditional screening techniques, which uncovered many known classes of bioactive metabolites, now often lead to the rediscovery of the same molecules. One potential solution lies in exploring "microbial dark matter," representing the vast fraction of natural biodiversity not cultivated under standard laboratory conditions in liquid media. To access this untapped diversity, we employ alternative cultivation methods such as microencapsulation of microbial communities and co-cultivation, thereby revealing a portion of the "dark metabolome." For metabolic profiling of these complex communities, we developed an integrated fractionation platform that includes low-scale solid-phase extraction, followed by nanofractionation, bioactivity mapping using GFP-producing test strains, and molecular networking for dereplication. This approach enables rapid assessment of various cultivation conditions effect on the microbial secretome and facilitates the analysis of complex mixtures in low quantities. The work was supported by the Russian Science Foundation (project no. 25-14-00281).

P-40-016
Chemokine proteolysis is facilitated by *Vibrio vulnificus* insulin-degrading enzyme to suppress host immune responses during infection

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Vibrio vulnificus is a facultative pathogen responsible for primary septicemia, a rapidly progressing and highly fatal bloodstream infection in humans. To establish infection and persist within the host, it employs multiple virulence strategies, including the insulin-degrading enzyme (vIDE). Previous studies have shown that vIDE is regulated by IIAGlc, a key component of glucose

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transport, which is essential for bacterial survival in mouse models. This suggests that *V. vulnificus* exploits host metabolism through vIDE for both nutrient acquisition and immune evasion. Our study investigates the role of vIDE in virulence, demonstrating that it disrupts metabolic homeostasis by significantly altering serum insulin and glucose levels in infected mice. This metabolic dysregulation likely facilitates bacterial access to essential nutrients while simultaneously impairing immune defenses, thereby promoting pathogen survival. Additionally, we identified a novel function of the vIDE-IIAGlc complex in degrading chemokines, which are crucial immune signaling molecules. Our experiments confirm that this complex efficiently degrades chemokines both *in vitro* and *in vivo*, thereby weakening host immune surveillance and facilitating bacterial dissemination. By targeting chemokine signaling pathways, *V. vulnificus* evades immune *detection*, promoting systemic spread and progression to septicemia. Our findings establish vIDE as a multifunctional enzyme central to *V. vulnificus* pathogenesis. By degrading both insulin and key immune molecules, vIDE disrupts host metabolic and immune regulatory processes, thereby enhancing bacterial virulence and increasing infection severity. These insights reveal a previously unrecognized immune evasion mechanism and underscore the potential of vIDE as a therapeutic target. Identifying vIDE's dual role in modulating host metabolism and immunity provides a foundation for developing novel intervention strategies to mitigate this life-threatening infection.

P-40-017
Exploring the gut microbiome's role in stroke recovery: the impact of functional disability

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Stroke, the second leading cause of death worldwide, results in significant neurological impairment and long-term disability, thereby imposing a substantial public health burden. Emerging evidence suggests that the gut microbiome influences stroke outcomes. To explore the potential link between the gut microbiome and post-stroke recovery, we conducted a longitudinal study involving 32 stroke patients assessed at two time points: stroke admission (T0) and three months later (T1). Clinical data, along with faecal and blood samples, were collected. Stroke severity and disability were evaluated using the National Institute of Health Stroke Scale (NIHSS) and modified Rankin Scale (mRS), respectively. Faecal microbiome profiling was conducted using 16S rRNA gene sequencing, while nuclear magnetic resonance was used for plasma metabolomic profiling. Gut microbiome variation was strongly influenced by inter-individual differences (49%), with smaller contributions from mRS (1.34%) and antibiotic history (0.87%). Stroke patients with higher disability (increased mRS) displayed augmented Blautia proportions and decreased plasma metabolites. The stroke patients' follow-up between T0 and T1 showed a higher prevalence of *Prevotella* and a lower prevalence of *Ruminococcus* enterotypes. Additionally, patients exhibited a higher prevalence of the dysbiotic Bacteroides 2 community type during stroke events than a healthy Western cohort. No association was found between NIHSS scores at T0 and mRS scores at T1, indicating that the severity of neurological deficits did not predict functional independence. Despite the small sample size and inability to assess causality, our study showed a potential link between gut dysbiosis and stroke, offering insights as potential biomarkers for improving stroke management.

P-40-018
Epiphytic bacteria of *Ulva rigida*: isolation, characterization, and potential agricultural applications

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Macroalgae, widely distributed in fresh and saltwater environments, have been the subject of numerous studies, and their essential roles are in the balance of the ecosystem. Algae are members of the plant taxa, although they do not have the tissue systems of plants, and they benefit from the symbiotic relationships seen in all other plants with their epiphytic flora. The main benefits of epiphytic flora are the conversion of unassimilable elements, stress resistance, growth promotion, and defense against pathogens. *Ulva rigida* is an essential species with different metabolic activities and a wide distribution in the marine environment. One of the most critical points for successfully cultivating this species, which is also consumed as food with high nutritional value, is understanding its bacterial flora and functions. Therefore, the study aimed to determine the heterotrophic bacterial flora of *U. rigida* samples collected seasonally from the Gulf of Inciraltı and their growth promoter profiles. Bacterial diversity was determined by metagenomic analysis, and culturable flora was isolated. The ability of the isolates to N-fixing, P and K solubility, and produce siderophore, ammonia, and indole-3-acetic acid (IAA) were analyzed to determine their growth-promoting potential. Thirty of the 225 isolates obtained were identified as Gram-negative rods, and 110 of the remaining isolates were identified as suspected actinomycetes. Our study is the first to investigate the epiphytic bacterial flora of the *U. rigida* population of the Inciraltı/Izmir coast. The results obtained will form the basis for further studies on the cultivation and morphogenesis of the alga. TUBITAK supported this study under the COST Working Group Support Programme with project number 4. *The authors marked with an asterisk equally contributed to the work.

P-40-019
Porphyromonas gingivalis lht system is involved in iron acquisition from heme

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Porphyromonas gingivalis is the main etiological agent responsible for the initiation and progression of periodontal diseases in

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humans. It does not synthesize heme *de novo* and does not produce and use siderophores, therefore it relies on heme as the main iron source. For heme uptake, *P. gingivalis* uses mainly the Hmu system, composed of a novel hemophore-like HmuY protein coupled with the heme outer membrane receptor HmuR, and the Hus system involved in heme and protoporphyrin IX uptake. Moreover, *P. gingivalis* produces an Iht system, putatively involved in iron uptake but its role is not known. The Iht system is composed of 5 proteins: IhtA, a TonB-dependent outer membrane receptor, IhtB, a heme-binding protein, showing homology to CbiK chelatases, and IhtC-E proteins form a typical ABC transporter system. Using bacterial and protein research models, this study aimed to characterize the role of the Iht system in *P. gingivalis*. First, we performed global gene expression analysis using RNA-seq, which showed that deletion of the IhtB-encoding gene influences the expression of ~100 genes, including a decrease in the expression of genes encoding components of the Hmu system and other transport proteins, and an increase in expression of genes encoding proteins involved in the production of the cell envelope. This indicates the importance of the Iht system for bacterial virulence. Using membrane-associated IhtB and IhtA proteins produced in *E. coli*, we confirmed that IhtB binds heme, whereas IhtA does not. Moreover, using spectrophotometric characterization of recombinant IhtB protein verified its ability to bind heme and a decrease in the heme content in the sample after reduction of the IhtB-heme complex. Our study demonstrates that the Iht system may be involved in iron acquisition from heme and this system may be important for *P. gingivalis* virulence. This study was supported by grant no 2021/41/B/NZ6/00702 received from the National Science Center (NCN) Cracow, Poland.

P-40-020
Evaluation of the MUS-3600 Urine Analyzer for the diagnosis of urinary tract infection; a comparitive study with urine culture

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In the laboratory, urine culture is considered the gold standard for diagnosing urinary tract infections (UTIs). Most specimens sent for urine culture are negative. A rapid and reliable screening method will shorten the analytic time for negative specimens, reduce laboratory workload, and lower costs. This study aimed to evaluate the performance of the MUS-3600 in patients with suspected UTIs. Urine samples sent to the clinical microbiology laboratory were analyzed immediately after culturing using the MUS-3600 Urine Analyser. The MUS-3600 uses flow-type micro-imaging technology. For detection and classification, the system uses artificial intelligence identification. Culture results were considered positive if pathogen colony counts were ≥104 cfu/mL and negative if < 104 cfu/mL. In our study, the cut-off value for WBC was 39, with a sensitivity of 63.4%, a positive predictive value (PPV) of 50.8%, a negative predictive value (NPV) of 90.1%, and an elimination rate of 67.5%. Cut-off values for bacilli (Gram-negative bacilli) and suspected cocci (cocci) were 103 and 859, respectively. The elimination rate for bacilli was 70.4%, sensitivity 62.1%, PPV 56.9% and NPV 90.2%. For suspected cocci, the elimination rate was 62.8%, sensitivity 44.4%, PPV 34.5%, and NPV 84.9%. When WBC (>39) and bacillus (>103) were evaluated together as a screening method, the highest elimination rate of 77.2% (NPV 87.9%) was achieved. The elimination rate for this combination was 87.4% (NPV 93.9%) in males and 68.5% (NPV 82.1%) in females. In our study, the MUS 3600 analyzer was good at predicting UTI. However, further studies are needed on its use as a screening test before it replaces culture.

P-40-021
Studies on the mechanism of antibacterial activity of natural membrane-active peptides

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Membrane-active peptides (MAPs) represent a unique class of molecules capable of interacting with lipid membranes and disrupting their integrity through various mechanisms, enabling their potential applications as therapeutic agents. They represent an appealing alternative to protein-targeting antimicrobials due to their minimal induction of resistance and broad-spectrum activity. We previously reported gausemycins – glycolipopetides with a membrane-associated mode of action – and examined their pore-forming ability (Tyurin et al, 2021, Angew. Chem., 60 (34), 18694–18703, Kravchenko et al., 2024, J. Nat. Prod., 87(4), 664–674). In this work, we comprehensively examined mode of action for several MAPs including non-ribosomal peptides (peptaibols and gausemycins) and nisin-like ribosomally synthesized and post-translationally modified peptides. First, gausemycin-resistant bacterial isolates (from both clinical and laboratory origins) were studied to elucidate their resistance mechanisms. Moreover, we evaluated the antimicrobial spectrum and assessed membrane permeabilization and depolarization induced by the studied peptides. Our findings suggest that the selected peptides interact with various membrane-associated molecules, including cell wall biosynthesis precursors and membrane proteins, as part of their mechanism of action. The insights into the specific mechanisms of these peptides pave the way for the targeted design of membrane-active bioactive molecules. This work was supported by the Russian Science Foundation (project no. 25-14-00281). *The authors marked with an asterisk equally contributed to the work.

P-40-022
Developing a mock microbial community for soil metagenomics

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Uncovering the interdependencies between individuals, ecosystems and their inhabiting microorganisms, microbiome research progressed with the fast development of high-throughput sequencing. Nonetheless, challenges arise as technical biases could be introduced in each step of microbiome analysis.

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Such variations occur from sample collection, nucleic acid extraction, sequencing, data analysis or due to the batch effect, all of which undermine the reliability of analysis as well as inter-study comparisons. By using proper extraction and sequencing controls, a considerable number of noteworthy variations can be corrected. The low number of standard microbial communities used as DNA extraction controls are designed for human microbiome studies, hence unfit to illustrate the microbial diversity of other environments, such as the soil or water. Here, we set out to design a microbial Mock Community (MC) composed of bacterial strains that portray the taxonomic diversity of soil microbiome and can evaluate the efficiency of to-be used extraction methods. Seven bacterial strains (*Paenarthrobacter* sp., *Bacillus* sp., *Klebsiella* sp., *Pseudomonas* sp., *Staphylococcus* sp., *Escherichia* sp., and *Enterococcus* sp.) were selected based on the following criteria: (1) the availability of sequenced genomes, (2) their relative abundance in soil populations or their close phylogenetic relationship with the most abundant genera in soil, and (3) the presence of key physiological features, such as a thick cell wall, distinct tinctorial properties, and the ability to form spores. Standard curves associating the microbial optical density measured spectrophotometrically at 600 nm with CFU/ml were created to estimate the number of bacterial cells. Work is underway to confirm the number of bacterial cells based on the number of bacterial genomes by measuring the amount of purines released after mild acid and alkali treatment using a Shimadzu UPLC system coupled with a Synergi Polar-RP column.

P-40-023
Gluten-degrading proteases of *Aspergillus* species

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Some of the most common food-associated health disorders are associated with gluten. These include celiac disease and gluten intolerance, which are congenital and incurable. The only way to manage these conditions is through a gluten-free diet. Therefore, the development of gluten-degrading agents is currently a relevant area of research. In this study, promising producers of glutenolytic enzymes were first selected using bioinformatics methods among species of the genus *Aspergillus*. Further, the dynamics of their overall proteolytic and glutenolytic activities were studied during cultivation. Additionally, zymography was used to demonstrate the presence of high proteases activity in the culture liquid and high activity of isolated protease complex. The representatives whose enzymes are most likely to have glutenolytic activity turned out to be the species *Aspergillus carbonarius*, *A. parasiticus* and *A. tubingensis*. The selected species were cultivated in submerged conditions with constant stirring, in a medium with gluten as an inducer of secretion of glutenolytic proteases. The activity of gluten hydrolysis and total proteolytic activity were evaluated based on the results of spectrophotometry of reaction mixtures after enzymatic reactions of culture liquid with gluten and azocasein, respectively. *A. parasiticus* showed the highest hydrolysis activity in dynamics and the earliest peak of glutenolysis, on the 4th day of cultivation. Zymography was performed in two variations of the separating gel composition: with gluten and gelatin. According to its results, in *A. parasiticus*, unlike other species, the presence of glutenolytic proteases was detected throughout the entire cultivation period. The high molecular weight protease complex of *A. parasiticus* hydrolyzed gluten most effectively. Thus, *A. parasiticus*, previously unknown as a source of glutenases, turned out to be the most promising producer of the studied species of the genus *Aspergillus*.

P-40-024
Type IV secretion systems in Gram-positive bacteria: understanding VirB8 assembly and its role in DNA translocation

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Conjugative Type IV secretion systems (T4SSs) are membrane-spanning multiprotein complexes specialized in the transfer of mobile genetic elements between bacteria. They are considered as main drivers for the spread of antibiotic resistance genes. While T4SSs are extensively studied in Gram-negative bacteria, their architecture and mode of function in Gram-positive bacteria remain poorly understood. According to the envelope structure in Gram-positive bacteria, it has been proposed that T4SSs are made by two functional complexes, the cell wall complex and the translocon. To bring new insights into the biology of these bacterial nanomachines, we focused on this study on characterizing VirB8-like proteins. Our funding revealed that the VirB8-like are cell wall components where they act as trimeric subunits, adopting a conserved assembly pattern, distinct from their counterparts in Gram-negative bacteria. Additionally, we identified the interaction of VirB8-like proteins with T4SS components, in particular the predicted translocon components, highlighting the central role of VirB8-like proteins in T4SS assembly. Notably, we revealed a direct interaction between VirB8-like proteins and DNA, indicating their direct involvement in DNA transfer during conjugation. Altogether, our data allowed us to propose a model of Gram-positive T4SSs, where VirB8-like proteins, assemble a channel-like structure in the cell wall, a model distinct from what it was described in Gram-negative bacteria[1]. References: [1] Maffo-woulefack, R., Mohamad Ali, A., Laroussi, H., CAPPELE, J., Romero Saavedra, F., Ramia, N., Robbert, E., Mathiot, S., Soler, N., Roussel, Y., Fronzes, R., HUEBNER, J., Didierjean, C., Favier, F., Leblond-Bourget. N., Douzi, B. 2025. Elucidating assembly and function of VirB8 cell wall subunits refines the DNA translocation model in Gram-positive T4SSs. Science advances. 10.1126/sciadv.adq5975.

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P-40-025
Microorganisms and plant essential oil-driven biocontrol – implications for hive and human health

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Apis mellifera are economically and ecologically essential insects that ensure biodiversity in the natural environment and contribute to human welfare. Unfavorable environmental conditions change the composition of the honeybees-associated microbiota and cause an increase in pathogenic microorganisms, posing a risk to bees and humans. Chemical antimicrobial therapy suffers from the spreading of antimicrobial resistance, leading to the contamination of bee products, and endangering people's health. Therefore, natural microbial or plant-origin compounds with antimicrobial activity featuring low health risks for humans and bees are in high demand. During this study, the metagenomics and culture technique-based analysis of microbial communities associated with honeybee drones was performed by focusing on the biocontrol potential of microorganisms. Next generation sequencing-based analysis revealed the prevalent bacterial (Acetobacteraceae Lactobacillaceae, and Orbaceae) and fungal (Saccharomycetaceae, Cladosporiaceae, and Debaromycetaceae) families. The molecular analysis of morphologically distinct microorganisms mainly detected *Starmerella* spp. and *Metschnikowia* spp. yeasts and bacteria from *Staphylococcus*, *Hafnia*, *Bacillus*, and *Micrococcus* genera. Antimicrobial assays demonstrated that *Metschnikowia* and *Starmerella* showed the highest biocontrol activity. The plant-origin essential oils were analyzed against honeybee contaminants and potential human pathogens. It was identified that oregano, lemongrass, and lemon balm EOs displayed the greatest antimicrobial potential. Yeast *Candida* sp. was more resistant to the effect of EOs than bacteria, of which *Staphylococcus* sp. was the most sensitive. The discovered broad-spectrum natural antimicrobials will open new horizons for sustainable control of hive health and have a high impact on the wellness of society. This study has received funding from the Research Council of Lithuania, agreement No S-MIP-24-55.

P-40-026
Investigating the semen microbiota: Insights into male infertility and potential underdiagnosis in fertile individuals

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Male factor infertility accounts for up to 50% of cases, despite the advancements in semen and genetic testing. Recent research highlights the microbiota's role in health and disease, including the study of semen microbiota through NGS technology, for its potential impact on male infertility. The study aimed to analyse the microbiota profile in semen samples from infertile patients versus control group. Semen samples were collected from 59 patients (aged 20–55 years) undergoing infertility evaluation, of which 21 patients (aged 20–45 years) were considered normal control group. DNA was isolated from semen samples according to manufacturer's recommendations and an input of 12.5 ng was used. The sequencing of the V3 and V4 regions of the 16S rRNA gene was performed according to the manufacturer's protocol. The results revealed that 45 samples (32 infertile patients and 13 control patients) exhibited an increased presence of Lactobacillaceae family species, averaging 86.47%, with *Lactobacillus iners* (42.79%) and *Lactobacillus jensenii* (5.39%) being the most prevalent. The same samples showed a low abundance of *Ureaplasma* sp. (3.56%) and *Gardnerella* (1.47%). The others 14 samples presented altered microbiota profile, with decreased abundance of Lactobacillaceae family species (33.15%), like *Lactobacillus iners* (14.62%) and *Lactobacillus jensenii* (1.52%). Moreover, other species from Streptococcaceae family, such as *Streptococcus thermophilus*, and *Streptococcus aureus* (one case – 40.93%) were found in 7/9 control samples (7%). Also, species from Enterobacterales family were found in 6 out of 14 samples, with abundances varying between 6.53% and 55%. These findings suggest that altered microbiota profiles observed in the control group may be influenced by the fact that infertile males undergo more extensive testing and investigation regarding reproductive health, leading to a potential underdiagnosis of microbiota imbalances in fertile individuals.

P-40-027
Characterization of the CPAR2_702260 gene in *Candida parapsilosis*: a link between temperature tolerance and sulfur metabolism

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Candidiasis represents a significant medical challenge, even in developed countries. In recent years, *Candida parapsilosis* has gained increasing attention, primarily due to its rising incidence, particularly within clinical environments. As a result, understanding the virulence, fitness, and underlying molecular mechanisms of this species has become increasingly important. The adaptation of *C. parapsilosis* to fluctuating environmental conditions is crucial for its survival, with stress tolerance playing a central role in its pathogenicity. Within the host organism, *Candida* cells are exposed to various types of stress, among which temperature is one of the most critical factors influencing their survival. Previously in our laboratory, we identified that the deletion of the *CPAR2_702260* ORF in *C. parapsilosis* resulted in a remarkable growth defect when the cells were cultivated at 37°C. The *C. parapsilosis CPAR2_702260* gene is orthologous to the *MET28* gene in *Saccharomyces cerevisiae*, which encodes a transcriptional regulator involved in sulfur metabolism. In *S. cerevisiae*, the absence of *MET28* disrupts the ability to utilize inorganic sulfur. Further characterization of the *CPAR2_702260* knockout mutant revealed a similar growth defect at 30°C, which could be rescued by the addition of methionine, but not cysteine. Notably, the growth defect observed at 37°C could be

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complemented by the supplementation of sorbitol, NaCl, EDTA, and methionine. Our findings suggest that *CPAR2_702260* plays a pivotal role in the interplay between temperature tolerance and sulfur metabolism, indicating its potential as a critical factor in the virulence and environmental adaptability of *C. parapsilosis*.
This project was funded by the Ministry of Culture and Innovation of Hungary from the National Research, Development and Innovation Fund, financed under the FK 22 funding scheme, project no. 143373.

P-40-028
Interkingdom communication via extracellular vesicles: indirect interactions between oral pathogenic bacteria and *Candida* species

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The human oral cavity is colonized by more than 700 microbes, such as bacteria, viruses, fungi, known as the oral microbiota. As a result of environmental effects, such as smoking or infections, the microbial composition may change, which can result in dysbiosis that may lead to diseases, such as oral candidiasis. Oral *candidiasis* is most commonly caused by *Candida albicans*, which can alter the bacterial diversity. To examine the nature of such fungal-bacterial interactions, we aim to investigate the interaction between *Candida* species- and oral pathogenic bacteria at the level of extracellular vesicles (EV). For our experiments we used the *C. albicans* SC5314 and *C. parapsilosis* CLIB214 strains, along with *Staphylococcus aureus* as pathogenic bacterial counterpart. We optimized the fungal and bacterial EV isolation protocol from solid media. The characterisation of the EVs by transmission electron microscopy and NanoSight showed round shaped particles with diameters between 50 and 250 nm. We examined the effects of EVs released by *C. parapsilosis* and the yeast and hyphae form of *C. albicans* on the growth and biofilm formation efficiency of *S. aureus* and vica versa. As a results, we found that EVs from *C. albicans* and *C. parapsilosis* had different effects on the growth and biofilm formation efficiency of *S. aureus*. Regarding the effect of bacteria, the *S.aureus* EV treatment can induce the hyphae formation of *C. albicans* cells. Altogether, these results suggest the presence of an active interaction between fungal and bacterial cells at the level of EVs.

P-40-029
RecN is one of the key proteins of the bacterial SOS response

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The SOS response is a process that occurs in a cell due to DNA damage. The result of the SOS response activation can be the exact restoration of damaged DNA regions, as well as the adaptation of the bacterium to the damaging effect. Due to this, the SOS response is an important mechanism for the adaptation of bacteria to the action of antibiotics. Today, the adaptation of bacteria to antibiotics is one of the most pressing problems in pharmacology and medicine. The SMC-like protein RecN is expressed and acts at an early stage of the SOS response and is one of the key factors in successful DNA repair. Current knowledge of the activity of the RecN protein is limited mainly to *in vivo* data, from which it follows that this protein is necessary for the correct implementation of the SOS response and successful DNA repair of bacteria, but the functions and mechanisms of action of this protein are still unclear. In this work, fluorescently labeled variants of the RecN protein were created and the features of its binding to DNA and localization inside cells were studied using standard biochemical methods, fluorescence microscopy and optical tweezers. Using these methods, we demonstrate the high affinity of RecN for DNA in single-stranded form. It was shown that outside the SOS response, RecN is localized at the poles and in the center of the dividing cell, and during the SOS response, its co-colonization with DNA was revealed. The study was supported by the grant of the Russian Science Foundation No. 24-74-00085.

P-40-030
Proteases with amidolytic and fibrinogenolytic activities from *Aspergillus tennesseensis*

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Micromycetes proteases, including those acquired from *Aspergillus* section *Versicolores*, were previously reported as enzymes with high anticoagulant and thrombolytic activities. The new species *A. tennesseensis* from this section was described in 2012. The analysis of proteases from *A. tennesseensis* with similar properties will help to establish the most potent enzyme producer among the mentioned section and describe the evolution of enzymes within the section and genus. Micromycete was cultured on a fermentation medium with peptone and fish hydrolysate as the main nitrogen sources. Amidolytic activity of culture liquid reached its peak on the 4th day of cultivation. Activity was measured with chromogenic peptide substrates of proteolytic enzymes of the hemostasis system: plasmin (substrate H-D-Val-Leu-Lys-pNa), thrombin (Tos-Gly-Pro-Arg-pNa), Xa factor (N-Z-D-Arg-Gly-Arg-pNa), activated protein C (pyro-Glu-Pro-Arg-pNa) and urokinase (pyro-Glu-Gly-Arg-pNa). We propose that this broad spectrum of enzymatic activity can be demonstrated by a single protease that cleaves peptide bonds after amino acid residues with positively charged side chains. Six proteins with fibrinogenolytic activity were identified using the zymography method: three of them had a low molecular weight (< 25 kDa), two proteins had a molecular weight of around 30-40 kDa and one had a high molecular weight (> 100 kDa). These enzymes did not demonstrate amidolytic activity with chromogenic peptide substrates. In contrast to previous studies of *Aspergillus* extracellular proteases where only a single active enzyme was found, we identified several active proteases with anticoagulant and thrombolytic activities. Isolating these proteases and studying their structure and properties may lead to the development of new antithrombotic drugs.

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P-40-031
Bacteriophage profiling: isolation and characterization of phage targeting *Pseudomonas aeruginosa*

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Pseudomonas aeruginosa is a Gram-negative opportunistic pathogen that causes wound and burn infections, pneumonia, sepsis, urinary tract infections and various hospital-acquired, acute and chronic infections in immunocompromised individuals. In addition to its natural resistance, *P. aeruginosa* can develop resistance to all types of antibiotics, especially fluoroquinolones, betalactams and aminoglycosides. Treating infections with antibiotics has become very difficult due to the resistance developed in bacteria. The increase in antibacterial resistance in bacteria has led researchers to discover and develop various antibacterial treatments. One of the alternative treatments is with bacteriophages, an old treatment method. Bacteriophages are viruses that enter the bacterial cell, multiply and eventually break down the bacteria to kill it. Bacteriophages were seen as a potentially useful treatment option because of their ability to target specific bacterial species, combat antibiotic-resistant strains, multiply at the site of infection, be effective against biofilms and not harm the normal flora. To reduce mortality from bacterial infections, it is important to investigate phages that are effective against MDR and XDR *P. aeruginosa* strains. In this study, virulent phages effective against *P. aeruginosa* were isolated from water samples. The physical properties, antibiofilm activity and whole genome sequencing of the phages obtained will be determined. The results of the study should contribute to the treatment of infections associated with antibiotic-resistant *P. aeruginosa*.

P-40-032
The preparation of Q fever diagnostic proteins for lateral flow assay

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The bacterium *Coxiella burnetii* causes a worldwide zoonotic disease, Q fever. *C. burnetii* infection displays a broad range of clinical manifestations, including self-limiting flu-like illness, fatigue syndrome, pneumonia, hepatitis, endocarditis, and vascular infections. However, most individuals remain asymptomatic. Although domestic ruminants serve as the main reservoirs of *C. burnetii*, they generally do not exhibit clinical signs of infection. Q fever diagnosis predominantly depends on serological methods. Lateral flow assay (LFA) is a rapid, portable, and inexpensive method for the detection and quantification of analytes from complex mixtures, which can be used in the field without trained technicians to use and interpret results. For the LFA preparation, it is necessary to have *C. burnetti* proteins available that have immunoreactive properties. In the study, we chose formerly confirmed immunoreactive proteins CBU_0307 and CBU_0092 to produce them in the *Escherichia coli* expression system in combination with pET22b (+) vector. We optimized the cell culture conditions to increase the expression level of the selected proteins. We focused on the inducer concentration, medium pH, temperature, and supplementation with additional compounds like glucose or glycerol. The His-tag was employed to purify the proteins via magnetic bead-based affinity chromatography. We used the MagneHis™ Protein Purification System kit (Promega, USA). We optimized the protocol by changing the concentration of NaCl. Western blot analysis using anti-His-tag antibodies was conducted to evaluate the success of the purification process. In summary, we obtained purified recombinant proteins, which will be further used in the LFA preparation. This work was supported by the following grant: SAS Return Project Scheme for Parents Returning to Work after Maternity or/and Parental Leave, VEGA 2/0125/24 and VEGA 2/0129/24.

P-40-033
Exploring the role of the iucA gene in hypervirulence and antibiotic resistance of *Klebsiella pneumoniae* using CRISPR/Cas9 genome editing system

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Klebsiella pneumoniae is one of the most prevalent hospital-acquired pathogens in Russian hospitals. The greatest danger to public health is posed by the combination of virulence genes (siderophore synthesis operons) and antibiotic resistance genes (metallo-β-lactamases) in a single strain. Siderophore genes are most often localized on the pLVPK and pVIR-CRHvKp4 hypervirulence plasmids. The aim of this study is to characterise the functional role of the plasmid-localised *iucA* gene of NIS synthetase, involved in the synthesis of the siderophore aerobactin, in the development of hypervirulence in *K. pneumoniae* strains with multiple antibiotic resistance. The *K. pneumoniae* genome was edited using the pUC19_CRISPR_ΔiucA system, which is based on the pUC19_CRISPR_ΔpmrA vector (Addgene #160903) encoding the *S. pyogenes* Cas9 nuclease, the genes of the Lambda red recombination system of the bacteriophage λ, as well as sequences encoding the sgRNA and the cassette for homologous recombination. Following the transformation of two pathogenic strains of *K. pneumoniae* (3911 and 1128) isolated from patients in St. Petersburg hospitals with the pUC19_CRISPR_ΔiucA plasmid, a targeted deletion (80 bp) in the *iucA* gene was obtained in strain 1128. This resulted in a shift in the reading frame and the formation of a premature stop codon. Furthermore, strain 3911 exhibited complete *iucA* gene knockout and loss of the hypervirulence plasmid. Inactivation of the *iucABCD* operon in strain 1128 results in blocked siderophore synthesis and acquisition of an avirulent pathotype but does not affect the growth rate or resistance to beta-lactam antibiotics. Conversely, the loss of the hypervirulence plasmid in strain 3911 did not affect siderophore synthesis, virulence, growth rate, or beta-lactam resistance, indicating the presence of other hypervirulence determinants. This work was supported by St. Petersburg State University, Project ID 129658320.

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P-40-034
Anticoagulant and fibrinolytic enzymes from *Aspergillus tabacinus*

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The micromycete *Aspergillus tabacinus* was described as the first microorganism producing an extracellular protease with protein C-like activity. It was also shown that the enzymes secreted by this producer demonstrated fibrinogenolytic activity. This property may be useful for the development of a drug with not only anticoagulant but also thrombolytic activity. For the subsequent development of a therapeutic agent, it is important to establish whether protein C-like and fibrinogenolytic activities are manifested by one enzyme or are characteristic of different secreted proteins of the micromycete. Fractional salting out of proteins from the culture fluid resulted in six different fractions and revealed several enzymes with target proteolytic activities. Desalting of all protein samples was performed using gel-filtration. Spectrophotometric analysis showed the presence of high amidolytic activity towards chromogenic peptide substrates of activated protein C, urokinase, and plasmin in all six protein samples. Zymography revealed several enzymes with fibrinogenolytic and fibrinolytic activities in different protein samples including 2 highly active low molecular weight proteins (≈12 and 18 kDa). The obtained data suggest that protein C-like and fibrinogenolytic activities are characteristic of different enzymes of *A. tabacinus*, since it was previously shown that protein C-like, urokinase-like and plasmin-like activities can be exhibited by a protease with a molecular weight of about 30 kDa but low-molecular proteases with such activities were not found. Further search for a method for the combined isolation of target enzymes from the culture fluid of the micromycete or the production of desired proteins in heterologous expression systems will allow the development of a pharmaceutical preparation that simultaneously exhibits anticoagulant and fibrinolytic activities. *The authors marked with an asterisk equally contributed to the work.

P-40-035
Exploring the biodegradation mechanisms of newly isolated *Pseudomonas veronii* SM-20: A potential candidate for PAH remediation

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This study examined the biodegradation capabilities and stress response mechanisms of *P. veronii* SM-20, a newly isolated strain from polluted soil. We explored its potential application as a bioremediation agent for restoring sites contaminated with polycyclic aromatic hydrocarbons (PAHs). Using phenotype microarray technology, we uncovered the strain's ability to utilize various carbon sources while exhibiting resistance to multiple stressors, including pH, metals, and antibiotics. Our comprehensive genomic analysis identified numerous genes associated with aromatic hydrocarbon metabolism, establishing a foundation for understanding the genomic biodegradation potential of this strain. In a series of biodegradation assays, *P. veronii* SM-20 was cultured with high concentrations of phenanthrene (PHE, 600 μg/mL) as the sole carbon and energy source. UPLC analysis revealed a significant ability to degrade approximately 25% of PHE over a 30-day period. The proposed degradation pathway involved the oxidative transformation of PHE into an unstable arene oxide and 9,10-phenanthrenequinone, followed by subsequent ring-cleavage. Furthermore, our comparative proteomics analysis provided insights into the strain's adaptive response to PHE exposure, highlighting changes in its proteomic profile related to aromatic hydrocarbon metabolism, surface remodeling, and stress response mechanisms. This multi-omics study highlights the exceptional genetic and metabolic attributes of *P. veronii* SM-20, positioning it as a promising candidate for the environmental restoration of PAH-contaminated sites.

P-40-036
Proteomic analysis of *Pseudomonas veronii* in response to phenanthrene exposure

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This study investigates the proteomic response of *Pseudomonas veronii* SM-20 to phenanthrene (PHE) exposure, revealing critical adaptations that enhance its survival and degradation capabilities. Proteomic profiling identified a differential abundance of proteins involved in bacterial surface remodeling, stress response, adaptation, and energy production. Key biological processes activated in *P. veronii* SM-20 following PHE exposure are illustrated, highlighting its metabolic flexibility. A diverse array of enzymes potentially involved in polycyclic aromatic hydrocarbon (PAH) degradation was uncovered, including catechol 1,2-dioxygenase, catechol 2,3-dioxygenase, and protocatechuate 3,4-dioxygenase, indicating the use of ortho- and meta-cleavage pathways. The presence of enzymes such as homogentisate 12-dioxygenase and 2,3-dihydroxybiphenyl 1,2-dioxygenase further underscores the strain's capability to process various aromatic compounds. Notably, the upregulation of TonB-dependent receptors suggests their role in the transport of aromatic compounds across the outer membrane. The enzymatic degradation pathway of PHE involves complex reactions initiated by cytochrome P450 monooxygenase, leading to the formation of degradation intermediates such as diphenic acid, which is significantly more soluble than PHE. Our findings elucidate the intricate enzymatic pathways utilized by P. veronii SM-20 for the catabolism of PHE, providing insights into microbial degradation mechanisms essential for bioremediation efforts. Funding: This research was funded by the Slovak Research and Development Agency (APVV 19–0519), Recovery and Resilience Plan of the Slovak Republic (09I03-03-V04-00556), and VEGA 2/0125/24.

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P-40-037
A new isolate with the ability to degrade plastics: *Trichoderma citrinoviride*

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In this study, a microorganism that can grow only in the presence of polyethylene glycol (PEG) as a carbon and energy source was isolated from contaminated water, soil and plastic samples collected from the work area of ATIKSAN recycling company. The microorganism, whose genomic DNA was isolated and sequenced, was identified as *Trichoderma citrinoviride*. Species belonging to the genus *Trichoderma* are known to be highly effective as biocontrol agents in soil ecosystems. The isolated microorganism was found to form biofilm, to produce various metabolites that have not yet been identified, and to have antimicrobial activity against some bacterial and fungal species. *T. citrinoviride* was found to grow in a mimimal medium that has LDPE, PE or acetate as the sole carbon and energy source. It was observed by SEM that the microorganism penetrated into the plastic and caused damage, cracks and thinning in LDPE, PE and acetate polymers, and it was determined by FTIR analysis that new bonds were formed in LDPE and PE plastics due to degradation. Characteristics such as growth at room temperature, antimicrobial activity, relatively rapid growth, production of different metabolites and biofilm forming ability make *T. citrinoviride* a potential candidate for plastic recycling. *The authors marked with an asterisk equally contributed to the work.

LB-R-40-01
The gut microbiota modulates the response to acupuncture treatment in an atopic dermatitis mouse model

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This study investigates whether the efficacy of acupuncture treatment for atopic dermatitis (AD) is influenced by gut microbiota composition. To explore this, fecal microbiota from AD patients – clinically assessed and categorized as responders or non-responders to acupuncture – were transplanted into antibiotic-treated mice. After inducing AD with topical MC903, mice underwent acupuncture at the LI11 acupoint. Treatment outcomes were compared between groups receiving microbiota from responders and non-responders. Key measures included skin severity, itching behavior, thymic stromal lymphopoietin (TSLP) expression, and the presence of mast cells and eosinophils in the skin. Additionally, intestinal barrier integrity was assessed through tight junction proteins (ZO-1 and Occludin). Acupuncture treatment was effective only in mice transplanted with microbiota from clinically assessed responders, resulting in significant reductions in scratching behavior, skin severity scores ($p < 0.001$), and TSLP expression (37.5%, $p < 0.001$). Increased expression of intestinal tight junction proteins ZO-1 and Occludin was also observed ($p < 0.001$). In contrast, mice receiving microbiota from non-responders showed no significant therapeutic response to acupuncture across these measures. These findings suggest that gut microbiota composition critically modulates the therapeutic response to acupuncture in AD, highlighting the potential for microbiota-based personalization of acupuncture therapy.

LB-R-40-02
Evaluation of inducible expression of Cas1-Cas2 from the chromosome on naïve CRISPR adaptation in *Escherichia coli*

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CRISPR-Cas is a prokaryotic adaptive immune system against mobile genetic elements (MGEs) that utilises a crRNA-mediated interference mechanism. Immunity against MGEs is established by integrating new DNA fragments or spacers from an MGEs into specific chromosomal sites called CRISPRs. The acquisition of new spacers is essential for CRISPR immunity, and this step is called "CRISPR adaptation". Depending on the mechanism of spacer generation, CRISPR adaptation is divided into two types – naïve and primed. For both types of adaptation, the Cas1–Cas2 complex is required for spacer capture and integration. In our previous work in *E. coli* we have shown that naïve adaptation requires the help of RecBCD activity and other host proteins to prepare suitable DNA substrates for integration. Naïve CRISPR adaptation is not a very efficient process, and insertion of new spacers can only be observed after overexpression of *cas1* and *cas2* genes from the plasmid. Plasmids are unstable in recB or recD and other mutants and are often lost, which affects the results and interpretation. In *E. coli*, *cas1* and *cas2* are the last two genes within an operon of seven genes (casABCDE12). Consequently, the *cas1* and *cas2* genes are the least expressed *cas* genes, which is probably the reason why naïve adaptation was never observed when these genes were expressed as polycistronic mRNA from a native or inducible promoter (using an empty vector as MGE). In this study, we have generated a strain in which the inducible pBAD promoter is placed upstream of the chromosomal *cas1* and *cas2* genes, at the exact locus. We present our new data on strain construction, strain verification and the effects of chromosomally expressed *cas1* and *cas2* genes on naïve CRISPR adaptation. We will discuss unexpected outcomes of this approach. *The authors marked with an asterisk equally contributed to the work.

LB-R-40-03
Novel tricyclic flavonoids as promising antimicrobial agents

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The rapid emergence and global spread of multidrug-resistant microorganisms threaten the effectiveness of current antimicrobial therapies, with severe medical and socioeconomic consequences. Although progress has been made in fighting antibiotic resistance, the dwindling pipeline of effective agents highlights the urgent need for novel antimicrobials. This study aimed to synthesize and evaluate the antimicrobial potential of a new series of tricyclic flavonoids bearing methyl, bromine and iodine substituents on ring A of the flavonoid scaffold. The structures of novel synthetic tricyclic flavonoids and their corresponding 3-dithiocarbamic flavanones were proven by X-ray structural analyses. Minimum inhibitory concentration (MIC) and minimum bactericidal/fungicidal concentration (MBC/MFC)

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were used to evaluate antimicrobial activity against WHO-priority pathogens. Growth kinetic and time–kill assays were employed to confirm the antimicrobial effectiveness. The mechanism of action was investigated using fluorescence microscopy. All tested compounds exhibited important antibacterial and antifungal activities, with MIC and MBC values as low as 0.48 μg/mL and 3.90 μg/mL, respectively, against *Staphylococcus aureus*. Several flavonoids showed superior bacteriostatic effects compared to chloramphenicol, maintaining growth inhibition for up to 24 h at 2×MIC. Notably, a strong bactericidal effect was observed against a MRSA strain, with no viable cells evidenced after 6 h of incubation and a total kill effect recorded up to 24 h. Microscopy revealed membrane disruption as a likely mechanism of action. These findings support the potential of tricyclic flavonoids as promising antimicrobial agents against resistant bacterial and fungal pathogens.

LB-R-40-04
Hemostatically active proteases of the micromycete *Aspergillus protuberus*: formation and properties

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Microbial fibrinolytic enzymes are being explored as new thrombolytic drugs due to their potential in the treatment of thrombotic diseases. *Aspergillus*, one of the most widespread fungal genera, rarely includes strains that produce fibrinolytic enzymes. This study aims to evaluate *Aspergillus protuberus* as a potential fibrinolytic enzyme producer and to systematically analyze the enzymatic properties and thrombolytic activity of its extracellular proteases, thereby contributing to the expansion of fibrinolytic resources within the genus *Aspergillus*. Preliminary screening showed that strong proteolytic activity on solid media containing casein and collagen, with enzyme indices of 1.21 and 1.32. Optimizing a fermentation medium with glycerol, glucose, GRM, $NaNO_3$, and $MgSO_4$ significantly enhanced enzyme production. During fermentation, dynamic monitoring revealed two activity peaks related to proteases with protein C-activating activity on days 4 and 6, and the 5th day was the critical time point for plasmin secretion. Isoelectric focusing of isolated extracellular proteolytic complex revealed four active protein components with isoelectric points (pI) of 4.78, 5.65, 7.85, and 9.95, and molecular weights ranging from 35 to 135 kDa, suggesting a complex composition. The enzymes showed optimal activity at 30–37°C and neutral to slightly alkaline pH, conditions suitable for human physiology. *In vitro* thrombolytic assays confirmed the rapid and efficient clot-dissolving capability of the fibrinolytic enzymes produced by *A. protuberus*, reducing residual thrombus to 37.7% in 10 minutes and to only 17.39% within 90 minutes. This study not only validates the feasibility of *A. protuberus* as a novel producer of fibrinolytic enzymes but also provides a theoretical and experimental foundation for the development of safe, efficient, and cost-effective microbial thrombolytic drugs.

LB-R-40-05
Prophage integrases of *Staphylococcus aureus*: global analysis of diversity and identification of integration sites

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Staphylococcus aureus is a major human bacterial pathogen responsible for skin infections as well as serious systemic diseases, including sepsis and endocarditis. The genetic plasticity of this species is determined by mobile genetic elements, in which prophages play a key role. The aim of this study is to analyze prophage regions in *S. aureus* genomes on a global scale, with a focus on the diversity of integrases and their integration sites. The analysis included complete genomes of *S. aureus* (n = 744) and temperate bacteriophages (n = 263) from the NCBI database. Prophage regions were predicted using PHASTEST and annotated with Pharokka. Nucleotide sequence similarity was assessed using the BLAST algorithm. Integration sites were refined using Vector NTI. A total of 2567 prophage regions ranging from 5 to 134.2 kb in length were identified in *S. aureus* genomes; 4 genomes were completely devoid of prophages (average number of prophages per genome: 3.33. In total, 2727 and 262 integrase genes were identified in bacterial and phage genomes respectively, of which 1320 and 262 were classified as classical. Among prophage integrases, the most abundant types were Sa3 (n = 540), Sa2 (n = 330), and Sa1 (n = 172); Sa10 and Sa11 types were not detected. Among temperate phage integrases, the most represented was Sa2 (n = 73). Both known and novel integration site variants were identified for all classical integrase types, with the highest diversity observed in Sa2 integrases (12 attL/attR site variants). Analysis of the remaining integrase genes revealed that a significant portion (n = 541) belonged to pathogenicity island integrases or two novel integrase types related to Sa2 and Sa9. The data demonstrate a high diversity of prophages and integrases in *S. aureus* genomes, highlighting their key role in the evolutionary plasticity of the species. The study was supported by Russian Science Foundation grant 22-15-00443- (https://rscf.ru/en/project/22-15-00443/).

LB-R-40-06
Mycobacteriophages: isolation, characteristics and analysis of therapeutic potential

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The growing resistance of *Mycobacterium tuberculosis* to antibiotics highlights the need for alternative treatments such as phage therapy. Despite over 11 000 known mycobacteriophages, Russian isolates remain largely unstudied. Here, we report the first Russian collection of mycobacteriophages and identify candidates with therapeutic potential. Phages were isolated from environmental samples using *Mycobacterium smegmatis* mc^{2}155

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as the host. Host range was assessed, and genome sequencing was performed using the Illumina platform. A total of 21 phages were identified. Electron microscopy revealed predominantly siphovirus morphology. The phages were assigned to clusters A, K, B, S, F, and the rare cluster Y (with only five known members). Phage Arbat showed < 5% nucleotide identity to known phages and was classified as a singleton with a unique genome (108.9 kb, 195 ORFs, 22 tRNA genes). Other genomes ranged from 45.7 to 75.3 kb. Five phages – Nadezda (Y), Yasnaya_Polyana (K), Vic9 (B), Cat (A), and Ksenia (S) – have been deposited in NCBI. Adsorption and one-step growth curves were obtained for Vic9 and Yasnaya_Polyana. Eight phages, including Vic9, Yasnaya_Polyana, Nadezda, and five cluster A phages, showed lytic activity against *M. tuberculosis* H37Rv. Yasnaya_Polyana showed the highest efficacy (EOP = 5×10^{-2}). Cluster A phages also exhibited activity against *M. abscessus*, *M. fortuitum*, and *M. avium*. Phages Cat and KV carried deletions in the integration module, affecting the integrase or repressor genes. A lytic derivative of the temperate phage Yasnaya_Polyana was generated via BRED (Bacteriophage Recombineering of Electroporated DNA) by deleting the repressor gene. This collection provides a valuable resource for fundamental research and clinical application. Phages Cat and Yasnaya_Polyana represent high-priority candidates for therapeutic development. This study was supported by the Russian Science Foundation (project no. 24-15-00514, https://rscf.ru/en/project/24-15-00514/).

LB-R-40-07
Study of bacteriolytic properties of a new extracellular amidase Ami produced by *Lysobacter capsici* XL1

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The isolation and characterization of new antimicrobial compounds are a global scientific challenge against antibiotic-resistant pathogens. Some of these promising lytic agents are extracellular bacteriolytic enzymes of bacteria that target various bonds in peptidoglycan. Bacteriolytic enzymes of *Lysobacter* have been studied by our team since 1975. Recent studies using omics technologies have identified a new extracellular bacteriolytic enzyme of *Lysobacter capsici* XL1 – the amidase Ami. The aim of this study was to investigate the bacteriolytic properties of a new bacteriolytic enzyme. The Ami enzyme exhibited activity against live pathogenic bacteria *Staphylococcus aureus*, *Micrococcus luteus*, *Enterococcus faecium*, *Bacillus anthracis*, including strains with virulence and immunogenic plasmids pXO1 and pXO2, as well as *B. cereus* strains. The specificity of Ami action was studied against peptidoglycans of different chemotypes: A1γ from *B. cereus* 217; A3α, from *S. aureus* 209P; A4α, from *E. faecium* FS86; A2, from *M. luteus* Ac-2230^T, using hydrazinolysis and dinitrophenylation methods to detect C-terminal a.a. and N-terminal a.a., respectively, followed by TLC analysis. The bacteriolytic enzyme Ami was found to hydrolyze the amide bond between N-acetylmuramic acid and L-Ala in all investigated peptidoglycans. Molecular docking was done using analogy with crystal structure of zinc protease AmpDh2 (PDB id 4BPA) to predict amino acid residues in the catalytic domain of the Ami that may be involved in binding to the carbohydrate moiety of the peptidoglycan. In the future, using molecular and genetic approaches, it is planned to experimentally study the mechanism of interaction of Ami with peptidoglycan. Thus, the bacteriolytic amidase Ami of *L. capsici* XL1 is a new promising antimicrobial agent with a broader range of antimicrobial action as compared to known bacterial and bacteriophage amidases. The work was supported by the Russian Science Foundation (No. 24-75-10078).

LB-R-40-08
Specificity of the action of the β-lytic protease of *Lysobacter capsici* against peptidoglycans of pathogenic bacteria

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The spread of pathogenic microorganisms in society that are multiresistant to all known antibiotics leads to an intensive search and isolation of new antimicrobial agents – antibiotics, peptides and bacteriolytic enzymes. Bacteriolytic enzymes of bacteria of the genus *Lysobacter* have been studied at IBPM RAS for many years. One of the most active bacteriolytic enzymes is the β-lytic protease (EC 3.4.24.32; family M23). It was shown that β-lytic protease hydrolyzes cells of living bacteria such as: *Staphylococcus aureus*, including MRSA strains, *S. haemolyticus*, *S. epidermidis*, *Micrococcus luteus* Ac-2230^T, *Kocuria rosea* Ac-2200^T, *Enterococcus faecium* FS86, *Streptococcus pyogenes*. The aim of this work was to study the specificity of β-lytic protease action on peptide bonds in peptidoglycans of pathogenic bacteria. The specificity of β-lytic protease action was studied against peptidoglycans of bacteria of different chemotypes: A3α in *S. aureus* 209P, A2 in *M. luteus* Ac-2230^T, A4α in *E. faecium* FS86 and A1γ in *Bacillus cereus* 217. After enzymatic hydrolysis of peptidoglycans, free C-terminal a.a. and N-terminal a.a. were determined using TLC. The peptidoglycan of *B. cereus* 217 β-lytic protease does not hydrolyse. Molecular docking was done using analogy with crystal structure of zinc protease pseudoalterin (PDB id 6IK4) to predict functionally significant sites in the structure of the β-lytic protease that may be responsible for binding to the cell surface of target cells. As a result, a.a. were predicted in the structure of the β-lytic protease that may be involved in interaction with the carbohydrate portion of the peptidoglycan. The obtained result is planned to be confirmed experimentally in the future. Thus, the obtained data expand our knowledge of the functioning of β-lytic protease and may be useful in the development of antimicrobial drugs. The work was supported by the Russian Science Foundation (Project No. 24-75-10078).

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LB-R-40-09
Bioinformatics analysis of antifungal mechanisms in *Serratia f*onticola: protein–protein interaction with *Botrytis cinerea* BAG1 and genome-encoded enzyme reportoire

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Botrytis cinerea, which causes gray mold, is a key fungal pathogen that develops resistance to traditional fungicides. This research examines the biocontrol potential of a rhizosphere-derived bacterial isolate, *Serratia fonticola* EBS19, with an emphasis on its protein-interaction and genomic properties. Genome sequencing and annotation identified glycoside hydrolase and other lytic enzyme encoding genes which have the potential to impart antifungal action. In order to reveal molecular interactions supporting suppression of pathogens, we performed *in silico* protein–protein docking of *B. cinerea's* principal stress regulator BAG1 with a glycoside hydrolase from bacteria. This showed a high-affinity binding interface, proposing a pathway for inhibition of fungi directly by intercession in stress pathways. Moreover, carbon source utilization profiling showed strain-specific metabolic traits which can promote adaptability as well as stability in plant-associated habitats. These bioinformatics results give information about the functional genomics of useful microbes and identify *S. fonticola* EBS19 as a potential candidate for future recombinant use as well as for the creation of targeted antifungal biopreparations. *The authors marked with an asterisk equally contributed to the work.

LB-R-40-10
Investigation of α-glucosidase inhibitory metabolites from *Paenibacillus* sp. JNUCC31: gene annotation, isolation, characterization, enzyme kinetics, and *in silico* analysis

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The rising global prevalence of type 2 diabetes emphasizes the need for safer and more effective α-glucosidase inhibitors. *Paenibacillus* species produce diverse secondary metabolites, yet their antidiabetic potential is underexplored. In this study, whole-genome sequencing of *Paenibacillus* sp. JNUCC31 revealed several biosynthetic gene clusters linked to secondary metabolism. GC-MS analysis identified anteiso-C15:0 (57.32%) as the dominant fatty acid, followed by iso-C16:0 (9.85%), C16:0 (7.94%), and anteiso-C17:0 (9.85%). Fermentation extracts (methanol, n-hexane, ethyl acetate) were tested for α-glucosidase inhibition, with ethyl acetate extract showing the highest activity (52.4 ± 0.7%). Five known compounds were isolated: adenosine, uridine, 4-hydroxybenzaldehyde, dibutyl phthalate (DBP), and 1-acetyl-β-carboline. Of these, adenosine, uridine, and DBP are known α-glucosidase inhibitors. Kinetic assays showed uridine acts as a competitive inhibitor, while adenosine and DBP displayed mixed-type inhibition. Molecular docking and MD simulations assessed the binding of active compounds and major fatty acids to human MGAM and microbial isomaltase. Uridine formed stable hydrogen bonds with both targets (−7.0 and −7.2 kcal/mol), while adenosine had stronger affinity for isomaltase (−7.5 kcal/mol). DBP bound mainly via hydrophobic interactions. Fatty acids showed weaker binding due to simpler structures. MD simulations confirmed stable ligand–protein complexes. MM/GBSA calculations showed adenosine and uridine binding energies between −14.18 and −28.63 kcal/mol; DBP showed stronger binding (−31.00 to −27.74 kcal/mol). Fatty acids also had notable affinity (−28 to −36.5 kcal/mol). Key binding residues included Trp406, Asp366, and Phe450 in MGAM, and Tyr158, Gln279, and Phe178 in isomaltase. Overall, *Paenibacillus* sp. JNUCC31 shows strong potential as a microbial source of α-glucosidase inhibitors, offering a structural basis for future inhibitor design. *The authors marked with an asterisk equally contributed to the work.

LB-R-40-11
Indole stimulates the physiology and pathogenicity of non-indole producing *Pseudomonas aeruginosa* ATCC 27853 by modulating distinctive metabolic and quorum sensing pathways

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Bacteria employ interspecies signalling molecules like indole – a widely produced microbial metabolite – to coordinate behaviour and adapt to multispecies environments by modulating neighbouring cells' physiology. Indole and its derivatives have been found to induce the physiological changes in non-indole-producing *Pseudomonas aeruginosa*, including drug resistance, biofilm formation, and virulence. We examined interactions between indole-producing *E. coli* K-12 MG1655 and *P. aeruginosa* strains. *E. coli* MG1655 increased pyocyanin production in clinical *P. aeruginosa* strains, including ATCC 27853, but not in PAO1. Exogenous indole supplementation confirmed this was indole-dependent pyocyanin stimulation. Moreover, indole enhanced the pathogenicity of *P. aeruginosa* ATCC 27853 in a *Caenorhabditis elegans* infection model. Biochemical and gene expression analyses revealed that indole-stimulated pyocyanin production depends on the Rhl and PQS quorum-sensing systems. Intriguingly, we observed a progressive decline in indole levels concurrent with increased pyocyanin in *P. aeruginosa* ATCC 27853 cultures, indicating a potential link between indole metabolism and pyocyanin production. Since anthranilate has been reported to link upstream and downstream indole metabolism in various bacteria, we hypothesized that indole may be degraded into anthranilate-a PQS biosynthesis precursor-thereby contributing to pyocyanin production. Consistently, indole treatment upregulated anthranilate catabolic genes (*antABC*, *catABC*). Further analysis revealed that indole-mediated stimulation of pyocyanin was independent of tryptophan catabolic genes *trpAB* but was dependent on the kynurenine pathway. These results suggest a model in which indole degradation via the kynurenine pathway supplies precursors for PQS biosynthesis, thereby enhancing pyocyanin production. This study advances understanding of interspecies signalling and polymicrobial infection mechanisms, offering potential clinical insights.

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LB-R-40-12
Distribution and functional characterization of the native Type I-F CRISPR-Cas systems in *Pseudomonas aeruginosa* suggests the prevalence of anti-CRISPRs

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CRISPR-Cas systems constitute the adaptive immune system in prokaryotes. They function to facilitate the recognition and defense against invading genetic elements. These systems have revolutionized genome editing by enabling precise and efficient genetic modifications. However, they are often neutralized by anti-CRISPR (Acr) proteins. Acrs are natural antagonists encoded by mobile genetic elements and bacterial genomes that can inhibit CRISPR-Cas immune functions at various stages, thus holding great potential for safer and more controllable genome editing. The class 1 type I CRISPR-Cas systems are the most abundant and diverse CRISPR-Cas systems found in nature. Among them, the subtype I-F represents a minimal type I CRISPR-Cas system with significant exploitation potential. The Gram-negative pathogen *Pseudomonas aeruginosa* is a key organism for studying the type I-F CRISPR-Cas system. We comprehensively analyzed the distribution and activity of native type I-F CRISPR-Cas systems in 145 clinical *P. aeruginosa* isolates. Genomic analysis revealed that among these isolates, 54 strains harbored a complete type I-F CRISPR-Cas locus, predominantly exhibiting a "sandwich" architecture (CRISPR array–Cas cascade–CRISPR array) with conserved repeat (28-29 bp) and spacer (31-33 bp) lengths. However, DNA interference activity assays showed that 54% of these CRISPR-Cas systems are inactive. Further analysis revealed that majority of these strains contain Acrs. A comprehensive analysis of Acr distribution in the entire 145-strain collection revealed that AcrIFs are prevalent in strains exhibiting inactive DNA interference activity and those lacking a type I-F CRISPR-Cas loci, suggesting an extensive evolutionary arms race between these systems. Additionally, these clinical isolates that carry a complete type I-F CRISPR-Cas system but without DNA interference activity and those carrying degenerated CRISPR-Cas systems, providing a reservoir for potentially novel Acrs.

LB-R-40-13
Astrocyte–macrophage crosstalk and antileishmanial activity of a boron-based small molecule in a CNS-inspired infection model

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Leishmania infantum, a protozoan parasite, rarely invades the central nervous system (CNS) yet can cause severe neuroinflammatory complications. To investigate the role of astrocytes in parasite control and evaluate the efficacy of a novel boron-based molecule (SPT), we established a co-culture model mimicking cerebral leishmaniasis. Bone marrow-derived macrophages (BMDMs) were infected with *L. infantum* and co-cultured with C8-S astrocytes at a 3:1 ratio. Parasite burden was analyzed at 0, 24, 48, and 72 h using gp63-FITC labeling and flow cytometry. The parasite population decreased significantly from 88.8% at 0 h to 73.9% at 24 h, 50.1% at 48 h, and 24.9% at 72 h ($p \leq 0.0001$). In contrast, astrocytes directly infected with parasites showed a parasite rate of only ~7% at 72 h, suggesting astrocyte-mediated parasite suppression. Treatment with SPT (75–200 μM) induced apoptosis-like cell death in *L. infantum* promastigotes (IC_{50}: 67–83 μM), disrupted mitochondrial membrane potential and caused cell cycle arrest in S and G2/M phases. SPT reduced Meta1 expression (↓0.5-fold, $p \leq 0.05$) and increased MCA5 expression (↑2.5-fold, $p \leq 0.05$), confirming inhibition of metacyclogenesis and activation of programmed cell death. In infected macrophages, SPT elevated IFN-γ and IL-12B expression (↑6.8-fold, ↑24.1-fold), while downregulating immunosuppressive cytokines IL-10 and TGF-β (↓0.4-fold, ↓0.3-fold). Similar pro-inflammatory responses were observed in astrocytes. Importantly, SPT showed no toxicity on healthy macrophages or astrocytes and decreased ROS levels in infected astrocytes by 40–80%, depending on dose ($p \leq 0.0001$). This is the first study to demonstrate both the immunoregulatory and antiparasitic efficacy of a boron-based therapeutic in a CNS-like *Leishmania* infection model.

Molecular basis of disease (excluding cancer)

P-41-001
The minor allele polymorphism in human alanine:glyoxylate aminotransferase modulates pathogenicity of Ile279Thr variant associated with primary hyperoxaluria type 1

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Human alanine:glyoxylate aminotransferase (AGT) is a liver peroxisomal enzyme that utilizes PLP to metabolize glyoxylate, to glycine. Inherited mutations of the AGXT gene reduce the amount of functional AGT resulting in oxalate accumulation and precipitation as calcium oxalate stones in the urinary tract. This condition is known as primary hyperoxaluria type 1. In humans, AGT is present as a major and a minor polymorphic form, the latter characterized by two point mutations, leading to the p.Pro11Leu and the p.Ile340Met amino acid changes. Although the presence of the minor allele is not pathogenic per se, it reduces the overall stability of AGT. Thus, it can synergize with other mutations and lead some mutations become pathogenic only when present on the background of the minor allele. Interestingly, most of the molecular effects of the minor allele are caused by the p.P11L substitution. We analyzed the peculiar behavior of the Ile279Thr variant, which displays high frequency in healthy populations, but has been found in PH1 patients associated with the p.P11L change in the absence of the p.Ile340Met substitution, thus suggesting that its pathogenicity could depend on the allelic background. To define the molecular effects of the p.Ile279Thr variant on AGT, and its possible synergy with polymorphic changes, we inserted the change on three different backgrounds, i.e. major, minor and p.P11L. We then performed biochemical analyses on the purified proteins as

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well as on the variants expressed in two different cellular models of disease. We found that the mutation of Ile279 causes a folding defect that is exacerbated in the presence of the Pro11 mutation, in agreement with clinical data. These data corroborate the idea that a correct diagnosis of PH1 should not be limited to the identification of pathogenic changes but also include a detailed analysis of the polymorphic background, which can strongly influence the clinical manifestations of a mutation.

P-41-002
Stressor-dependent effects in a mouse model of post-traumatic stress disorder: down-regulation of immune response with minimal impact on the antioxidant system

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Post-traumatic stress disorder (PTSD) is a chronic mental disease that can arise in people who have experienced traumatic, shocking, or dangerous events and is especially high in people from countries at war. The long-term molecular consequences associated with PTSD are poorly understood. Our study examined long-term effects of different types of traumatic factors such as physical (2-hour restraint), social (social isolation), and psychological (predator sound) in male mice C57BL/6J. In the open field (OF) test, the following parameters were tested: average speed, time spent in the inner zone, and the number of mice defecation. All treatments explored in the present study led to behaviors that indicate anxiety. However, every treatment conferred specific pattern of behavioral outcomes tested in the OF. All stressors induced long-term changes, on the 156th day, in hematological parameters and mRNA levels in the cerebral cortex. Mice of all stressed groups showed lower levels of *CCL2* and *CYP1A1* mRNA and higher levels of *PPARGC1A*, *SHANK1*, and *SGK1*. Socially isolated mice additionally showed higher *FKBP5* mRNA levels in the cerebral cortex. Lower blood paraoxonase (PON) and myeloperoxidase activities (MPO) were observed in plasma of stressed mice. Thus, our results suggest that exposure to traumatic factors usually used for induction PTSD in rodent models leads to long-term consequences as suppression of inflammation response (*CCL2*, MPO), dysregulation of detoxication (*CYP1A1*, PON) and glucocorticoid-mediated stress response (*SGK1*, *FKBP5*) and elevation of metabolic regulator *PGC-1α* in the cerebral cortex and mice. Moreover, we suggest immune markers, such as levels of *CCL2* and IL-6 mRNA, MPO, PON, and leukocyte count, as good markers of a stress disorder. Considering a complex pathophysiology of PTSD, we suggest testing PTSD on a system level that integrates multiple biochemical markers underlying highly diverse behavioral consequences.

P-41-003
Investigating histone-homocysteine interactions in disease progression

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Extranuclear histones (EnH) may play a role in the progression of various diseases, including neurodegenerative disorders (ND), where histone H1 is released into the extracellular space, affecting neurons and microglia. Elevated homocysteine (Hcy) have been linked to more than 100 disorders. Hyperhomocysteinemia (HHcy, Hcy>15 μM) is a risk factor for cardiovascular and neurodegenerative diseases. The objective of this study was to evaluate the potential influence of HHcy on EnH, which could have implications for disease progression. The study was conducted on plasma, brain, liver and spleen from the Apoetm1Un (ApoE KO) and SOD1-G93A (SOD1) mouse models, human plasma from patients with thromboembolism (VTE) and HUVEC and N2A AD cell lines grown in hyperhomocysteinemic conditions. Hcy was quantified by HPLC, while levels of EnH were analyzed by western blotting and immunohistochemistry. The cell viability and size were assayed with Countess 3 instrument, while histone PTMs were examined via LC-MS/MS. The results showed an increase in histone EnH1, H3, H3K9me2 and Hcy levels in plasma, brain, liver and spleen in ApoE KO and SOD1 mice compared to WT, except in SOD1 spleens where a decrease in Hcy was observed. Correlation between plasma EnH1 and Hcy, and En histones between different organs was demonstrated, suggesting the existence of a potential organ-organ axis. Human VTE plasma samples revealed higher levels of H1 and anti-H1 antibodies, which correlated with Hcy levels. HUVEC cells treated with Hcy, Hcy thiolactone (HTL) and histones showed an increase in EnH and disruption of one-carbon metabolism. N2A AD cells, which serve as a model for ND, exhibited altered histone PTMs following treatment with Hcy and HTL, suggesting an impaired cellular functions. These findings suggest a potential correlation between Hcy and EnH, which could be used as a marker of disease progression. *The authors marked with an asterisk equally contributed to the work.

P-41-004
Impact of myopathy-linked TPM2 mutations on troponin-mediated actomyosin regulation

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In striated muscle, the regulatory complex of tropomyosin (Tpm) and troponin (Tn) governs the Ca^{2+}-dependent interaction of myosin heads with actin, thereby regulating muscle contraction. The Tpm regions critical for Tn binding include its N-terminal and central segments, although their specific roles in contraction regulation remain incompletely understood. To explore the impact of Tpm-Tn interactions on the mechanism of contraction regulation, we analyzed four disease-associated missense mutations in *TPM2*, the gene encoding the skeletal muscle-specific Tpm2.2 isoform. The mutations result in amino acid substitutions in the N-terminal (D20H and E41K) and central (E181K and N202K) segments of Tpm2.2 and are associated

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with either hypercontractile (D20H and E181K) or hypocontractile (E41K and N202K) phenotypes. Recombinant, bacterially expressed, wild-type and mutant Tpm2.2 variants were analyzed using a series of *in vitro* biochemical assays. At low, non-activating [Ca^{2+}], all Tpm2.2 mutants exhibited wild-type-like behavior, effectively inhibiting the Tn-regulated steady-state actomyosin ATPase activity in solution. However, the EC_{50}^{inh} values for inhibition were elevated by two- to four-fold. At high [Ca^{2+}], both hypocontractile mutants suppressed actomyosin ATPase activity and speed of actin filaments that are propelled by myosin. The hypercontractile Tpm2.2 mutants increased actin translocation speed by myosin, but exhibited diverse effects on actomyosin ATPase activity, showing either hyperactivation (Tpm2.2-D20H) or hypoactivation (Tpm2.2-E181K). Mutations D20H and E41K located in the N-terminal segment enhanced direct interactions between Tpm2.2 and Tn, while mutations E181K and N202K located in the central segment of Tpm2.2 had no detectable effect. In conclusion, the substitutions in the N-terminal and central segments of Tpm2.2 disrupt the Tn-dependent regulation of actomyosin contractility, albeit through different mechanisms.

P-41-005
Innovative host-directed therapies against *Staphylococcus aureus*: targeting intracellular infections with miRNAs

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The overuse of antibiotics has contributed significantly to the global crisis of multidrug-resistant (MDR) pathogens. Despite intensive efforts, the last three decades have seen no discovery of novel antibiotic classes. Most newly proposed therapies target bacterial mechanisms, inadvertently selecting for resistant strains. Contrary to earlier assumptions, mounting evidence identifies *Staphylococcus aureus* as a facultative intracellular pathogen. This capability allows the bacterium to evade antibiotic therapies, many of which fail to reach effective intracellular concentrations, and circumvent immune defenses. Once internalized, *S. aureus* can disrupt phagosomal membranes and replicate within the cytoplasm of host cells, further complicating its eradication. Addressing this challenge, host-directed therapies targeting key host-pathogen interactions offer a promising alternative. Notably, human microRNAs (miRNAs), which regulate gene expression, have emerged as potential candidates. However, efficient miRNA delivery to target cells remains a hurdle. Recent advances in exosome-based delivery systems show promise in overcoming this limitation by ensuring miRNA stability and targeted delivery. In this study, a high-throughput screening of 2798 miRNAs identified 10 candidates capable of inhibiting intracellular *S. aureus* USA300 infections in A549 cells. These miRNAs also reduced infection progression in primary bronchial epithelial cells. RNA-Seq analysis of infected A549 cells pretreated with the most effective miRNAs revealed key host genes and pathways modulated during infection. While further validation is required, these findings underscore the potential of miRNA-based therapies to combat intracellular *S. aureus* infections and address the MDR crisis by offering a host-centered therapeutic approach.

P-41-006
The role of X-inactive specific transcript (XIST) in neuronal development, neuroinflammation, myelination, and therapeutic responses in cerebral organoids

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XIST, a long non-coding RNA, regulates X chromosomal inactivation and has been indirectly linked to autoimmunity. This study investigated how XIST affects developmental processes, neuroinflammation, myelination, and response to therapeutics. In this context, myelinated and inflammation-induced cerebral organoids were produced from XIST-silenced and control H9 cells, subsequently treated with the disease-modifying drugs DMF and FTY720. Myelination and inflammation-based gene expression and α-synuclein levels were analyzed by qRT-PCR and ELISA, respectively. Immunofluorescence staining was employed to assess the levels of inflammation, myelination, and development-related proteins. Transcriptome and secretome analyses with bioinformatic assessments, were conducted to explore the XIST's comprehensive role. Silencing resulted in a 2-fold increase in IL-10 levels, while MBP and PLP1 levels increased by 2.3- and 0.6-fold, respectively. The Iba1 protein levels increased 2.7-fold in the XIST-silenced tissues; however, silencing did not affect myelination. The response to FTY720 showed distinct characteristics in XIST-silenced tissues regarding inflammation and myelination, whereas no significant alterations were noted in DMF-treated organoids. The numbers of neural stem cells, microglia, oligodendrocytes, and neurons were also doubled in the XIST-silenced tissues. Silencing reduced the α-synuclein concentration by about 3-fold. The XIST's role in neuronal development and immune response were elucidated by transcriptome and gene enrichment analysis. Nonetheless, the secretome analysis did not reveal a sufficient quantity of secreted protein. The study demonstrated that XIST regulates anti-inflammatory functions via controlling microglia activation and alpha-synuclein levels. Last but not the least, its role in cerebral organoid development and immune response regulation spots XIST as a promising therapeutic target. This study was supported by TUSEB 28520 and TUBITAK 119Z389.

P-41-007
Pitpnm3, a new target of the HGF/Met pathway in hepatic progenitor cells

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Hepatic progenitor cells (HPC) are bipotential cells involved in liver regeneration after chronic damage. Their fate and role

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during the development and progression of chronic liver disease (CLD) relies on their intrinsic plasticity and the diversity of signals from their niche, having been described as having both pro-fibrogenic and pro-regenerative actions. Hepatocyte growth factor (HGF) is among the key signals involved in liver regeneration and, as a component of the HPC niche, modulates HPC behavior. However, the molecular mechanisms responsible for HGF-mediated regulation of HPC are only partially understood. To address this question, a transcriptomic study using MACE (Massive Analysis of cDNA ends = 3'mRNA-Seq) technology has been performed to identify target genes of the HGF/Met pathway in HPC and, consequently, potential targets for modulation of liver regeneration in CLD. Among these genes, Pitpnm3, a member of a family of membrane-associated phosphatidylinositol transfer proteins, was selected for further validation and study. The HGF-mediated induction of Pitpnm3 mRNA and protein levels in HPC was validated *in vitro*. More importantly, by using siRNA and CRISPR Cas9 KO-based approaches we show that both pro-proliferative and pro-migratory/invasive actions of HGF in HPC are abolished upon Pitpnm3 silencing. Pitpnm3 silencing also interferes with HGF-triggered signaling pathways in these cells. Altogether, results point to Pitpnm3 as a critical mediator of HGF-triggered signaling and biological activity in HPC. As a next step, we are currently evaluating its role in HPC under specific pathological contexts, such as chronic treatment with TGF-β, emulating a fibrotic liver damage; or HPC that have undergone a malignant transformation by Ras constitutive activation; or HPC pretreated with bile salts, emulating a cholestatic context. Finally, we will deepen in Pitpnm3 pathophysiological implications in CLD using experimental models and human patients' samples.

P-41-008
Elucidating the molecular mechanisms of human smooth muscle cell plasticity: a translational *in vitro* platform for therapeutic target discovery in coronary artery disease

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Smooth muscle cell (SMC) dysfunction is pivotal to coronary artery disease (CAD) progression. SMCs support vascular stability and regulate vascular tone in their contractile state. However, in response to vascular injury, secreted growth factors such as platelet-derived growth factor B (PDGF-BB) induce a transition to a synthetic phenotype. This transition destabilises the vessel wall and exacerbates disease progression. Despite its significance in CAD, the underlying molecular mechanisms of SMC plasticity remain poorly understood, hindering therapeutic development. A human coronary artery SMC-based *in vitro* model was developed to study PDGF-BB-induced phenotypic plasticity. Bulk RNA sequencing identified key markers and regulators of the transition. Translational relevance of the model was validated through benchmarking against published scRNA-seq data from diseased human coronary arteries. Candidate regulators were prioritised by filtering for CAD-associated GWAS loci. Their functional roles are being evaluated using a medium-throughput siRNA screening pipeline. PDGF-BB treatment induced hallmark features of the synthetic SMC phenotype, including enhanced proliferation, migration, and ECM remodelling. Bulk RNA sequencing identified novel regulators of the transition, with significant overlap observed with CAD GWAS loci. Comparison with scRNA-seq data from CAD patients highlighted congruous expression patterns, validating the model's translational relevance. Preliminary siRNA screening of prioritised candidate genes revealed significant modulatory effects on SMC phenotype, yielding novel insights into the underlying mechanisms of SMC plasticity. This study establishes a robust translational platform to investigate SMC plasticity. By integrating transcriptomics, GWAS loci, and functional validation, novel regulators of SMC plasticity were identified, providing a foundation for therapeutic strategies to mitigate CAD progression.

P-41-009
MKRN3 ubiquitin ligase associated with central precocious puberty plays a key role in translation

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MKRN3 belongs to the Makorin RING finger ubiquitin ligase (MKRN) family, all members of which have C3H, Cys-His and RING finger motifs in their structure. Mutations that disrupt this gene are associated with a variety of disorders. For example, point mutations affecting the ubiquitin ligase activity of MKRN3 lead to the development of central precocious puberty, as MKRN3 inhibits the production of gonadotropin-releasing hormone, which in turn directly triggers the onset of puberty. At the same time, large deletions in this gene cause Prader-Willi syndrome, in which the functional role of MKRN3 is poorly understood. To study molecular roles of MKRN3 and identify its new targets, we overexpressed a fusion of MKRN3 and the TUBE domain (a component that stabilizes the complex of the ubiquitin ligase and its target) in a human neuroblastoma cell line. We were able to significantly expand the spectrum of putative MKRN3 partners during the co-immunoprecipitation experiments. We observed that a significant number of proteins associated with translation, endoplasmic reticulum transport and nonsense-mediated degradation co-precipitated with MKRN3. However, we suspected that some of the interactions detected were RNA-mediated, since MKRN3, like other members of the family, may have an RNA-binding function. Repeating this experiment with RNAse treatment revealed that MKRN3 binds directly to ribosomal proteins. Further investigation of the effect of MKRN3 on translation revealed that it interacts with the large subunit of the ribosome in the p-stalk region. Finally, analysis of MKRN3 and ribosome complexes revealed enrichment of translation initiation factors and 3'UTR processing proteins. Our sequencing data of RNAs co-immunoprecipitating with MKRN3 also confirm the interaction of MKRN3 with mRNAs in the 3′UTR region. This suggests that MKRN3 plays a key role in 3'UTR mRNA processing and translation-associated processes. This work was supported by RSF grant 24-14-00048.

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P-41-010
Pathway dynamics in progeroid DNA-repair-deficient mice: a multi-tissue analysis

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The rise in the elderly population in industrialized countries is increasing cases of age-related diseases, with the number of people over 60 expected to double by 2050. Understanding aging mechanisms is crucial for managing these diseases, and growing evidence suggests that DNA damage plays a key role in aging. The Ercc1Δ/- mouse model, which exhibits accelerated aging due to a DNA repair defect, serves as a valuable system to study this interplay. This study investigates the systemic effects of DNA repair deficiency across multiple tissues in Ercc1Δ/- mice by integrating RNA-seq data to identify common cellular and molecular changes. Through Gene Set Enrichment Analysis (GSEA) and Overrepresentation Analysis (ORA), we analyze transcriptomic data to gain insights into gene expression changes. Our findings reveal a significant systemic dysregulation of immune system pathways, suggesting that persistent DNA damage signaling leads to chronic immune activation. Additionally, we identify other pathways previously linked to aging, further validating the model. This study presents a pipeline for identifying shared and systemic responses to DNA repair deficiency, contributing to our understanding of DNA damage as a major driver of aging and age-related diseases.

P-41-011
Fibroblast growth factor-12 regulates vascular smooth muscle cell stiffness and mechanosignaling, driving aortic aneurysm development in Marfan syndrome mice

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Emerging evidence suggests that dysregulated mechanosignaling in vascular smooth muscle cells (VSMCs) and increased VSMC stiffness play a critical role in the aortopathy of Marfan syndrome (MFS). However, the key mediators driving these alterations in mechanosignaling and cellular stiffness during thoracic aortic aneurysm (TAA) formation in MFS remain largely undefined. Here, this study shows that fibroblast growth factor 12 (FGF12) is highly expressed in VSMCs within the aneurysmal walls of MFS ($Fbn1^{C1039G/+}$) mice and MFS patients. In human aortic SMCs, FGF12 is upregulated by transforming growth factor-β and cyclic mechanical stretch. FGF induces aberrant mechanosignaling, as evidenced by increase in RhoA-GTP level, actin polymerization, and focal adhesion assembly, leading to significant elevation in VSMC stiffness, via Angiotensin II (AngII)/AngII type 1 receptor (ATIR) signaling pathway. In an AngII/β-aminopropionitrile-induced aneurysm model, $Fgf12^{+/-}$ mice exhibit significant reduction of TAA formation and arterial stiffness, along with a marked attenuation of ATIR hyperactivation and aberrant mechanosignaling in the arterial walls compared to wild-type mice. Furthermore, $Fgf12^{+/-}Fbn1^{C1039G/+}$ mice display substantial decrease in both TAA formation and arterial stiffness compared to $Fbn1^{C1039G/+}$ mice. These findings establish FGF12 as a novel factor mediating aberrant mechanosignaling and elevated stiffness of VSMCs, contributing to TAA pathogenesis in MFS.

P-41-012
Could high serum CRP levels serve as a biomarker for children and adolescents diagnosed with generalized anxiety disorder?

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Generalised anxiety disorder (GAD) is a common anxiety disorder characterized by persistent and uncontrollable worry and anxiety. Recently, the role of neuroinflammation in the pathogenesis of psychiatric disorders has been increasingly discussed. Our study aims to detect levels of serum C-reactive protein (CRP), neopterin, TWEAK, IFN- γ, TNF- α, and IL-6 in patients with GAD and thereby contribute to existing evidence about inflammation and GAD. The study included 25 treatment-naive GAD patients aged 8-18 and 30 healthy controls. The criteria for diagnosis were established by the Schedule for Mood Disorders and Schizophrenia for Children (K-SADS-PL-DSM-5) and the Revised Child Anxiety and Depression Scale (RCADS). The study participants' serum CRP, neopterin, TWEAK, IFN- γ, TNF- α, and IL-6 levels were analyzed. The results were compared between the children and adolescents diagnosed with GAD and controls. Serum CRP levels of GAD patients 1.7 [1.2–2.94 mg/L] were significantly higher than healthy controls 1.3 [1.2–1.7 mg/L] ($p = 0.034$). There was no difference, which was statistically significant, between groups in terms of other biomarker levels ($p > 0.05$). Based on the ROC curve analysis, the AUC was determined as 0.665 (95% CI: 0.511–0.820) for CRP (p: 0.036). Positive correlations were found between RCADS-CV total anxiety score ($r = 0.729$, $p = 0.001$) and RCADS-CV GAD score ($r = 0.642$, $p < 0.001$) with the illness duration. Our results showed that the levels of CRP, an indicator of inflammation, were higher in children and adolescents diagnosed with GAD. The results are consistent with a role of inflammation in anxiety disorders. CRP may have increased due to neuroinflammation, which is thought to play a role in GAD pathophysiology. On the other hand, the increase in CRP levels may have triggered GAD pathophysiology. CRP contribution to the pathophysiology of GAD should be investigated in greater detail. *The authors marked with an asterisk equally contributed to the work.

P-41-013
Tight junction proteins and inflammation in obsessive-compulsive disorder severity: the role of MarvelD3 and tricellulin in children and adolescents

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Obsessive-compulsive disorder (OCD) is a chronic neuropsychiatric condition in which individuals experience

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involuntary, unwanted thoughts (obsessions) and repetitive acts (compulsions) that are directed at avoiding these. Although the exact aetiopathogenesis of OCD is unknown, along with genetic factors, processes related to neuro-inflammation and barrier integrity have emerged as prominent in recent years. This study aims to investigate the association of serum levels of tight junction (TJ) proteins (occludin, tricellulin, MarvelD3) and inflammatory markers (CRP, TNF-α, IL-6) with drug-naïve children and adolescents and OCD severity. Forty-three children and adolescents with OCD and 37 age-matched controls were recruited for the study. ELISA was employed to quantify the levels of serum TJ proteins and inflammatory markers in the subjects. MOCI was employed to quantify OCD severity. Statistical analysis determined associations between clinical features, biochemical results, and disease duration. The OCD group had significantly higher rates of psychiatric comorbidity and family history of OCD. Tricellulin, MarvelD3, and TNF-α levels were positively correlated with MOCI scores. MarvelD3 levels were also found to be associated with disease duration. MarvelD3 and TNF-α levels were significantly higher in patients with moderate-severe OCD. No significant differences were found between the groups regarding CRP and IL-6 levels. MarvelD3 and tricellulin are expected to play a role in the pathophysiology of OCD and modulate the disease severity by way of barrier function and inflammation. The correlation of MarvelD3 with disease duration suggests that the protein is a chronicity marker. These findings support the mechanistic role of TJ proteins in OCD and their potential as therapeutic targets. *The authors marked with an asterisk equally contributed to the work.

P-41-014

Abstract withdrawn.

P-41-015

Transthyretin variants differentially activate cell-specific pathways and biomarkers in transthyretin amyloidosis target cells

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Transthyretin (TTR) amyloidosis (ATTR) is a protein-misfolding disorder characterized by systemic deposition of TTR aggregates in various tissues and organs, resulting in dysfunction. The clinical manifestations of ATTR include cardiomyopathy (CM) and polyneuropathy (PN) caused by specific mutations in the *TTR* gene, leading to TTR variants (TTRv). ATTR-CM arises primarily from age-related failure of wild-type (wt) TTR but can also result from TTRv. This study aimed to identify cardiac-specific biomarkers and contribute to the design of targeted therapeutics for ATTR. Different TTRs (wt, V30M, S52P, T119M, and V122I) were subjected to physiological pH-mimicking conditions, and their aggregation states were monitored until oligomer/pre-fibril formation, using dynamic light scattering, ThT-binding assays, and transmission electron microscopy. Then, ATTR-target cells, including cardiac AC16, primary mouse cardiac fibroblasts (mPCF), and neuronal SH-SY5Y cells, were exposed to soluble and aggregated TTRs. Cellular effects were assessed through viability, cytotoxicity, and caspase 3/7 activity (ApoTox-Glo Triplex assay), metabolic activity via mitochondrial function (MTT assay), and extracellular flux (Seahorse XFe24 assay). Additionally, biomarkers of endoplasmic reticulum (ER) stress (BiP) and autophagy (p62 and LC3) were analyzed by western blotting of cell lysates using specific antibodies. Cell exposure to TTRs did not alter cellular metabolism, indicating no impact on this pathway. The differential regulation of p62 and LC3 in AC16 and SH-SY5Y cells suggests autophagy impairment. Elevated BiP levels in AC16 cells and mPCFs exposed to aggregated V122I, a potent CM TTRv, confirmed the induction of ER stress. These results show that tissue-specific pathways and biomarkers are differentially activated by aggregated TTRs, with TTRv exerting cell-specific effects, correlating with their pathological impact, thus highlighting the potential of targeted therapies for ATTR.

P-41-016

Endoplasmic reticulum stress and ferroptosis: effect of 7,8-dihydroxyflavone

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Endoplasmic reticulum (ER) stress is a condition triggered by the accumulation of unfolded and misfolded proteins in the ER lumen, leading to disruptions in ER homeostasis. Prolonged ER stress leads to cell death such as apoptosis and ferroptosis. Ferroptosis is characterised by iron accumulation in tissue and is closely associated with intracellular oxidative stress. The beneficial effects of 7,8-dihydroxyflavone (7,8-DHF), a plant flavonoid and brain-derived neurotrophic factor (BDNF) mimetic, such as decreasing body weight, increasing systemic energy consumption and protecting cells against various oxidants have been demonstrated. The aim of this study was to investigate the effect of 7,8-DHF on ferroptosis and oxidative stress markers in tunicamycin (TUN)-induced ER stress model. For this purpose, three groups were formed with 10 C57BL/6 male mice in each group. To induce ER stress, two groups were injected with a single dose of 0.5 mg/kg TUN. Then 5 mg/kg/day 7,8-DHF was administered to one group for 21 days. The other group was administered solvent for the same period. Iron, total oxidant status (TOS), total antioxidant status (TAS) levels in serum and liver tissue samples obtained at the end of the experiment were determined by commercial colourimetric kits and malondialdehyde (MDA) level was determined by manual method. In tunicamycin-induced ER stress model, tissue iron levels and MDA levels were significantly higher than the control group ($p = 0.01$ and $p = 0.001$), while TAS levels were significantly lower ($p = 0.004$). In 7,8-DHF treated group, tissue iron and MDA levels were significantly decreased ($p = 0.001$ and $p = 0.0013$), while TAS levels were increased ($p = 0.019$) compared to TUN treated group. In conclusion, ER stress induces ferroptosis and oxidative stress, whereas 7,8-DHF exhibits protective effects by decreasing tissue iron levels and oxidative stress and increasing antioxidant levels.

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P-41-017
An advanced *in vitro* model of skin fibrosis for drug discovery

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Skin fibrosis remains a significant clinical challenge due to the ineffectiveness of current therapeutic interventions. A critical barrier to advancing treatment lies in the scarcity of physiologically relevant, reproducible, cellular *in vitro* models that reflect as best as possible dermal fibrosis. This study aims to optimize an *in vitro* skin fibrosis model by utilizing dextran sulfate as a macromolecular crowding (MMC) agent in combination with TGF-β_1 or a cocktail of pro-inflammatory cytokines (IL-6, IL-13). The objective is to enhance the efficiency of TGF-β_1-induced fibroblast-to-myofibroblast transition (FMT) and better mimic the excessive extracellular matrix (ECM) proteins deposition characteristic of fibrotic disorders. Human dermal fibroblasts (HDFs) serve as the model system, with FMT efficiency assessed through gene expression profiling (qRT-PCR) and protein-level analyses (western blot and ELISA). In addition, the metabolic activity and cytotoxicity of cells exposed to the new fibrosis model will be assessed using MTT and LDH assays to ensure their physiological relevance and suitability for drug screening. Preliminary results indicate that an optimized fibrosis model in synergy with cytokines, accelerates ECM protein deposition and enhances αSMA (alpha smooth muscle actin), fibronectin, and collagen expression. This optimized model will serve as a valuable tool for screening anti-fibrotic drugs and developing targeted therapies for pathological skin scarring. Acknowledgments: this work was financially supported by the National Science Centre project agreement number UMO-2024/53/N/ST5/00539.

P-41-018
Effects of cigarette smoking on atherogenic plasma index, lipid profiles, and systemic immune-inflammation index in chronic patients

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Cigarette smoking is a well-established risk factor for cardiovascular disease and systemic inflammation. An increase in chronic diseases such as obesity, diabetes, metabolic syndrome, and thyroid dysfunction has been observed worldwide, contributing to the growing prevalence of cardiovascular disease and imposing significant costs and burdens on healthcare systems. This study investigates the effects of cigarette smoking on lipid profiles, the Atherogenic Plasma Index (API), and the Systemic Immune-Inflammation Index (SII) in chronic patients. A total of 29 chronic patients (83.3% female, 16.7% male) with average age of 49.6 were divided into two groups: smokers (n = 18) and non-smokers (n = 11). Lipid profiles including total cholesterol, LDL cholesterol, HDL cholesterol, triglycerides, API, and SII were measured and compared between groups. Smokers had slightly higher total cholesterol (208.88 mg/dL vs. 207.55 mg/dL), LDL cholesterol (132.71 mg/dL vs. 130.45 mg/dL), and triglycerides (160.88 mg/dL vs. 149.82 mg/dL) than nonsmokers. Conversely, HDL cholesterol was lower in smokers (44.18 mg/dL vs. 48.70 mg/dL). Similarly, smokers had a higher mean API (0.5382 vs. 0.4303) and SII (473.57 vs. 401.60) than nonsmokers. Despite these observed differences, statistical analysis revealed no significant differences between the two groups for any of the measured parameters. These results indicate that smokers tend to have slightly altered lipid profiles, API, and SII compared to non-smokers, but the differences were not statistically significant. The trends observed, particularly elevated triglycerides, API, and SII in smokers may have clinical significance and warrant further investigation. Our study is ongoing and we aim to include more patients to provide a broader and more robust evaluation of the effects of cigarette smoking on inflammatory and cardiovascular risk markers in chronic disease management.

P-41-019
Chemokines CCL2 and CX3CL1 in alcohol use disorder

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Chronic alcohol consumption induces inflammatory responses that are manifest in the stimulation of immune cells to secrete peripheral pro- and anti-inflammatory cytokines. Acute and moderate alcohol consumption is believed to be associated with suppression of inflammatory responses, while chronic alcohol abuse increases inflammation. The aim of the study was to investigate polymorphisms of chemokine genes, and the content of its proteins in peripheral mononuclear cells in patients with alcohol use disorder. The study involved 201 patients with alcohol use disorder (F10.2, ICD-10). The comparison group included 205 healthy individuals. The study was conducted on the basis of the Core Facility "Medical genomics". Genotyping of polymorphism of chemokine genes (CCL2: rs4795893, rs2530797; CX3CL1: rs170364) was performed by real-time PCR using DNA-Synthesis kits (Russia). The expression of proteins – products of the studied genes in peripheral blood mononuclear cells was assessed using Magnetic Lumine Assay Kit (Cloud-Clone Corp., China). No statistically significant differences in the frequency distribution of genotypes and alleles of the studied polymorphic variants were found between patients with alcoholism and the control group. At the same time, rs4795893*GG of the CCL2 gene is associated with a higher severity of the anhedonia symptom (SHAPS) upon admission to the clinic ($p = 0.029$), and rs4795893*AA is associated with a more pronounced pathological craving for alcohol (OCDS) ($p = 0.034$). The content of CCL2 in peripheral mononuclear cells of patients before treatment was statistically significantly higher than in the control group ($p = 0.001$) and did not decrease during therapy. The association of CCL2 with alcohol use

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disorder is shown. The degree of its involvement in pathogenesis remains to be studied. This work was financially supported by the Russian Science Foundation (project no. 23-15-00338).

P-41-020
Antioxidant enzyme gene polymorphic variants: relation with metabolic syndrome in patients with schizophrenia receiving long-term antipsychotic therapy

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Second-generation antipsychotics (SGAs) have been linked to a rise in metabolic syndrome (MetS) and cardiovascular diseases in people with schizophrenia. Somatic illness can lead to a drop in quality of life, less compliance, and a shorter life expectancy. MetS involves increased lipid peroxidation, and SGA treatment has been shown to cause oxidative stress. Enzymes NQO1, CAT, and SOD are crucial for prooxidant-antioxidant balance. This study assessed the association of NQO1, CAT, and SOD gene polymorphisms with MetS in patients with schizophrenia undergoing SGA therapy. The study included 603 patients with schizophrenia (302 men and 301 women, median age 39 years). MetS was diagnosed in 156 (25.9%) individuals. Genotyping of polymorphisms of the studied genes was performed by real-time PCR on a QuantStudio 5 amplifier (Applied Biosystems, USA) at the Center for Collective Use "Medical Genomics" of the TNRMC. Statistical analyzes were performed using Statistica V.12.0. The blood serum of schizophrenia patients with MetS had higher levels of thiobarbituric acid-active products, which are indicative of lipid peroxidation intensification, compared to those without MetS ($p = 0.042$). We found associations between the NQO1 rs1800566 polymorphism and MetS among patients with schizophrenia. The NQO1 rs1800566*A allele was found to be a predisposing factor (OR 1.63, 95%CI 1.01-2). The NQO1 rs1800566*G allele was identified as a protective factor against MetS (OR 0.61, 95%CI 0.38-0.99). Carriers of the TT genotype had higher blood serum triglyceride levels compared to those with the CC or CT genotype ($p = 0.049$). No association was identified between CAT (rs1001179) and SOD2 (rs4880) gene polymorphisms and risk of MetS in schizophrenia patients. The results suggest a potential role of NQO1 in the pathogenesis of SGAs-induced MetS. The study is supported by the Russian Science Foundation, project #23-75-10088.

P-41-021
Apoptosis modulation by the antidepressant escitalopram in alemtuzumab-treated multiple sclerosis patients: an *ex vivo* approach

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Multiple sclerosis (MS) is a chronic inflammatory disease characterized by neurodegeneration. Alemtuzumab, a third-line immunosuppressive drug, is used when first- and second-line treatments fail, reducing lymphocyte counts through apoptosis. Depression is common in MS patients, and escitalopram, a selective serotonin reuptake inhibitor (SSRI), is frequently prescribed. While escitalopram has demonstrated apoptotic effects in cancer cell lines and genotoxicity in lymphocyte cultures, its combined effect with alemtuzumab on lymphocyte apoptosis remains unclear. This study examined the apoptotic effects of escitalopram on peripheral blood mononuclear cells (PBMCs) from MS patients receiving alemtuzumab, comparing them to healthy controls and naïve MS patients. Blood samples were collected from these groups, and PBMCs were isolated and cultured. Escitalopram dose optimization was conducted using the Alamar Blue test. Apoptosis markers (Caspase-3 and Annexin V) were analyzed via flow cytometry, and *CASP3* gene expression was determined using qRT-PCR. No significant differences were observed in *CASP3* gene expression or Annexin V activation between escitalopram treated and untreated groups. However, Caspase-3 activation, while not statistically significant ($P = 0.066$), showed a trend toward significance, suggesting a potential increase in apoptosis. These findings highlight the need for further investigation into the underlying mechanisms and clinical implications, as well as a careful evaluation of drug interactions in MS treatment to optimize patient outcomes. This study was supported by TUBITAK 2209-A-1919B012211950. *The authors marked with an asterisk equally contributed to the work.

P-41-022
The protective effects of aurantiamide acetate on a cellular model of hypertrophic cardiomyopathy

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This study aimed to evaluate the protective effect of aurantiamide acetate (AA) in angiotensin II (Ang II)-induced cellular hypertrophic cardiomyopathy through the mitogen-activated protein kinase (MAPK) signaling pathway. The potential of AA to alleviate ER stress was also investigated in H9C2 cardiomyocytes. Hypertrophy was induced using Ang II, and the angiotensin type-1 receptor (AT1R) antagonist losartan was used to treat negative controls. Tunicamycin (TM) generated ER stress, and the ER stress blocker tauroursodeoxycholic acid (TUDCA) was applied to negative controls. Cell viability was assessed using the MTT assay. Changes in cell surface area were quantified following phalloidin staining using ImageJ software. Levels of atrial natriuretic peptide (ANP), glucose-regulated protein 78 (GRP78), p38 mitogen-activated protein kinase (p38 MAPK), extracellular signal-regulated kinase (ERK) and c-jun n-terminal kinase (JNK) were evaluated using immunofluorescence and ELISA. Treatment with 1 μM Ang II successfully induced hypertrophy in H9C2 cells. Cells treated with 6.25 μM AA exhibited a significant reduction in cell surface area and decreased protein levels per cell, like the effects observed with 10 μM losartan. ANP levels were significantly reduced in AA-treated cells. TM did not induce hypertrophy, however both 0.5 μg/ml TM and Ang II treatment significantly increased levels of the ER stress marker GRP78. Both AA and TUDCA treatment

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markedly reduced ER stress in cardiomyocytes. Ang II treatment also increased p38, ERK, and JNK phosphorylation levels, while AA and losartan treatment effectively reduced protein phosphorylation in these markers. In conclusion, aurantiamide acetate significantly attenuated cellular hypertrophy. The observed reduction in the phosphorylation of MAPK markers and ER stress following AA treatment suggests its potential protective role in the induction of hypertrophic cardiomyopathy.

P-41-023
Evaluation of serum synaptophysin-like protein-1 and glutathione peroxidase 4 levels in epilepsy patients

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Epilepsy is a chronic, non-contagious brain disorder that affects around 50 million people worldwide. It is characterised by recurrent seizures, which are brief episodes of involuntary movements that can affect part or all of the body, sometimes accompanied by loss of consciousness and control of bowel or bladder function. Synaptophysin-like protein 1 (SYPL1) of the synaptophysin family is a vesicular membrane protein found in neuronal and non-neuronal tissues. The selenoprotein glutathione peroxidase 4 (GPX4) is one of the major antioxidant enzymes in the human body that reduces complex hydroperoxides to their respective alcohols using reduced glutathione as the reducing agent. In this study, we aimed to investigate the serum levels of SYPL1 and GPX4 in epilepsy and control groups. Thirty-eight patients with epilepsy and 30 healthy volunteers were included in the study. SYPL1 and GPX4 levels were measured by enzyme-linked immunosorbent assay (ELISA). Statistical analyses were performed using Graphpad Prism. SYLP1 levels were significantly higher in epilepsy patients (35.27 ± 17.16) compared to controls (26.34 ± 10.19; $p = 0.0033$). GPX4 levels were significantly lower in epilepsy patients (4.58 ± 3.29) compared to controls (7.41 ± 3.91; $p < 0.001$). ROC curves were designed for SYPL1 and GPX4 in epilepsy patients. According to the ROC analysis, SYPL1 and GPX4 were found to be diagnostically sensitive parameters for epilepsy. These results suggest that SYPL1 and GPX4 can be used as useful biomarkers in the diagnosis of epilepsy, providing valuable information for early diagnosis and potential clinical applications. Further studies are needed to confirm these findings and to investigate their utility in clinical practice. This study was supported by the TUBITAK 2209-A programme (project number: 1919B012318716).

P-41-024
COPreDict: a genetic algorithm constructs a drug-target panel for a personalized chronic obstructive pulmonary disease (COPD) RT-PCR blood test

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Chronic obstructive pulmonary disease (COPD) is a heterogeneous disorder characterized by progressive airflow limitation and chronic inflammation, with variable responses to treatments. Current therapeutic strategies rely on broad clinical classifications rather than personalized molecular profiles, often leading to suboptimal outcomes. To address this, we developed a targeted PCR panel coupled with COPreDict bioinformatics software tool to prioritize COPD drugs based on patient-specific transcriptomic signatures. The panel counts 93 genes and is based on QIAGEN RT2 qPCR Primer Assays. The panel was designed on the basis of publicly available datasets analysis, mainly GSE60399, which compares COPD acute exacerbation and patient stabilization states. Data processing was performed using Genome Enhancer (Kel AE et al. (2019) BMC Bioinformatics 20, 119). Key transcriptional regulators were found by promoter analysis of differentially expressed genes with the TRANSFAC database and ML algorithm. Consequently, druggable master regulators were identified by network analysis using the TRANSPATH and HumanPSD databases. The final panel includes 48 master-regulators and drug target genes implicated in COPD pathophysiology, 15 internal control genes for normalization, and 30 regulatory molecules exhibiting gradual expression changes between COPD exacerbation and stabilization states. The panel was validated in an independent patient case study, supporting the COPD molecular mechanism model developed for the COPreDict system. Using input expression values from a patient's blood sample, the COPreDict system ranks COPD drugs for that patient by identifying the most active master regulators in the sample. This approach could potentially advance precision medicine in COPD by tailoring drug selection to patient-specific gene expression profiles. This work was funded by 29th IraSME Call for Transnational Collaborative R&D Project Proposals, grant number KK5297004LU2. *The authors marked with an asterisk equally contributed to the work.

P-41-025
Understanding MPS-plus: insights into the molecular basis of the disease

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Mucopolysaccharidoses (MPS) represent a subset of rare genetic disorders, with an estimated prevalence of 1 in 44 000 individuals. Despite the identification of the primary genetic

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defect—mutations leading to the deficiency of specific enzymes responsible for glycosaminoglycan (GAG) degradation—the precise molecular mechanisms underlying disease pathogenesis remain largely elusive. MPS-plus syndrome (MPSPS) is an exceptionally rare variant of MPS, with approximately forty reported cases worldwide. The "plus" designation refers to an atypical genetic anomaly that results in GAG accumulation, coupled with additional clinical manifestations. Unlike conventional MPS types, where lysosomal GAG accumulation results from the absence or severe dysfunction of a specific lysosomal enzyme, MPSPS presents a unique pathophysiological scenario in which no known enzymatic deficiency has been identified. To date, the molecular pathomechanism of MPSPS remains undefined, and no targeted therapy has been established. Recent studies have suggested that ambroxol and genistein, compounds known for their efficacy in reducing intracellular deposits of toxic macromolecules and promoting autophagy, may hold therapeutic potential. This study aims to characterize the molecular defects in cells derived from MPSPS patients. Our analyses of MPSPS fibroblasts confirmed significantly increased levels of heparan sulfate relative to control cells. Fluorescence and electron microscopy, combined with western blotting, revealed alterations in marker protein levels, localization, and structural organization in affected cells. Furthermore, treatment with ambroxol and genistein facilitated the clearance of accumulated macromolecules and enhanced the expression of LC3-II, a key autophagy marker. Preliminary identification of molecular defects in MPSPS cells was achieved, and ambroxol and genistein demonstrated potential in improving organelle morphology and inducing autophagy.

P-41-026
Zinc-dependent pathways mediating retinal neurodegeneration

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Oxidative stress is considered a shared factor inducing retinal damage in age-related macular degeneration (AMD), diabetic retinopathy (DR), and glaucoma, prevalent blind-causing diseases that affect different retinal neurons. Oxidative conditions are associated with the release of mobile zinc due to the oxidation of zinc buffer proteins (metallothioneins). In this study, we examine whether excess zinc is a common mediator of neurodegeneration in various retinal diseases and what are the mechanisms underlying its effects. Animal studies demonstrated elevated levels of mobile zinc associated with ocular hypertension, photodamage and inflammation, key drivers of glaucoma, AMD, and DR. Zinc was shown to exert a rapid and pronounced cytotoxic effect in retinal cell models. Molecular fishing in retinal extracts followed by mass spectrometric identification revealed candidate proteins that may mediate pro-apoptotic zinc signaling, among which were components of the retinoic acid (RA) cascade that regulates gene expression in the retina. Western blotting and targeted lipidomics analysis demonstrated activation of RA signaling in response to zinc stress. Knockdown of RA cascade genes affected the susceptibility of retinal model cells to zinc-induced cytotoxicity. Transcriptome analysis of cells subjected to zinc stress revealed upregulation of zinc transporters and chaperones, followed by downregulation of neurotrophic factors and triggering of apoptotic pathways. These processes were associated with activation of RA-dependent genes, including transcription factors that play a critical role in the regulation of neuronal proliferation and apoptosis in the retina. Based on these results, it is suggested that retinal cells are sensitive to zinc stress and the action of zinc is mediated by the RA cascade, which may be a promising target for the treatment of retinal degenerative diseases. This work was supported by the Russian Science Foundation Grant No. 24-15-00171.

P-41-027
Microarray transcriptome analysis and profiling of angiogenesis-related gene expression reveals an increase in angiogenesis in chronic rhinosinusitis with nasal polyps

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Chronic rhinosinusitis (CRS), classified as with nasal polyps (CRSwNP) and without NPs (CRSsNP), is associated with symptoms such as nasal congestion, nasal secretion, etc. CRS is a chronic inflammatory disease with inflammation of the nasal cavity and paranasal sinuses. However, the role of angiogenesis in CRS remains to be explored. This study aimed to investigate angiogenesis-related gene expression profiles and identify the genes and status associated with angiogenesis in CRS. Six controls with nasolacrimal duct obstructions and 6 patients with CRSwNP and -sNP were recruited. The 84 angiogenesis-related gene expression was analyzed by real-time PCR microarray in the nasal mucosa tissues of control and CRSsNP and the NPs of CRSwNP. The differentially changed genes (DEGs) in CRS were regarded as statistically significant when the changes were more than control 2 folds and $p < 0.05$. It was found that 7 and 27 DEGs were upregulated and downregulated, respectively, in CRSwNP. In contrast, only 3 downregulated genes were observed in CRSsNP. Among these DEGs, 12 genes per 34 DEGs including some *cxcl* genes, *mmp-9*, *angiopoietin-1*, *adhesion G protein-coupled receptor B1*, *endothelin-1*, etc., were altered more than 4 fold in CRSwNP, whereas *endothelin-1* was the only >4-fold changed gene in CRSsNP. Their mRNA and protein expressions were further confirmed by RT-PCR, Western blotting, and immunohistochemistry. Moreover, the overall angiogenesis status in the nasal tissues was obtained by detecting the expression levels of the endothelial biomarker through IHC, showing that the number of small vessels was apparently increased in the NPs of CRSwNP but not in the nasal mucosa of CRS. Collectively, we showed here the first evidence of a distinct gene expression profile related to angiogenesis between CRSwNP and -sNP through a microarray transcriptome analysis, suggesting that angiogenesis may play a certain role in CRSwNP, but not CRSsNP, during disease progression.

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P-41-028
Serum thioredoxin and lactoferrin in rheumatoid arthritis and their associations with disease markers and acute phase proteins

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Thioredoxin (Trx) and lactoferrin (Lf) are multifunctional proteins part of the body antioxidant defence and counteract the oxidative tissue damage. The aim of our study is to investigate Trx and Lf levels in serum of rheumatoid arthritis (RA) patients and establish the association of these proteins with disease markers such as rheumatoid factor (RF), anti-CCP antibodies and acute phase proteins such as fibrinogen, CRP, and ferritin. The study included 114 patients with RA and 42 healthy subjects. Serum Trx, Lf, RF, anti-CCP antibodies, IL-6, CRP, and ferritin were determined using commercially available ELISA kits. Fibrinogen levels were measured by the Clauss fibrinogen assay in platelet-poor plasma. Serum Trx levels in RA were significantly higher compared to the control group, 36.4 (29.6-40.2), ng/mL versus 19.0 (16.3-26.8), ng/mL, $p < 0.0001$. Serum Lf levels were elevated in RA compared to the control group, but the difference was not statistically significant. We found significant negative correlations of Trx and Lf with RF in RA, $r = -0.254$, $p = 0.05$ and $r = -0.238$, $p = 0.014$, respectively. The correlations of Trx and Lf with other disease markers such as anti-CCP antibodies, DAS28, ESR, and IL-6 were not statistically significant. A strong positive correlation between Trx and Lf was observed in RA, $r = 0.519$, $p < 0.0001$ [results previously published in: Delcheva GT et al. (2024) J Med Biochem 43, 1-7]. Trx and Lf were positively correlated with fibrinogen in RA patients, $r = 0.410$, $p = 0.002$ and $r = 0.394$, $p < 0.0001$, respectively. The correlations of Trx and Lf with other acute phase proteins such as ferritin and CRP were not statistically significant. In conclusion, Trx and Lf were associated with RF and fibrinogen, but not with anti-CCP antibodies, systemic disease activity and other acute phase proteins. Therefore, the two proteins may serve as new biomarkers for assessment of pathological changes and monitoring of disease progression in RA and may reflect higher levels of oxidative stress in RA patients.

P-41-029
VDAC1-targeted peptide rescues mitochondrial dysfunction, counteracting amyloid-β oligomer toxicity in Alzheimer's disease

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Mitochondrial dysfunction is implicated in a wide range of pathologies and has been proposed as contributing factor to the onset and progression of Alzheimer's disease (AD), the most common form of dementia in the elderly. Analysis of post-mortem brains from AD patients revealed increased levels of voltage-dependent anion-selective channel 1 (VDAC1) in the dystrophic neurites surrounding amyloid-β (Aβ) deposits, providing strong evidence for the physical association between VDAC1 and Aβ. VDAC1 is the most abundant pore-forming protein of the outer mitochondrial membrane. As a channel, it allows the continuous exchange of ions (Mg^{2+}, Na^{+}, Cl^{-}, Ca^{2+}), ATP/ADP, NAD^{+}/NADH and Krebs cycle intermediates between the cytosol and mitochondria, thus playing a crucial role in the regulation of cellular homeostasis and bioenergetics. In this work, we report that Aβ oligomers significantly alter the conductance, voltage-dependency and kinetic features of VDAC1 reconstituted in artificial membranes, as well as its slight selectivity for anions, leading to a marked preference for cations. Given that VDAC1 is mainly involved in the trafficking of charged molecules into and out of mitochondria, a general reduction in cell viability and mitochondrial respiration was observed in neuroblastoma cells and primary cortical neurons from rat embryos exposed to Aβ oligomers. Interestingly, the toxic effect mediated by Aβ oligomers was counteracted by NHK1, a small synthetic, cell-penetrating peptide based on the sequence of N-terminal domain of Hexokinase 1, the most common physiological interactor of VDAC1. On these results, VDAC1 emerges as a crucial molecule in mitochondrial dysfunction in AD and as a promising pharmacological target for the development of new therapeutic avenues for this devastating neurodegenerative disease still without a cure. This work was financially supported by Airalzh, AGYR 2022, grant no. PEPTIDALZH to A. Magrì. *The authors marked with an asterisk equally contributed to the work.

P-41-030
Investigation the effect of 7,8-dihydroxyflavone on hippocampal endoplasmic reticulum stress

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The accumulation of unfolded or misfolded proteins within the endoplasmic reticulum (ER), due to genetic and environmental factors, leads to endoplasmic reticulum stress. Most neurodegenerative diseases are pathologically characterized by the abnormal aggregation of misfolded proteins and the formation of neuronal inclusion bodies. The toxic accumulation of protein aggregates leads to progressive neuronal damage and neurodegeneration. Mesencephalic astrocyte-derived neurotrophic factor (MANF) is a neurotrophic factor and studies have shown that it protects cell against to ER-stress. In this study, we aimed to determine the effects of 7,8-dihydroxyflavone (7,8-DHF) on MANF and inflammation in tunicamycin (TUN)-induced ER stress in the hippocampus. 6-8-week old male C57BL/6 mice were used for experimental studies. Three groups were formed with 6 mice in each group. Two groups were injected with a single dose (0.5 mg/kg) of TUN i.p. to induce ER stress. Then, solvent was administered to one group and 7,8-DHF (5mg/kg/day) to the other group for 21 days. The other group received only solvent. At the end of the experimental period, the animals

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were sacrificed by decapitation method. Hippocampus tissues were removed. Glucose-regulated protein 78 (GRP78) as a marker of ER stress, IL-18 and MANF levels for inflammation were determined by commercial ELISA kits. GRP78 level was found to be higher only in the TUN group compared to the control group ($p = 0.007$). While the IL-18 level increased in the TUN group compared to the control group ($p = 0.001$), it decreased significantly in the 7,8-DHF treated group ($p = 0.018$). MANF level increased in 7,8-DHF treated groups compared to TUN and control groups ($p = 0.023$ and $p = 0.021$, respectively). In conclusion, it is thought that 7,8-DHF may exhibit protective effects against TUN-induced hippocampal ER stress by reducing inflammation and increasing MANF levels. *The authors marked with an asterisk equally contributed to the work.

P-41-031
In silico analyses of rare CFTR variants from The Turkish National Cystic Fibrosis Patient Registry to predict their functional effects

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Cystic fibrosis (CF) is an autosomal recessive disease caused by mutations in the cystic fibrosis transmembrane conductance regulator (CFTR) gene. Although the F508del variant has the highest frequency (~22%) in our population, its prevalence is significantly lower compared to that in the US and Europe. This indicates the presence of diverse and relatively rare variants among CF patients in Türkiye. This genetic heterogeneity also creates uncertainties regarding the eligibility of Turkish CF patients for modulator drugs that are essential for effective treatment. After genotyping our patients, 279 distinct CFTR variants were identified. We prioritized the variants with a frequency of less than 0.2%, which correspond to 253 variants and collectively account for 20.2% of all alleles. Our study aimed to classify these rare variants of unknown significance using *in silico* tools, such as MutationTaster, Sorting Intolerant from Tolerant (SIFT), MutPred-LOF, Functional Analysis Through Hidden Markov Models, Polymorphism Phenotyping v2, GOR IV, SNPeffect 4.0, Have (y)Our Protein Explained, SpliceAI and ChimeraX. A total of 40 missense mutations with unknown effects were analyzed using multiple in silico tools, of which 26 were classified as pathogenic or damaging. While determining the pathogenicity of missense mutations, three programs with different algorithms were considered. If at least two of them classified a variant as damaging, we accepted it as damaging. Notably, SIFT was often the program that did not classify some variants as damaging, highlighting potential discrepancies in prediction algorithms among *in silico* tools. Therefore, we employed multiple algorithms to ensure a more reliable and comprehensive assessment. After that, we tried to classify these pathogenic variants into the seven known mutation classes of the CFTR gene. The findings of this ongoing study will contribute to addressing the unmet treatment needs of CF patients in Türkiye.

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P-41-032
Molecular genetic study of ZDHHC19 gene

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Palmitoylation is a reversible, dynamic post-translational modification characterized by the covalent attachment of palmitic acid residues to proteins. It is responsive to extracellular stimuli and has been implicated in several pathological conditions. This process is mediated by enzymes from the zinc finger DHHC-domain containing (ZDHHC) protein family, which consists of 23 members in humans, including ZDHHC19. Although the specific biological functions of ZDHHC19 remain largely unexplored, current evidence suggests that its substrates might influence critical cellular processes, such as proliferation and cell motility, highlighting ZDHHC19 as a promising candidate in disease research. This study aimed to investigate the expression levels of ZDHHC19 and its regulating microRNAs in sepsis and to explore their associations with clinical variables. Peripheral blood samples (N = 146) were collected, followed by RNA isolation, reverse transcription to circular-DNA (cDNA), and quantification by real-time polymerase chain reaction (PCR). Single nucleotide polymorphisms (SNPs) in ZDHHC19 were examined using genotyping assays. Optimal internal controls were selected using NormFinder, identifying GAPDH for normalizing ZDHHC19 and RNU6 for microRNA expression. Statistical analysis was performed using SPSS software with false discovery rate (FDR) correction. Results showed a significant alteration of ZDHHC19 expression in sepsis patients compared to controls, and expression levels correlated with clinical variables. However, no association was found between ZDHHC19 polymorphisms and phenotypic data. These findings suggest a potential role for ZDHHC19 in the pathogenesis of sepsis, necessitating further research to establish causality and clarify its cellular functions. Funding: Supported by the National Research, Development, and Innovation Office (grant: K-1316).

P-41-033
MicroRNAs in Alzheimer's disease: regulators of neuroinflammation and potential biomarkers

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Alzheimer's disease (AD) is a progressive neurodegenerative disorder characterized by amyloid-β aggregation, tau hyperphosphorylation, and chronic neuroinflammation. Circulating microRNAs (miRNAs) are emerging as promising biomarkers due to their stability in blood, brain specificity, and regulatory role in neuroinflammatory processes. Dysregulated miRNA expression in AD affects immune responses, glial activation, and inflammatory cytokine production. Our previous research identified several human plasma miRNAs as potential AD biomarkers [1]. However, their validation in AD animal models remains incomplete. This study analyzed plasma levels of miRNAs involved in neuroinflammation, including hsa-miR-146a-5p, a key regulator of astrocyte-driven inflammatory responses, and hsa-miR-29b-3p, associated with cytokine modulation, in the triple-transgenic (3xTg-AD) mouse model, and compared them with non-transgenic controls. Plasma miRNA levels were quantified using real-time quantitative PCR (RT-qPCR) in 16–20-month-old 3xTg-AD and wild-type (WT) mice (n = 17 per group; 8 females, 9 males). Spike-in miR-39-3p was used as an exogenous control, while miR-192-5p served as a stable normalizer. Significant differences in miRNA expression were observed between WT and 3xTg-AD mice, mirroring previously reported alterations in human AD plasma samples. Notably, miR-146a-5p was significantly upregulated in 3xTg-AD mice, consistent with its role in pro-inflammatory signaling. Also, miR-29b-3p was upregulated, supporting its involvement in immune dysfunction. These findings suggest that circulating miRNAs not only serve as potential biomarkers for early AD detection, but also actively regulate neuroinflammatory pathways. Understanding their mechanistic contribution to AD may facilitate the development of novel RNA-based diagnostic and therapeutic strategies. Study supported by the Polish NCN grant 2022/47/B/NZ7/03005.
Reference: [1] Nagaraj S et al. https://doi.org/10.1016/j.arr.2018.10.008.

P-41-034
Molecular mechanisms of endothelial cell damage in sepsis: the interplay between extracellular histones and HMGB1

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Sepsis, a widespread global ailment, involves an exaggerated immune response to an infection, leading to both hyperinflammation and immunosuppression. Within the hostile microenvironment in which sepsis develops, multiple pro-inflammatory molecules are released into the bloodstream, causing various physiological changes such as the secretion of chromatin-derived nuclear proteins like histones or high-mobility group box 1 (HMGB1). These play a crucial role in compromising pathogen viability; however, they also exert detrimental effects on the body by mediating inflammatory responses, coagulation defects, organ injury and cell death, particularly affecting the vascular endothelium. Nevertheless, many questions remain regarding the specific mechanisms leading to cell death mediated by these proteins. Therefore, understanding the release processes of these mediators holds the potential to improve the management of sepsis in patients. Here, using a human umbilical vein cell (HUVEC) line, we focused on characterising the potential pathways involved in the internalisation of histones into endothelial cells, as well as the cellular responses that they may trigger, including those involving the autophagic machinery and response to oxidative stress. Our results revealed that extracellular histones are internalized through clathrin-mediated endocytosis; moreover, when combined with other nuclear proteins such as HMGB1, the cytotoxic effects of

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histones on endothelial cells were enhanced, and additionally, expression of antioxidant enzymes involved in the detoxification of reactive oxygen species (ROS) was altered. Taken together, these findings suggest that histone-mediated cytotoxicity may be intertwined with the actions of HMGB1, providing a more comprehensive view of inflammatory responses in sepsis and paving the way for the development of novel therapeutic strategies to mitigate the cytotoxicity associated with nuclear proteins.

P-41-035
Inhibition of fibrin formation prevents fat embolization in lungs

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Fat embolism is a well-known example of the embolization process, often leading to vessel blockage by emboli. While it is hypothesized that fibrin polymerization may play a role in the pathogenesis of fat embolism, this has yet to be conclusively proven. Therefore, the aim of this study was to investigate the effect of inhibiting fibrin polymerization on the embolization process. The study involved the simulation of embolism in 3-month-old California rabbits weighing 2.5 kg. A single infusion of 2.5 ml of a lipophilic solution was administered into the ear vein at a rate of 10 ml/min. Calix[4]arene C-145 was used to inhibit fibrin polymerization in the experimental group, while the control group received an injection of an equal volume of saline solution instead of the lipophilic solution. Blood samples were collected at 1, 12, and 24 hours after the injection, and histological analysis of the lungs was performed the following day to directly detect emboli. Analysis of blood plasma samples revealed a decrease in protein C levels (less than 80%) and an accumulation of soluble fibrin monomeric complexes (up to 35 μg/ml) after the injection of the lipophilic solution, indicating the initiation of blood clotting. Histological examination showed significant fat embolization under the conditions of lipophilic solution injection. However, the addition of calix[4]arene C-145 significantly reduced these manifestations, resulting in fewer fatty inclusions with altered localization. Our study suggests that fibrin polymerization plays a crucial role in embolization by providing a rigid framework for the emboli. Inhibiting fibrin formation may therefore slow down the embolization process and mitigate its consequences.

P-41-036
Mitochondrial translocator protein (TSPO) upregulation correlates with impairment of respiration and mitophagy in a mouse model of ALS

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Amyotrophic lateral sclerosis (ALS) is a late-onset, progressive and fatal neurodegenerative disease that primarily affects the upper and lower motor neurons in the brain, brainstem and spinal cord, ultimately leading to muscle weakness and paralysis. Several genetic factors underlying ALS have been identified that drive motor neuron degeneration, increase susceptibility to the disease or influence its rate of progression. Although the mechanisms leading to selective motor neuron death are heterogeneous and not yet fully understood, mitochondrial dysfunction and loss of mitophagy represent crucial events in onset of ALS and, more generally, of all neurodegenerative diseases. In this study, we analyzed mitochondrial function in the spinal cords of symptomatic transgenic mice expressing the human SOD1 G93A mutant. We found that impaired motor skills correlated with a significant reduction in mitochondrial respiration. Indeed, oxygen fluxes related to ATP production and maximal capacity are altered, as a result of partial inhibition of respiration related to electron transport chain (ETC) complexes I and II. This correlates well with the accumulation of dysfunctional mitochondria in the motor neurons of ALS transgenic mice. However, we ascertained that this does not depend on downregulation of ETC protein expression, nor on a reduction in overall mitochondrial mass. To investigate this dysfunctional condition, we focused on translocator protein (TSPO), a small mitochondrial multi-drug binding protein whose upregulation has recently been linked to loss of mitophagy in a Parkinson's disease model. We observed that, in our ALS mouse model, TSPO levels were increased in correlation with a decrease in the expression of ATG12, a key marker of mitophagy. Overall, our data suggest that TSPO is a key regulator of mitochondrial homeostasis in ALS.

P-41-037
Identifying the molecular causes of neurodegenerative diseases using new animal models created with CRISPR-Cas gene editing

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Human neurological disorders SCAN1 (spinocerebellar ataxia with axonal neuropathy type 1) and SCAR23 (spinocerebellar ataxia, autosomal recessive 23) are caused by mutations in tyrosyl-DNA phosphodiesterases 1 and 2 (TDP1 and TDP2). These enzymes remove crosslinked topoisomerases from the DNA backbone and thus prevent genomic instability. Topoisomerases 1 and 2 (TOP1 and 2) are essential for cell survival as they release the torsional stress of DNA during transcription and replication. On the other hand, given the high cellular demand for TOPs, they are the second most abundant DNA-protein crosslinks (DPCs) under physiological conditions. If crosslinked topoisomerases are not repaired, accumulated TOP-DPCs lead to replication and transcription arrest and eventually to DNA breaks. Exactly how the impairment of enzymatic TDP activity leads to neurological dysfunction and why the effects only occur in the brain is still unknown. To address these questions, we have used CRISPR-Cas editing to create new zebrafish lines which are deficient in TDP1 and/or TDP2. I will present our recent discoveries where we biochemically characterized DNA protein crosslinks in mutant strains. We have found that (1) TDP1 is a key factor for the repair of TOP1- and histone H3-DPCs; (2) TDP2 is crucial for the removal of TOP2 and KU70/80-DPCs; (3) TDP1 and TDP2 deficiency causes increase of multiple DPCs and not just crosslinked topoisomerases as previously thought; (4) TDP1 and SPRTN protease function in separate pathways in the repair of

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endogenous TOP1-DPCs and in the same pathway after TOP1-DPC induction by the exogenous agents; and (5) TDP2 deficiency leads to an increase in DSBs, likely due to its role in the error-free NHEJ. In summary, we have identified some of the molecular causes of the neurodeficient phenotypes caused by TDP deficiencies, while our ongoing work will provide further in-depth analyses of neurological disorders in the aging brain.

P-41-038
Exploring putative regulatory mechanisms of human PHGDH

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Unbalanced L-serine (L-Ser) levels have been implicated in cancer development and associated with neurological dysfunctions in serine deficiency disorders and Alzheimer's disease (AD). Recent research has highlighted sex-dependent variations in serine metabolism during healthy aging and AD [previously published in Maffioli et al. (2022) Cell Rep. 40, 111271]. Sex-specific differences in post-translational modifications of D-3-phosphoglycerate dehydrogenase (PHGDH), a critical enzyme for the *de novo* L-Ser synthesis through the phosphorylated pathway (PP), could drive metabolic adaptations by altering its properties. To explore this hypothesis, we identified five phosphorylation sites (S55, T60, T78, S383, and S473) in PHGDH from both healthy and AD-affected subjects using immunoprecipitation of hippocampal tissues followed by nano-liquid chromatography tandem mass spectrometry. Biochemical analyses, including alanine-scanning and phosphomimetic mutagenesis, revealed that secondary and tertiary structures were preserved in all PHGDH variants. However, the oligomerization state was significantly altered, particularly for variants at the C-terminal region (S383A/D and S473A/D). Phosphomimetic substitutions near the substrate binding site, namely S55 and T78, drastically impaired enzymatic activity, reducing catalytic efficiency by 40-fold and 450-fold, respectively, compared to wild-type PHGDH. The NAD^+ binding process was altered for S55D, S383A/D, and S473D variants, while the binding of NADH remained unchanged. Furthermore, since PHGDH phosphorylation could led to altered compartmentalization [as previously published in Ma et al. (2021) Nat Metab. 3, 1357-1371], we are investigating the subcellular localization of PHGDH variants mimicking the phosphorylation in the U251 cells line. Overall, these studies blaze a path for exploring the regulatory processes of the PP, with the aim to extend its relevance in fields such as neurodegeneration and metabolic disorders. *The authors marked with an asterisk equally contributed to the work.

P-41-039
Neutrophil extracellular traps and sphingosine-1-phosphate: a pro-fibrotic loop in endometriosis

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Endometriosis is a chronic inflammatory/fibrotic disease affecting ~190 million women of reproductive age worldwide, characterized by ectopic endometrial-like tissue. Although its pathogenesis remains unclear, recent evidence highlights a pivotal role for neutrophils. Increased neutrophil infiltration and extracellular trap (NET) formation are linked to lesion progression (1). However, the molecular mechanisms orchestrating neutrophil activation and their involvement in the endometriotic lesion progression remain uncertain. In the lesion microenvironment, the signaling of the bioactive lipid sphingosine 1 phosphate (S1P) has been implicated in fibrosis development (2). Here, we investigated the role of S1P in neutrophil-endometriotic cell cross-talk. Differentiated HL60 cells (dHL60) were used to assess NET production via SyberGreen staining. S1P enhanced NET release at baseline and upon Phorbol 12-myristate 13-acetate stimulation. NET, isolated and characterized, were used to treat human epithelial endometriotic cells. NET treatment upregulated different markers of epithelial mesenchymal transition (ZEB1, Snail), demonstrating NET involvement in the establishment of fibrosis. To assess reciprocal signaling, sphingosine kinase isoforms (SK1, SK2), which catalyze S1P synthesis, and the S1P transporter, Spns2, were silenced in endometriotic cells. Conditioned media from Spns2-silenced cells did not affect NET release. Interestingly, SK silencing significantly reduced NET release, with SK2 depletion exerting stronger effects. Overall, these findings reveal an interplay between neutrophils and endometriotic cells that promotes fibrosis in an S1P rich microenvironment. Funding from European Union-Next-Generation. EU National Recovery and Resilience Plan (PNRR)–MISSION 4 COMPONENT 2, INVESTIMENT N. 1.1, PRIN2022PNRR–P20222A8HL PROSPECT CUP I53D23005890001 Previously published in: 1. Gardella B et al. (2022) Sci Rep 12, 3227. 2. Bernacchioni C et al. (2024) Fertil Steril 121, 631-641 *The authors marked with an asterisk equally contributed to the work.

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P-41-040
Postoperative alterations in BDNF, OPG, and oxidative stress markers in cardiopulmonary bypass patients: potential implications for diagnosis and treatment

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Cardiopulmonary bypass (CPB) is essential in cardiac surgery but induces oxidative stress, inflammation, and endothelial dysfunction, contributing to complications. Osteoprotegerin (OPG) regulates vascular inflammation, brain-derived neurotrophic factor (BDNF) supports neurovascular health, and CX3CL1 mediates immune responses. Sirtuins (SIRT family) regulate oxidative stress and metabolism. This study examines perioperative changes in these biomarkers and their clinical significance. We analyzed 9 male and 10 female CPB patients with blood samples collected preoperatively and postoperatively with healthy controls. OPG and BDNF were measured by ELISA, while SIRT2 and CX3CL1 expression was assessed via western blot. Oxidative stress was evaluated with total antioxidant status (TAS) and total oxidant status (TOS). Statistical analyses were performed using SPSS v26, with Shapiro-Wilk test for normality. Paired comparisons used paired t-tests or Wilcoxon signed-rank tests, while ANOVA or Kruskal-Wallis tests were applied for group comparisons ($p < 0.05$). Postoperative OPG increased in males ($p = 0.0391$), suggesting vascular inflammation. BDNF declined in males but increased in females, indicating sex-specific neurovascular responses. TOS significantly increased postoperatively ($p = 0.0009$ in males, $p = 0.0088$ in females), while TAS remained unchanged, highlighting oxidative stress.CX3CL1 was absent preoperatively but strong postoperatively, indicating CPB-induced inflammation. SIRT2 was detected preoperatively but lost postoperatively, suggesting reduced protection against oxidative damage. These findings suggest CPB induces oxidative stress, vascular inflammation, and neurovascular alterations. CX3CL1 and OPG may serve as inflammatory biomarkers, while SIRT2 depletion could indicate endothelial dysfunction. Future research should explore therapies enhancing SIRT2 activity, modulating CX3CL1-driven inflammation, or boosting BDNF signaling to improve post-CPB recovery.

P-41-041
Truncating variants in TTN M4 disrupt skeletal and cardiac function and impair mitochondrial activity

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Titin (TTN) is a structural protein in the sarcomere, essential for maintaining sarcomere integrity and providing passive tension for muscle contraction. Genetic disorders caused by *TTN* mutations are collectively referred to as titinopathies. While truncating mutations in the M-line region are often associated with skeletal muscle disorders such as tibial muscular dystrophy (TMD) and limb-girdle muscular dystrophy (LGMD), mutations affecting constitutively spliced exons are believed to cause more severe consequences, including dilated cardiomyopathy (DCM). Zebrafish possess two TTN paralogs, *ttn.1* and *ttn.2*. To investigate the impact of truncating variants (TTNtv) in constitutively spliced exons within the M-line region, we generated genomic lesions in the M4 domain of *ttn.1* and *ttn.2*. Behavioral analysis revealed that adult *ttn* mutants exhibited reduced skeletal muscle function, with significant declines in fast-twitch muscle performance in aging *ttn.1* mutants, resembling the late-onset features of TMD/LGMD. Mutant larvae showed impaired and delayed motor function development. Additionally, microvideography analysis demonstrated decreased cardiac function in mutant larvae, particularly in *ttn.2* mutants, mimicking early-stage DCM-like symptoms. Ultrastructural analysis identified sarcomeric disorganization, mitochondrial abnormalities, and increased autophagosome formation in both fast-twitch and cardiac muscle. A human rhabdomyosarcoma cell model carrying M4 TTNtv exhibited significantly reduced maximal respiration, indicating mitochondrial dysfunction. Taken together, our findings suggest that M4 TTNtv is pathogenic, leading to both skeletal and cardiac phenotypes as well as mitochondrial abnormalities. Our zebrafish *ttn.1* and *ttn.2* mutants serve as potential models for studying skeletal and cardiac manifestations of titinopathy, respectively. *The authors marked with an asterisk equally contributed to the work.

P-41-042
Gene expression dysregulation in Sanfilippo and Morquio syndromes: the impact of PFN1 on glycosaminoglycan accumulation

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Mucopolysaccharidosis (MPS) is a group of inherited metabolic disorders characterized by the accumulation of incompletely degraded glycosaminoglycans (GAGs). MPS diseases, including Sanfilippo syndrome (MPS III) and Morquio syndrome (MPS IV), are classified based on the type(s) of GAG(s) stored and the specific enzymatic deficiencies. Although the genetic defects causing MPS are well-known, the underlying molecular mechanisms remain incompletely understood. This study aimed to investigate gene expression patterns in MPS III and IV

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subtypes to identify dysregulated genes that could provide insight into the molecular mechanisms of these disorders. Transcriptomic analyses were conducted to assess gene expression in MPS and control cells. Western blotting and immunohistochemistry were used to measure protein levels of the most dysregulated genes and small interfering RNA (siRNA)-mediated gene silencing was used to examine the impact of reduced gene expression on GAG(s) accumulation. Our analysis identified 45 commonly dysregulated genes in MPS III subtypes and 150 in MPS IV subtypes. Notably, *PFN1*, *MFAP5*, and *MMP12* showed significant dysregulation. Elevated levels of PFN1 were observed in MPS cells, which could be reduced by lowering GAG levels with genistein treatment. Silencing PFN1 led to a decrease in GAG accumulation, suggesting a strong correlation between PFN1 expression and GAG levels. In conclusion, a large number of commonly dysregulated genes were identified across MPS III and IV subtypes, with PFN1 emerging as a key gene correlated with GAG accumulation. These findings suggest that PFN1 could be a promising new therapeutic target for both MPS III and IV, offering potential for future treatment strategies.

P-41-043
Decoding cardiac calsequestrin: from calcium handling to arrhythmogenesis

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Calsequestrin-2 (CASQ2) is the primary Ca^{2+}-binding protein within the junctional sarcoplasmic reticulum (jSR) of cardiac muscle. Traditionally regarded as a Ca^{2+}-specific biological sensor for the excitation-contraction coupling process, its critical role is closely tied to its Ca^{2+}-dependent polymerization and tremendous Ca^{2+}-binding capacity (up to 60 ions per 45-kDa monomer). Missense mutations in CASQ2 cause life-threatening arrhythmias and cardiac arrest in children and young adults and exhibit unpredictable hereditary penetrance. Our multidisciplinary approach combining turbidimetric analysis with mass photometry (MP), size-exclusion chromatography (SEC), qualitative cryo-EM imaging and dynamic light scattering (DLS), uncovers a previously unrecognized role of Mg^{2+} and K^+ ions in modulating CASQ2's polymerization response to Ca^{2+}. Surprisingly, MP, SEC, and microscale thermophoresis (MST), also reveal that CASQ2 forms dimers with nanomolar affinity independently of Ca^{2+}. Mg^{2+}, a key regulator of cardiac contraction, maintains a pool of highly Ca^{2+}-responsive CASQ2 units by stabilizing a highly Ca^{2+}-sensitive dimeric state which is in conformational competition with other oligomeric species. Our findings also intriguingly suggest that the intrinsically disordered, highly acidic C-terminus of CASQ2 modulates the transition between alternative dimeric conformations and distinct Ca^{2+}-dependent polymerization mechanisms. We propose that the dimeric state of CASQ2 represents the fundamental physiological unit and the key step for integration of signals from distinct physiological ions (Ca^{2+}, Mg^{2+}, and K^+) into CASQ2 dynamic regulatory mechanism. Coupled with published data on pathological CASQ2 trafficking, this model provides a new mechanistic framework for arrhythmogenic mutations. We predict that the dimerization capacity of pathological mutants is the molecular discriminant between the dominant and the recessive form of CASQ2-specific disorders.

P-41-044
Soluble receptor for advanced glycation end products and soluble lectin-like oxidized low-density lipoprotein receptor-1 in the knotty journey from metabolic syndrome to its most common complications

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Metabolic syndrome (MetS) increases significantly the risk of type 2 diabetes mellitus (T2DM) and coronary artery disease (CAD). On one hand, this cannot be totally explained by the traditional cardiometabolic risk factors. On the other hand, soluble receptor for advanced glycation end products (sRAGE) and soluble lectin-like oxidized low-density lipoprotein receptor-1 (sLOX-1) have emerged as perspective serum markers of vascular inflammation and atherosclerosis, but the data on their role in the pathophysiology of nascent MetS and its further complications are rather discrepant. Hence, the present study aimed to explore the association of the serum levels of sRAGE and sLOX-1 with newly diagnosed MetS and MetS most common complications. Additionally, the relationship between sRAGE, sLOX-1 and classic cardiometabolic risk factors was investigated. The serum levels of sRAGE and sLOX-1 were analysed by ELISA in 37 heathy controls, 50 subjects with uncomplicated newly diagnosed MetS, 40 MetS + T2DM patients and 34 MetS + CAD patients. Lower sRAGE, but higher sLOX-1 concentrations were found in the MetS cohort in comparison to the control group ($p < 0.001$ and $p < 0.01$, respectively). In the newly diagnosed MetS subjects, sRAGE levels correlated statistically significantly and negatively with body mass index, waist circumference and high-sensitivity C-reactive protein (hs-CRP), while sLOX-1 correlated positively with fasting glucose, HDL-cholesterol, systolic blood pressure and hs-CRP. There were no significant differences in the levels the two soluble receptors between MetS and MetS + T2DM groups. Nevertheless, lower sRAGE levels and higher sLOX-1 levels were significantly associated with an augmented risk for CAD in MetS subjects ($p < 0.01$). We could conclude that the measurement of the serum levels of sRAGE and sLOX-1 might help in the identification of a subset of patients at increased risk of MetS and its further complication by CAD.

P-41-045
Efficacy of surfactant therapy and its impact on selected gene expression from lung tissue in experimental ARDS

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The complex pathophysiology of acute respiratory distress syndrome (ARDS) remains poorly understood due to limitations of animal models and challenges in human studies. Processes in

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ARDS can further lead to the accumulation of inflammatory cells, edema fluid, leakage of plasma proteins into the alveolus, inactivation surfactant and disruption of surfactant production, resulting in alveolar-capillary membrane deterioration and ultimately impaired gas exchange. Substitutional surfactant therapy (SST) could represent a promising treatment strategy for ARDS patients, as it may contribute to lung function recovery. The aim of this study is to analyze changes in the expression of selected genes associated with ARDS following SST administration in an animal model of acute lung injury. Adult rabbits with severe ARDS were divided according to surfactant treatment; the natural surfactant poractant alfa and the synthetic surfactants CHF5633 and Combo, which contain synthetic analogs of surfactant proteins B and C. After SST the animals were protectively ventilated for 4 hours. Post mortem, total RNA was isolated from lung tissue and analyzed by RT-qPCR. Data were corrected for the signal from three endogenous controls used as reference genes (*B2M*, *HPRT1*, and *18S rRNA*) and further analyzed using the $2^{-\Delta\Delta Ct}$ method. Statistical analysis and comparison of changes in the expression of selected genes (*SFTPA*, *SFTPD*, *SFTPB*, *SFTPC*, *ABCA3*, *VEGFA*, *IFIT3*, *RAB11A*, *IL-6*, *EGLN1*, *FGF7*, *LCN2*, *CLDN-18.1*, *UGRP1*, *NAPSA*, *MMP28*, *LTB4R*, *S100A12*, *NKX2-1*, *CAV1*) were performed against healthy controls. The gene expression patterns associated with ARDS in the untreated group corresponded to findings reported by other authors. Both synthetic surfactants influenced the expression of selected genes comparably with natural one, which may suggested their impact on the regulation of molecular processes related to the pathogenesis of ARDS. This study was supported by VEGA 1/0097/23, VEGA 1/0026/25.

P-41-046
Two-year follow-up of CD40 expression in classical monocytes in relapsing-remitting multiple sclerosis subjects: preliminary findings

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During neuroinflammation, CD40L+ CD4+ T cells invade the central nervous system (CNS) and activate CD40+ monocytes, macrophages, B cells, endothelial cells, and other resident CNS cells, thereby promoting inflammation [Peterson JW et al. (2005) Neurol Clin 23, 107–29]. Surface expression of CD40 (receptor for CD40 ligand, CD40L) in classical CD14++CD16- monocytes has been reported to be altered in multiple sclerosis (MS) disease. The study objective was to investigate the expression of the CD40 marker on the classical (CD14++CD16−) peripheral blood monocyte subpopulations of RRMS in a two-year follow-up. Patients receiving teriflunomide (Aubagio) treatment for at least 12 months were eligible for inclusion in the study. Three RRMS with alterations in subclinical neurophysiological findings (absent or prolonged latency of motor evoked potential from extremity muscles) with a mean age of 50 ± 2, and three of them with no neurophysiologic alteration with a mean age of 47 ± 3.2 were included [Režić Mužinić N et al. (2023) Biomedicines 11, 2870]. One hundred microliters of blood samples were incubated with anti-human CD14, CD16, and CD40 fluorochrome conjugated antibodies and detected with flow-cytometry. The results show that RRMS with alterations in subclinical neurophysiological findings had significantly higher mean fluorescence intensity of CD40 in classical monocytes than the RRMS with no neurophysiologic alteration ($p = 0.036$). The RRMS subjects had an EDSS score of 2.80 at baseline and an EDSS 3.68 in a two-year follow-up. RRMS with neurophysiological alterations and EDSS progression showed the higher expression of CD40 on classical monocytes after two-year follow-up in comparison to RRMS without neurophysiologic alterations. These preliminary findings suggest that longitudinal monitoring of CD40 on classical monocytes might be a good marker to monitor MS disease progression utilizing neurophysiologic examination of motor status and EDSS evaluation.

P-41-047
Detection of the mutations in telomere related genes in children diagnosed with interstitial lung disease (ILD)

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Childhood interstitial lung diseases (chILD) are a rare, heterogeneous group of disorders that are often associated with high morbidity and mortality. Diagnosis is frequently missed or delayed due to nonspecific clinical findings. Reports suggest that patients with telomere-related gene (TRG; *STN1*, *TERC*, *TERT*, *RTEL1* and *PARN* as selected) mutations are at higher risk of developing ILD. The prevalence of chILD is increasing every day, yet there is limited research on TRG mutations in pediatric patients. Among TRGs, STN1 is a critical protein of telomere replication, specifically for the release of replication stress and lagging strand synthesis. Variants in the *STN1* gene, especially rs11191865 (NC_000010.11:g.103913084G>A) in intron 2, are one of the most prominent groups among telomere associated variants. Our project aimed to characterize TRG mutations of 20 patients diagnosed with chILD who were regularly followed up at our pediatric pulmonology department. DNA of the patients were isolated from peripheral blood samples. After PCR amplification of the exons of selected genes, Sanger sequencing and/or RFLP method were performed. For *STN1* gene analysis, HpyCH4III restriction endonuclease enzyme was used. As a result of our preliminary study, the distribution of rs11191865 was A/A: 25%, G/A: 62.5% and G/G: 12.5% in ILD patients, and the allele frequency was determined as 56.25% for A allele, and 43.75% for G allele. Two patients carrying A/A genotypes were presented with severe telomeropathy, one with usual interstitial pneumonia and the other with hypersensitivity pneumonia. In addition, 4 patients carrying G/A genotype were presented with pulmonary fibrosis. The findings of this ongoing study will contribute to the diagnostic and treatment approach

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for chILD patients by pointing out TRG genotypes and enlighten the pathophysiology of the disease.

P-41-048
Formyl peptides receptors–NADPH oxidase axis drives sex dimorphism in idiopathic pulmonary fibrosis through oxidative stress induced by Nrf2 dysregulation

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Idiopathic pulmonary fibrosis (IPF) is a chronic, progressive, interstitial lung disease associated with a poor outcome. IPF is a well-established male disorder, with men accounting for roughly 70% of all IPF cases and showing the worst prognosis. The pathogenesis of IPF is still poorly understood, resulting in a lack of effective treatment. Hence, identifying the molecular signatures involved in IPF pathogenesis could be relevant in discovering novel targets for IPF therapy. Emerging evidence indicates that IPF may result from a continuous loop of inflammation promoted by the release of damage-associated molecular patterns (DAMPs). DAMPs exert their inflammatory effects through pattern recognition receptors (PPRs), including formyl peptides receptors (FPRs). FPRs are a small family of PRRs comprising three members (FPR1, FPR2, and FPR3) widely expressed in several tissues and cell types, regulating different biological processes, including the development and resolution of inflammation. At the molecular level, FPRs stimulation can lead to the activation of NADPH oxidase (NOX) enzymes, thus generating reactive oxygen species (ROS). Given the role of NOX-dependent ROS production in contributing to IPF, we sought to examine the expression levels of different isoforms of FPRs and NOXs by using a bleomycin model of PF. Herein, we observed a significant upregulation in the expression of FPR1 and FPR2 receptors as well as NOX3 and NOX4 enzymes in treated male mice as compared to their female counterparts. According to earlier research showing FPR2-mediated activation of Nrf2 transcription factor, we detected a strong increase in Nrf2 levels in bleomycin-treated male mice. Notably, male mice were also characterized by a significant dysregulation of some key Nrf2 target genes involved in oxidant defense. Altogether, these results highlight novel mechanisms driving sex dimorphism in IPF, thus providing potential targets for the identification of personalized drug therapies. *The authors marked with an asterisk equally contributed to the work.

P-41-049
Long-term changes in neuropilin-1 (NRP-1) and angiotensin-converting enzyme 2 (ACE-2) levels in COVID-19 patients

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Neuropilin-1 (NRP-1) and angiotensin-converting enzyme 2 (ACE-2) cell membrane receptors are key in COVID-19 infection. ACE-2 allows SARS-CoV-2 entry into cells, while NRP-1 may affect disease progression. Changes in their levels in patient's cells may contribute to long-term side effects after COVID-19, like neurological and cardiovascular issues. We determined the concentration of soluble NRP-1 and ACE-2 in the serum and their expression on T-lymphocytes, B-lymphocytes, neutrophils and monocytes in 38 COVID-19 patients hospitalized in the Military Institute of Medicine in Warsaw, Poland, in 2021–2022. Analyses were performed during hospitalization and prospectively at 3-, 6-, and 9-months post-infection. 25 healthy individuals served as a control group. NRP-1 and ACE-2 expression was assessed by immunophenotyping peripheral blood mononuclear cells (PBMC) using CytoFLEX Flow Cytometry. All examined leukocyte populations expressed both receptors. Soluble NRP-1 and ACE-2 levels in the serum were measured with ELISA kits. NRP-1 levels were lower in COVID-19 patients compared to controls during hospitalization. After 3 months, NRP-1 levels increased and were higher than those of healthy controls. A similar increase was observed at 6 months. By 9 months, NRP-1 levels returned to the level of healthy individuals. ACE-2 concentrations did not differ from healthy controls during hospitalization and remained stable over time. These results show that NRP-1 levels decrease during COVID-19, then rise after recovery, remaining elevated up to 6 months. These fluctuations potentially might impact angiogenesis and neuroplasticity. Research funded by: 1. "Innovative integrated system for field and stationary diagnosis of parasitic, bacterial, and viral invasions in PKW areas (Diagnotrop)" DOB-BIO-12-04-001-2022; 2. "Assessment of the course of COVID-19 based on clinical presentation and immune response parameters – long-term observation" SZPITALE-JEDNOIMIENNE/43/2020. *The authors marked with an asterisk equally contributed to the work.

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P-41-050
Midkine fails to be served as a novel biomarker for unexplained female infertility

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Cumulus cells (CCs) surrounding the oocyte support oocyte maturation, fertilization, and hormone production. Midkine (MK), a growth factor, expressed in CCs, promotes CC proliferation and growth. Unexplained female infertility (UFI) occurs when no specific cause for a couple's inability to conceive can be identified despite normal test results. This condition remains unclear, which can be frustrating for both couples and clinicians seeking answers. This study aimed to explore whether MK could serve as a novel biomarker for diagnosing and treating UFI. Serum (S), follicular fluid (FF), and CCs were analyzed from patients aged 20–42 years diagnosed with male factor (MF) and UFI. Patients who underwent ICSI were evaluated for Anti-Müllerian Hormone (AMH) and MK levels, along with other hormone levels (FSH, LH, E2, PRL, INHB, TSH). Oocyte and embryo quality, fertilization and pregnancy rates, and CC parameters (Cell count, ultrastructure, and apoptosis rate) as well as MK gene mutations were analyzed. The Student's t-test was performed, with $p < 0.05$ considered statistically significant. The lowest CC number was observed in the UFI group ($p < 0.05$). The MF group exhibited the lowest CC apoptosis rate and the highest CC viability rate ($p < 0.05$). AMH and MK levels (S, FF) were significantly lower in the UFI group compared to the MF group ($p < 0.05$). Additionally, MK and AMH levels were significantly lower in non-pregnant subjects than in pregnant subjects ($p < 0.05$) and were also reduced in individuals over the age of 35 ($p < 0.05$). Structural analysis of CCs revealed a higher number of lytic cells, cell remnants, and apoptotic bodies in non-pregnant subjects [Previously published in: Erguven et al. (2024) TJB 48, 698-708]. No MK gene mutation was detected in the CCs of either MF or UFI patients. MK did not exhibit resistance to either AMH activity or apoptosis, and therefore, cannot be considered a novel biomarker for diagnosing or monitoring the treatment of UFI cases.

P-41-051
Evaluation of the association between KEAP1 rs1048290 G/C genetic polymorphism and relapsing-remitting multiple sclerosis

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Multiple sclerosis (MS) is a chronic autoimmune disease characterized by inflammation and neurodegeneration within the central nervous system, primarily affecting young adults. Most MS patients have the relapsing-remitting MS (RRMS) type, which causes worsening of neurological dysfunction through temporary attacks. Oxidative stress plays a significant role in the etiology of MS. Kelch-like ECH-associated protein 1 (KEAP1) – nuclear factor-erythroid 2-related factor 2 (Nrf2) pathway is critical in regulating the cellular response to oxidative stress. One of the single nucleotide polymorphisms (SNPs) in the *KEAP1* gene, rs1048290 G>C, located in exon 4, can alter the function of *KEAP1*, thereby affecting Nrf2 activation and influencing antioxidant defense. The association of rs1048290 G>C with MS has not been studied in the Turkish population, so this study aimed to investigate its potential relationship with MS. Blood samples were obtained from 189 RRMS patients and 190 healthy controls at the Neurology Clinic of Ankara Bilkent City Hospital. Following DNA isolation, genotyping of the rs1048290 G>C polymorphism was conducted using the TaqMan assay. Frequency of the polymorphic C allele was found to be 0.394 in patients with RRMS and 0.439 in the control group (OR = 0.83, p = .21). Additionally, the polymorphic CC genotype was present in 14.8% of RRMS patients and 22.1% of the control group. When comparing the frequency of the CC genotype to the combined GC + GG genotypes (recessive model), the OR was 0.61 (95% CI: 0.36–1.04, p = .07). In the dominant model, comparing the combined CC + GC genotypes to the GG genotype, the OR was 0.93 (95% CI: 0.61–1.41, p = .72). These findings showed no connection between MS disease and the rs1048290 G>C SNP. These are the initial results of a large-scale, ongoing study. This study is supported by TÜBITAK (323S265).

P-41-052
Boron and hBN nanoparticle-supplemented fecal microbiota transplantation as a potential therapy for testicular injury

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The gut microbiota plays a crucial role in the development and functioning of various body systems, contributing to metabolic, physiological, and protective functions. In this study, we hypothesized that changes in the microbiota could be associated with sperm quality and fertility in males. Our research aimed to enhance the effects of fecal microbiota transplantation (FMT) in testicular injury treatment by supplementing it with boric acid (BA) and boron nitride (hBN NPs) nanoparticles. A total of 70 Wistar albino rats were used and divided into seven groups (n = 10): Control, LPS (100 μg/kg), LPS + FMT (2 ml), LPS + hBN NPs (100 μg/kg), LPS + BA, LPS + FMT + hBN NPs, and LPS + FMT + BA. For the FMT procedure, fecal pellets collected from donor rats in the control group were dissolved in 3 mL of sterile PBS, centrifuged at 500 g for 30 s, and the soluble fraction was administered via oral gavage (2 mL/rat). At the end of the experimental period, biochemical and pathological analyses were conducted on testicular and sperm samples. Sperm motility, cellular apoptosis, and inflammation were assessed using ELISA. Epididymal sperm viability was measured, revealing a significant decrease in sperm motility in the LPS group ($p < 0.001$), whereas treatment groups exhibited motility levels comparable to the control group. Inflammatory, oxidative stress, and apoptotic markers were also analyzed. Compared to the control group, the LPS group showed a significant increase in the inflammatory markers TNF-α and IL-6. However, the LPS + BA, LPS + FMT + hBN NPs, and LPS + FMT + BA groups exhibited a marked reduction in these markers ($p < 0.001$). Similarly, ROS levels were significantly lower in the LPS + FMT + hBN NPs and LPS + FMT + BA groups compared to the LPS group ($p < 0.001$).

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These findings suggest that FMT supplemented with BA and hBN NPs may have potential therapeutic effects in reducing inflammation and oxidative stress in testicular injury. *The authors marked with an asterisk equally contributed to the work.

P-41-053
Soluble receptor for advanced glycation end products (sRAGE) levels and RAGE rs1800624 polymorphism may affect the inflammatory cytokine profile in coronary artery ectasia

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The activation of advanced glycation end products (AGEs) and their receptor RAGE is a key promotive factor in chronic inflammation. Cytokines play a significant role in the pathogenesis of coronary artery ectasia (CAE), with elevated levels of inflammatory mediators such as INFγ, TNFα, IL-1β, and IL-8. The AGEs-RAGE pathway further amplifies inflammation by inducing cytokine release, including TNFα, IL-1β, and IL-6. The RAGE -374T>A (rs1800624) genvariation has been suggested to modulate its transcriptional activity. This study aimed to investigate the impact of rs1800624 on inflammatory cytokine levels in Turkish CAE patients. A total of 49 CAE (+) coronary artery disease (CAD) patients and 30 CAE (-) CAD patients as controls were included in the study. Serum cytokine and soluble-RAGE (sRAGE) levels were measured using Luminex technology and ELISA, respectively. The RAGE rs1800624 genotyping was performed using a real-time PCR TaqMan®assay. The A allele frequency (91.8%) was higher than the TT genotype frequency (8.2%) in CAE patients ($p = 0.002$). PECAM, IL-33, and TNFα levels were significantly elevated in CAE patients compared to controls ($p = 0.02$, $p = 0.002$, and $p = 0.034$, respectively). CAE patients with sRAGE levels below the cut-off value (≤1300pg/ml) had a relative increase in the whole cytokine profile, which was significant for P-Selectin and TNFα levels ($p < 0.05$). Additionally, CAE patients with rs1800624 A allele exhibited higher HbA1C ($p = 0.002$) and lower IL-8 levels ($p = 0.048$) compared to non-carriers (TT genotype), indicating a potential link between this genetic variation and altered cytokine dynamics. Our findings suggest that the rs1800624 A allele may contribute to CAE pathophysiology by influencing inflammatory responses. These results highlight the role of RAGE gene polymorphism in chronic inflammation and their potential implications in CAE progression. Further studies are warranted to elucidate the mechanistic pathways underlying these associations.

P-41-054
Investigation of the relationship between NFE2L2 rs6721961 T/G genetic polymorphism and relapsing-remitting multiple sclerosis

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Multiple sclerosis (MS) is a chronic inflammatory disease of the central nervous system. Relapsing-remitting multiple sclerosis (RRMS) is characterized by relapses followed by periods of remission without neurological deterioration. Oxidative stress has been associated in MS pathophysiology through its role in neuroinflammation and neurodegeneration. One of the single nucleotide polymorphisms (SNP), rs6721961 T>G, located in the promoter region of *NFE2L2* (Nuclear Factor Erythroid 2 Like 2) gene, may influence gene expression and the cellular response to oxidative stress. To the best of our knowledge, no study has investigated the association of this SNP with MS. Therefore, this study aimed to investigate whether the rs6721961 T>G SNP is associated with susceptibility to MS in a Turkish cohort. For this investigation, a total of 189 RRMS patients and 186 controls were recruited from the Neurology Clinic of Ankara Bilkent City Hospital. Genomic DNA was extracted from peripheral blood samples, and the rs6721961 T>G genotypes were determined using the TaqMan genotyping assay. The frequency of the polymorphic T allele was calculated as 0.183 in RRMS patients and 0.167 in the control group (OR = 1.12, $P = 0.57$). The frequency of the polymorphic TT genotype was calculated as 3.7% in the RRMS patients and 3.8% in the controls. When the frequency of TT genotype was compared with that of TG + GG genotypes, the OR value was 0.98 (95% CI = 0.34-2.86, $P > 0.999$) (recessive model); when TT + TG genotype was compared with GG genotype (dominant model), the OR value was 1.16 (95% CI = 0.75-1.80, $P = 0.50$). The analysis of the data revealed no significant association between the rs6721961 T>G and MS in this cohort. These preliminary findings represent the initial results from a larger ongoing study that aims to include additional participants. This study is supported by TÜBITAK (323S265). *The authors marked with an asterisk equally contributed to the work.

P-41-055
Investigation of RHOA and ROCK1 gene expression in coronary artery disease

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Cardiovascular diseases (CVDs) encompass a broad group of disorders affecting the heart and vascular system and are among the leading causes of death worldwide. Conditions such as hypertension, atherosclerosis, vasospasm, ischemic heart diseases, and heart failure are associated with various molecular

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mechanisms involved in vascular tone regulation and cellular signal transduction. GTP-binding protein RhoA and Rho-kinase (ROCK1 and ROCK2) play critical roles in key biological processes, including vascular smooth muscle cell contraction, cell motility, proliferation, inflammation, and oxidative stress. Since ROCK inhibition has shown promising therapeutic effects in various diseases, including cardiovascular, renal, and diabetes-related conditions, this study aimed to investigate the expression levels of RHOA and ROCK1 genes in coronary artery disease (CAD). The study included 40 individuals diagnosed with CAD and 38 healthy individuals with no systemic diseases. RNA was isolated from blood samples collected in EDTA tubes using the innuPREP Blood RNA kit, followed by cDNA synthesis. RHOA and ROCK1 gene expression levels were analyzed using RT-PCR (Roche LightCycler 480; Roche Diagnostics, Mannheim, Germany). Both genes were found to be significantly downregulated in the patient group compared to the healthy group ($p = 0.03134$ for RHOA, $p = 0.01215$ for ROCK1). This study suggests that these genes may have prognostic or predictive significance and may contribute to the discovery of new targeted treatment models.

P-41-056
Effect of microtubule stability on SMN secretion in spinal muscular atrophy model

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Spinal muscular atrophy (SMA) is an autosomal recessive disease caused by the deficiency of survival motor neuron (SMN) protein. In addition to its nuclear and cytoplasmic localization, SMN protein is also secreted from the cells via exosomes and microparticles. The cytoskeleton, particularly microtubules, plays a role in extracellular protein secretion by facilitating intracellular vesicle trafficking. Microtubules are dynamic filaments composed of alpha and beta tubulin proteins, both of which possess several post-translational modifications (PTM). Some of the alpha tubulin PTMs are associated with stabile microtubule structures, including detyrosination. In an *in vitro* SMA model, we previously demonstrated a decreased level of detyrosinated α-tubulin together with reduced microtubule stability. We found that the level of tubulin tyrosine ligase (TTL) –the enzyme catalyzing α- tubulin tyrosination – was upregulated in Smn deficient cells [1]. In this study, we investigated the effects of microtubule stability on extracellular SMN secretion. We used an siRNA-mediated gene silencing approach and increased microtubule stability of SMN knock-down motor neuron-like NSC34 cells by suppressing TTL expression. Western blot studies demonstrated a significant upregulation in α-tubulin detyrosination in co-knockdown cells compared to SMN deficient cells. To analyze the amount of extracellular SMN, we collected culture media and examined the protein contained in TCA precipitates by western blot. We showed that extracellular SMN levels were significantly increased in co-knockdown cells compared to the cells with SMN knockdown only. Our results indicate that SMN secretion is affected by microtubule dysregulations and modifying alpha tubulin PTMs could be an approach to restore microtubule-dependent cellular pathologies in SMA. Studies on motor proteins are ongoing to mechanistically explain intracellular SMN transport. Reference: [1] Bora G et al. (2021) Hum Mol Genet 29:3935–3944.

P-41-057
The effects of PCSK9 (proprotein convertase subtilisin/kexin type 9) on human endothelial cells

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The role of proprotein convertase subtilisin/kexin type 9 (PCSK9) in cholesterol metabolism is well established. However, PCSK9 also affects vascular wall cells independently of its hyperlipidemic effects. Endothelial cells are crucial in maintaining vascular function and play a key role in the development of atherosclerosis. Understanding PCSK9's effects on vascular endothelial cells is vital to elucidating the mechanisms underlying atherosclerosis Endothelial cell lines were used in experiments. Cells were transfected with a PCSK9 overexpression plasmid and PCSK9 siRNA. The effects of PCSK9 upregulation and the protective effect of PCSK9 silencing in cells exposed to atherogenic substances (oxidized low-density lipoprotein [OxLDL] and hydrogen peroxide [H_2O_2]) were examined by analyzing inflammation- and apoptosis-related markers using western blots. Additionally, a wound healing assay was conducted to assess the impact of PCSK9 expression on cell migration. PCSK9 overexpression increased the expression of inflammatory and apoptotic markers while reducing endothelial function proteins, similar to OxLDL and H_2O_2. Silencing PCSK9 reversed the effects of OxLDL and H_2O_2. In the wound healing assay, PCSK9 and PCSK9 siRNA had opposing effects on cell migration and proliferation. Moreover, PCSK9 increased the expression of MTOR, one of the key proteins involved in cell proliferation. PCSK9 affects vascular endothelial cells through various pathways, including inflammation, apoptosis, proliferation, and endothelial function. Understanding these mechanisms is crucial for developing targeted therapies for atherosclerosis. *The authors marked with an asterisk equally contributed to the work.

P-41-058
Co-occurrence of two rare syndromes with different pathogenetic mechanisms within the same family

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The 22q11.2 deletion syndrome (DiGeorge Syndrome) has a broad spectrum of phenotypic features. Common clinical signs are palatal and congenital heart defects, thymic aplasia/hypoplasia, hypoparathyroidism, and mild intellectual disability. Angelman syndrome (AS) is a neurodevelopmental delay caused by maternal deletion, paternal uniparental disomy, imprinting

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defect, and UBE3A gene mutations on chromosome 15 (15q11.2-q13). Although normal at birth, the clinic occurs in childhood with developmental delay, ataxia, absence of speech, severe intellectual disability, and seizures. We present a very rare co-occurrence of Angelman and DiGeorge syndromes in two sisters referred to our department with seizures and dysmorphic features. An 8-year-old elder sister was previously diagnosed with Angelman syndrome by MS-MLPA and applied for genetic counseling. FISH analysis confirmed that it is a maternal deletion (15q11-q13). Upon the family's statement that the younger sibling also had occasional seizures, both siblings were evaluated together. In the physical examination, the younger sister (5 years old) had mild dysmorphic findings with a narrow forehead, edematous eyelids, hypertelorism, flat nasal bridge, prominent lips, brachydactyly, and obesity. Denver developmental screening test results demonstrated mild motor and intellectual delay. The chromosome analysis was normal. The microarray analysis showed 22q11.2 deletion and was confirmed by FISH analysis. The karyotypes of both parents were normal. Array analysis demonstrated that DiGeorge syndrome was maternally transmitted. Psychiatric and cardiological follow-ups were recommended for the mother. The co-occurrence of two rare syndromes with distinct pathogenetic mechanisms in a single family complicates the determination of inheritance patterns, the calculation of risk for subsequent pregnancies, and providing effective genetic counseling. *The authors marked with an asterisk equally contributed to the work.

P-41-059
The expression levels of miR-181a-3p and TGF-β in aortic tissues of patients with ascending aortic aneurysms have changed, whereas the levels of H19 have remained unchanged

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Aortic aneurysms are severe vascular conditions characterized by progressive dilation of the aorta, which can lead to rupture and life-threatening hemorrhage. The pathogenesis of aortic aneurysms involves extracellular matrix (ECM) degradation, chronic inflammation, oxidative stress, and smooth muscle cell (SMC) apoptosis, weakening the aortic wall and increasing the risk of rupture. Non-coding RNAs, such as lncRNA H19 and miR-181a, play a role in the regulation of vascular remodeling and inflammation through the TGF-β pathway. This study compares the expression of H19, miR-181a-3p, and TGF-β in aortic tissue from patients undergoing surgery for ascending aortic aneurysms (AsAA) and those undergoing multivessel coronary artery bypass grafting (mCABG) to investigate their involvement in aneurysm pathogenesis. Blood and aortic tissue samples were collected intraoperatively. RNA extraction and qRT-PCR analysis were conducted, normalizing expression to U6 and GAPDH. Statistical tests included Mann-Whitney U, ANCOVA, Spearman's correlation, and ROC analysis performed with SPSS ($p < 0.05$). Among the 24 patients in each group, miR-181a-3p was significantly lower in AsAA ($p = 0.004$), while TGF-β was elevated ($p = 0.022$). After adjusting for DM, only miR-181a-3p remained significant ($p = 0.023$). H19 showed no significant difference ($p = 0.091$). miR-181a-3p negatively correlated with aortic diameter ($p = 0.047$), while TGF-β exhibited a positive correlation ($p = 0.037$). ROC analysis indicated that miR-181a-3p could distinguish AsAA from mCABG, with an AUC of 0.74, sensitivity of 85%, and specificity of 57%. miR-181a-3p is decreased in AsAA and correlates with aortic diameter, indicating its potential as a biomarker. Further studies are necessary to validate these findings. *The authors marked with an asterisk equally contributed to the work.

P-41-060
GC contents of the genes may imply associations with coronary artery disease, autism, obesity, and type 2 diabetes

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The guanine (G) and cytosine (C) contents of human genes influences the mutation rates and expressions of the genes, as well as the DNA-hybridization energies. Elucidating the details of GC content's relation with diseases is still a rather obscured field. Accordingly, we started calculating the GC contents of the genes in the human reference genome. We calculated GC contents of around 8000 genes so far. We performed a comparative analysis of the GC contents of the disease-associated genes within them, as a preliminary study on a possible disease relevance of the GC contents, in certain directions. The disease-associated genes that we have utilized were based on literature [i.e., disease-related gene sets previously published in: Baker BH et al. (2023) Front Neurosci 17, 1106573]. The GC contents of a group of genes with specific disease-associations were compared. The number of genes in each group was expected to be more than or equal to 30. Their GC contents were compared to the remaining genes' GC contents. The numbers of the respective disease genes, which fitted to this criteria, were as follows: 30 of the 80 coronary artery disease-related genes, 38 of the 91 autism-related genes, 37 of the 104 schizophrenia-related genes, 102 of the 253 congenital heart disease-related genes, 33 of the 96 obesity-related genes, and 34 of the 81 type 2 diabetes-related genes. The GC contents of those genes were compared with that of the remaining genes, which we calculated so far. Comparisons were performed through t-test, by assuming unequal variances. The compared genes' GC contents were significantly different for all, except for that of the schizophrenia, and the congenital heart disease. We also compared the data of additional gene-groups obtained through gene ontology analysis (Baker BH et al. 2023). It led to significant results only for the data of that of the adenyl-nucleotide binding, and the ribonucleotide binding. We will further this research with the gene-length comparisons.

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P-41-061
Estrogen has a neuroprotective effect by preventing iron accumulation in Kufor Rakeb syndrome

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Estrogen influences susceptibility to a variety of neurodegenerative disorders. Kufor Rakeb syndrome (KRS) is a rare neurodegenerative disorder associated with mutations in ATP13A2 gene encoding a protein playing roles in the transport of intracellular inorganic cations such as iron. KRS exhibits severe symptoms – including dementia, supranuclear gaze palsy, spasticity – as the loss of ATP13A2 function leads to iron accumulation in the basal ganglia. Previously, we identified mutations leading to a premature stop codon in the ATP13A2 and nonsense mediated decay (NMD) of the mRNA in two siblings exhibiting different clinical presentations [Kırımtay et al. (2021) Brain Research, 1750, 147167]. The female patient, having a later onset and slower progression, led us to hypothesize that the KRS progression may be slowed down by protective effects of the estrogen that can also inhibit NMD, which would potentially trigger intracellular stress due to the production of truncated ATP13A2 protein. To identify these contradicting effects of estrogen, we studied ER stress behind estrogen's protective effect on KRS in fibroblasts of these patients. By qPCR and western blot (WB) analyses, the truncated ATP13A2 protein expression was found to be triggered in fibroblasts treated with estradiol, which was more prevalent in the female patient. Upon estradiol application, an increase, varying by gender, in the expression of ER stress proteins was found. However, CHOP expression was decreased. Thereupon, we investigated iron accumulation in fibroblasts treated with estrogen by Prussian blue staining and revealed that iron accumulation was decreased, particularly in female fibroblasts. Finally, FPN and HMOX expressions regulated by NRF2 responsible for iron metabolism, were increased in response to estrogen. These findings demonstrated that, while estrogen causes ER stress by inhibiting NMD and causing non-functional ATP13A2 protein expression, it displays neuroprotective properties via decreasing iron accumulation.

P-41-062
Mitochondrial effects of huntingtin polyglutamine expansion in a CRISPR-edited HEK model

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Huntington's disease (HD) is an autosomal dominant neurodegenerative disorder characterized by an expansion of CAG repeats in the *HTT* gene. One of the key mechanisms of HD pathogenesis is mitochondrial dysfunction, leading to energy metabolism disturbances and cellular apoptosis. This study explored the relationship between CAG repeat length and mitochondrial dysfunction in HEK cells edited using CRISPR-Cas technology. Three cell lines with different CAG repeat numbers – 41, 53, and 84 – were used. Mitochondrial functionality was assessed using enzymatic assay kits with colorimetric measurements to determine the activity of individual respiratory chain complexes. Mitochondrial membrane potential was evaluated using the JC-1 dye and fluorescence-based measurements. MitoTracker dye was used to visualize mitochondria, and immunofluorescence was employed to detect cytochrome c release, a key marker of apoptosis. The results indicate that the cell line with 84 CAG repeats exhibits the most significant mitochondrial dysfunction compared to the control. Reduced activity of respiratory chain complexes, increased cytochrome c release, and a decline in mitochondrial membrane potential were observed. These results suggest that progressive mitochondrial dysfunction is closely linked to CAG repeat expansion, emphasizing the importance of targeting mitochondrial pathways in HD treatment strategies.

P-41-063
Vascular imbalance in polycystic ovary syndrome

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Polycystic ovary syndrome (PCOS) is a multifactorial endocrine disorder associated with vascular dysfunction and increased cardiovascular risk. This study aims to investigate the dysregulation of vascular tone in PCOS, focusing on the imbalance between vasodilators (nitric oxide [NO] and apelin) and vasoconstrictors (noradrenaline and reduced prostacyclin). By examining these factors, the study seeks to elucidate their contribution to endothelial dysfunction and cardiovascular complications in PCOS patients. Forty-four patients diagnosed with PCOS according to the 2003 Rotterdam Criteria, along with 44 healthy controls, were included in the study. Ultrasound evaluations were performed on all volunteers. Serum nitric oxide (NO), apelin, noradrenaline, and prostacyclin levels were measured using commercial enzyme-linked immunosorbent assay (ELISA) kits. Additionally, routine biochemical, hormone, and glycated hemoglobin analyses were conducted on all samples. There was no statistically significant difference between the PCOS and control groups in terms of marital status, age, and body mass index (BMI) ($p > 0.05$). Compared to the control group NO, apelin, and noradrenaline concentrations were significantly higher in patients with PCOS ($p < 0.01$). In contrast, prostacyclin concentrations were significantly lower in patients with PCOS compared to the control group ($p = 0.011$). Additionally, a statistically significant difference was found between the PCOS and control groups in FSH, LH, testosterone, SHBG, glucose, and HDL levels ($p < 0.05$). The disrupted balance between vasodilation and vasoconstriction in PCOS, driven by altered levels of NO, apelin, noradrenaline, and prostacyclin, contributes to endothelial dysfunction and increased cardiovascular risk. These molecular disturbances underline the need for targeted therapeutic strategies aimed at restoring vascular homeostasis in PCOS patients.

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P-41-064
Conformational consequences of beta-catenin mutations causing CTNNB1 neurodevelopment syndrome

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CTNNB1 syndrome is a rare neurodevelopmental disorder, which manifests in children, with frequent symptoms including intellectual disability, motor and speech delay, microcephaly, visual defects and autism-like behavioural problems. It is caused by germline *de novo* mutations in the CTNNB1 gene, which codes for β-catenin, an elongated protein built of α-helical repeats. β-catenin is critical in embryogenesis, particularly of the nervous system, as a central hub in the Wnt/β-catenin signalling pathway, which through transcription regulation controls the balance between cell proliferation and differentiation. Furthermore, it is part of the adherens junctions (AJ) complexes between cells, which provide mechanical support in epithelial and neuronal tissues and are critical for brain development, as well as synapses formation and plasticity. Multiple CTNNB1-associated mutations, distributed throughout the gene, have been described. The most prevalent ones generate truncated variants of β-catenin that may be unstable, unable to fold, or display altered functions i.e. probably resulting in loss of protein function. However, the precise consequences of the pathogenic mutations on the structure and functionality of β-catenin are presently unknown. To explore the effects of CTNNB1 mutations on β-catenin folding, stability and interaction with certain ligands, we are expressing recombinant variants corresponding to various patients, and characterizing their conformational properties through circular dichroism spectroscopy and size exclusion chromatography. Our data indicate that the truncated variants can adopt, to some extent, a native-like secondary structure but their thermal stability is significantly impaired. Moreover, we are evaluating the consequences of CTNNB1-linked mutations on β-catenin interaction with N-cadherin, to explore the putative misregulation of cell adhesion during brain development in CTNNB1 syndrome.

P-41-065
Serum and tear biochemical correlations in hypertensive retinopathy: insights into oxidative stress and the renin-angiotensin system

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Hypertensive retinopathy (HR) is associated with oxidative stress (OS), antioxidant defense mechanisms, and the renin-angiotensin system (RAS). This study analyzed correlations between serum and tear biochemical markers to evaluate their role in HR progression. Weak but significant correlations were found between serum and tear levels of malondialdehyde (MDA) ($r_s = 0.277$, $p = 0.008$), total antioxidant activity (TAA) ($r_s = -0.226$, $p = 0.032$), superoxide dismutase (SOD) ($r_s = 0.336$, $p = 0.001$), glutathione (GSH) ($r_s = -0.361$, $p = 0.000$), and angiotensin II (Ang II) ($r_s = -0.323$, $p = 0.045$). Serum Ang II levels correlated positively with HR severity ($r_s = 0.413$, $p = 0.009$), while tear Ang II levels showed a negative correlation ($r_s = -0.357$, $p = 0.026$), suggesting the involvement of a local RAS. Antioxidant system markers in tear fluid, including TAA ($r_s = 0.357$, $p = 0.001$), catalase (CAT) ($r_s = 0.261$, $p = 0.013$), glutathione peroxidase (GPx) ($r_s = 0.299$, $p = 0.004$), and glutathione reductase (GR) ($r_s = 0.252$, $p = 0.017$), were positively correlated with HR grade, except for SOD ($r_s = -0.284$, $p = 0.007$), which decreased as HR progressed. These findings suggest that local antioxidant mechanisms are activated but may be insufficient to counteract oxidative damage. Additionally, a correlation was observed between OS and lipid metabolism markers, particularly between MDA and triglyceride levels ($r_s = 0.464$, $p = 0.000$), supporting the role of dyslipidemia in HR pathogenesis. The study highlights the potential of Ang II as a candidate biomarker for HR evaluation, emphasizing the need for further research to confirm its diagnostic and prognostic value. Previously published in part in: Pavlovschi E et al. (2021) Rom J Ophthalmol 65, 46–53.

P-41-066
A reference genetic diagnosis center experience of individuals with gender dysphoria in Turkiye

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Gender dysphoria (GD) is defined as distress resulting from the incompatibility between an individual's gender identity and the biological/physical sex assigned to that individual at birth. Genetic analysis, including chromosome analysis, is still not informative about the causes of GD. While there are some studies that explore the possible genetic and chromosomal influences on gender identity, it is important to understand that gender dysphoria is a complex and multifaceted condition influenced by a combination of biological, environmental, and psychological factors. Here, we expand our previously published study with additional cases and new data. The study samples consisted of 1330 individuals having GD with more than 400 new cases. Out of these cases 945 (71%) of them had female-to-male (FtM) and 385 (29%) had male-to-female (MtF) GD. The karyotype analysis demonstrated that 373 out of 385 (96.8%) individuals with MtF GD had the 46,XY karyotype and 3 cases had Klinefelter syndrome (0.7%), while 933 out of 945 (98.7%) individuals with FtM GD had the 46,XX karyotype, 8 cases had mosaic Turner syndrome (0.84%); the remaining were polymorphic variant carriers in both groups. In the Y chromosome microdeletion analysis, azospermic factor c (AZFc) deletion was detected in only two individuals with MtF GD. Our findings suggest that chromosomal abnormalities are rare in individuals with GD. In our center, karyotype analysis and Y

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microdeletion testing are routinely performed to rule out disorders of sex development and to identify any structural or numerical chromosomal anomalies in individuals experiencing GD. According to our data, no significant alterations have been observed at the chromosomal level or in Y chromosome microdeletions. It is crucial to evaluate further genetic and epigenetic factors together in the molecular pathogenesis of GD.

P-41-067
New therapeutic approaches for refractory rheumatoid arthritis

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Rheumatoid arthritis (RA) is a chronic autoimmune disease characterized by hyperplastic synovium, cartilage destruction and bone erosion. Methotrexate (MTX) is used on early stages of RA but it has numerous side effects while Janus kinase (JAK) inhibitors, like baricitinib (BAR), have shown efficacy in refractory patients. In this context, nanohydrogels (NHs), obtained through the self-assembly of functionalized polymers, represents a promising approach. These innovative nanomaterials can encapsulate drugs, improving their biodistribution and therapeutic efficacy. The aim of this study is the development of targeted and personalized therapies, to reduce the numerous and well-known systemic side effects resulting from the oral administration of drugs conventionally used for RA, particularly for patient's refractory to therapies. The project aimed to evaluate the efficacy of NHs on the SW982 and RAW 264.7 cell lines, to calculate the IC_{50} value. Quantitative PCR and ELISA analyses have been performed to evaluate the inflammatory pathways and the wound healing assay to assess the antiproliferative effects of the NHs. The next phase involved the evaluation of NHs in collagen-induced arthritis (CIA) rat model. Animals received intra-articular treatment with NHs, monitoring clinical signs, pain assessment and histological alterations. Oxidative stress markers and pro-inflammatory cytokines also have been evaluated. To assess the better efficacy of NHs formulations compared to classic oral therapies, oral doses of MTX and BAR have been administered to control groups. On-site administration allows for a targeted effect on the disease since we are far from a cure today. Chronic diseases require continuous drug administration that causes systemic damages, with further global deterioration of patient's health. Being able to avoid oral administration with *in-situ* treatment represents a potential alternative for the treatment of the disease and the improvement of patient's life.

P-41-068
The role of ESCRT-complex subunit SNF8 in brain development

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The endosomal sorting complex required for transport (ESCRT) machinery plays an essential role in membrane remodeling, recognition and sorting of ubiquitinated cargo and autophagy, and comprises three multi-subunit complexes (ESCRT I–III). The cell biology of these functions has been very well studied, but little is known regarding to their role in neuron biology. Previously, we reported nine individuals from six families presenting with a spectrum of neurodevelopmental/neurodegenerative features caused by biallelic variants in *SNF8*, encoding the ESCRT-II subunit SNF8 (also known as EAP30/VPS22) (1). The phenotypic spectrum included four individuals with severe leukoencephalopathy, neurodevelopmental arrest and secondary microcephaly without additional major congenital anomalies. Examination of brain tissue in one individual revealed a massive reduction of white matter substance, hypo-/aplasia of the corpus callosum as well as signs of neurodegeneration. We have provided evidence that biallelic SNF8 variants cause loss of ESCRT-II subunits by proteomics in patient-derived fibroblasts. In addition, these cells display an accumulation of autolysosomes and abnormal lysosomes. Aberrant autophagy was also detected by immunohistochemistry in affected brain tissue samples. To understand the functions of SNF8 in brain development, we have now established mouse and iPSC models for further mechanistic studies. Reference: Brugger M, Lauri A, Zhen Y, Gramegna LL, Stenmark H, Tartaglia M, Wagner M. Bi-allelic variants in SNF8 cause a disease spectrum ranging from severe developmental and epileptic encephalopathy to syndromic optic atrophy. Am J Hum Genet. 2024;111(3):594-613. doi: https://doi.org/10.1016/j.ajhg.2024.02.005.

P-41-069
Targeted *KCNA1* gene editing in mice for epilepsy modelling

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KCNA1 gene encodes potassium voltage-gated channel subfamily A member 1 subunit (kv1.1 protein). This subunit consists of six transmembrane domains, with the fourth domain acting as a voltage sensor, while the fifth and sixth domains, along with the loop between them, form the pore. Functional potassium voltage-gated channels are composed of four alpha subunits. Mutations in the *KCNA1* gene in humans are associated with various channelopathies, the most common being Episodic Ataxia Type 1 (EA1) and epilepsy. Mouse lacking kv1.1 protein has been described as a valuable epilepsy model with spontaneous seizures, but also show high postnatal mortality rate. Our primary goal was to create novel epilepsy mouse model by CRISPR/Cas editing of *KCNA1* gene. We have designed two single guide RNAs (sgRNAs) with different binding sites for developing mice strains with different mutated kv1.1 by zygote microinjection of sgRNA with Cas9 mRNA construct. First sgRNA targeted the region encoding sixth transmembrane domain. We obtained five mosaic pups with different deletions size from 1 to 19 nucleotides, in 3 out of 5 mice multiple mutation variants were detected. We observed spontaneous epileptic seizures at an early age (1–2 weeks). Unfortunately, all animals died before reaching puberty (between 2 and 3 weeks of age). The mortality of the animals we obtained was higher than in the previously described model. Hence, we have changed sgRNA binding site therefore possible indels would affect the C-terminus part of the protein (with all six transmembrane domain remains intact). There is evidence, that early stop-codon

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mutations on the C-terminus lead to EA1 and epileptic phenotype in human. Thereby we obtained mouse with single nucleotide insertion, which leads to early stop-codon mutation. We have managed to get F1 generation, but further research is needed for phenotype analysis. This work was supported by St. Petersburg State University, Project ID 129658320.

P-41-070
New catechol-O-methyl transferase knockout (COMT-KO) mouse strain with no visible signs of preeclampsia

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Catechol-O-methyl transferase (COMT) is the main enzyme that plays an important role in the metabolism of catechol substrates such as catechol estrogens, catecholamines and L-dopa. COMT exists in two forms: membrane-bound and soluble. Disturbances in the COMT function are associated with neuropsychiatric, cardiovascular and other diseases and, in particular, with preeclampsia, which is possibly caused by a deficiency of the product of catechol estrogen metabolism, 2-methoxyestrogen. A well-studied mouse strain with null *comt* allele (obtained by replacing a gene fragment with a Neo^R cassette) are considered as a model for preeclampsia[1], since in the homozygous state they demonstrate the main symptoms of this disease – high blood pressure and proteinuria during pregnancy, as well as fetal death. Using CRISPR/Cas9 technology on a C57Bl/6 background, we developed a new mouse strain with a *comt* gene knockout. These mice lacks both forms of COMT due to a 20-base pair deletion located in the start codon region of the soluble form, which also disrupts the open reading frame of the membrane-bound isoform. High-performance liquid chromatography analysis confirmed the absence of COMT enzymatic activity in these mice[2]. Interestingly, in contrast to previous models, we did not observe reduced viability of COMT-KO pups when fed a standard diet, suggesting the absence of preeclampsia-like symptoms. This discrepancy with earlier findings may reflect differences in genetic background, the method used to generate the knockout, or the conditions under which the animals were maintained. Given the complexity of preeclampsia, these factors could significantly influence disease manifestation in mouse models. This work was supported by St. Petersburg State University, Project ID 129658320. References: [1]J.A. Gogos et al. (1998) Proc. Natl. Acad. Sci. USA 95 (17) 9991-9996. [2]Previously published in Akhmarov I.I. et al. (2024) Laboratory Animals for Science. 2024; 4: 4–13.

P-41-071
The importance of serum apelin-13, apelin receptor, nitric oxide and nitric oxide synthase levels in the differentiation of patients with ST elevation and non-ST elevation myocardial infarction

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Apelin, a peptide hormone, binds to the angiotensin II protein J receptor (APJ), increasing endothelial nitric oxide synthase (eNOS) and inducing vasodilation. This study examines the diagnostic value of serum apelin-13, APJ, NO, and eNOS in ST-elevation MI (STEMI) and non-STEMI (NSTEMI). A total of 225 adults (40-80 years) were included: STEMI (n = 45), NSTEMI (n = 45), unstable angina pectoris (USAP) (n = 45), stable angina pectoris (SAP) (n = 45), and healthy controls (n = 45). Apelin-13 and APJ levels were significantly lower in acute NSTEMI and STEMI patients compared to controls ($p < 0.001$, for all). Both NSTEMI and STEMI groups had remarkably reduced serum NO and eNOS activity than controls ($p < 0.001$). Apelin-13, APJ, NO and eNOS were lower in STEMI than in NSTEMI subjects ($p < 0.001$, $p = 0.019$, $p = 0.003$ and $p < 0.001$, respectively). Altered apelin-13 and APJ levels showed significant discrimination with high sensitivity and specificity between healthy individuals with NSTEMI (AUC = 0.900 and AUC = 0.928) and STEMI (AUC = 0.970 and AUC = 0.985). Strong positive correlations between serum apelin-13 and APJ, NO and eNOS activity were observed in STEMI patients ($p < 0.001$, for all). The study results highlight a significant difference in the levels of apelin-13, APJ, NO, and eNOS between patients with acute NSTEMI, STEMI and healthy individuals. Serum apelin-13 and APJ might be used as a clinical biomarker for early recognition of NSTEMI and STEMI, while lower NO and eNOS levels indicate endothelial dysfunction. Correlation findings support apelin-13's role in STEMI pathophysiology and its potential as a marker of endothelial dysfunction or myocardial damage. These results highlight apelin-13 and APJ as valuable diagnostic biomarkers for distinguishing between these two forms of acute coronary syndrome, emphasizing their potential in early detection and risk stratification of AMI. Larger multicenter studies are needed for validation.

P-41-072
Disruption of ferroptosis pathways in the mouse model of mucopolysaccharidosis type I

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Hurler disease, known also as mucopolysaccharidosis type I (MPS I), is a rare genetic metabolic disorder caused by a deficiency of the enzyme alpha-L-iduronidase, which is responsible for the breakdown of glycosaminoglycans (GAGs) in lysosomes. Then, GAGs accumulate in various tissues and organs, leading to their damage. The symptoms of MPS I are

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diverse and may include enlargement of the liver and spleen, bone deformities, breathing difficulties, impaired heart function, and neurological symptoms. Current treatments have proven insufficient to fully reduce all symptoms, even though GAG levels may normalize. Therefore, there is a significant need to explore new aspects of the pathogenesis of this disease, which could contribute to the identification of new therapeutic targets. Recent discoveries regarding the role of lysosomes in ferroptosis highlight the potential involvement of this process in the pathogenesis of MPS. Ferroptosis is a form of programmed cell death dependent on high iron concentrations and lipid peroxidation. Dysregulation of ferroptosis has been linked to the development of various neurodegenerative diseases and cancers, but this phenomenon has never been studied in the context of lysosomal storage disorders. Thus, the aim of this study was to assess the dysregulation of ferroptosis and to determine the molecular mechanism of this process. Studies conducted using a mouse model of MPS I indicated an increase in iron levels and lipid peroxidation, indicating enhanced ferroptosis activity. This process may be regulated in the cell through different pathways. Our research indicated disturbances in levels of proteins responsible for maintaining proper iron metabolism and changes in abundance of proteins involved in autophagy-dependent ferroptosis. In summary, disruptions in iron trafficking and autophagy-dependent ferroptosis may be responsible for an increased intensity of ferroptosis, representing a new aspect of MPS I pathogenesis.

P-41-073
The role of the estrogen receptor GPER in ferroptosis regulation in mucopolysaccharidosis type I

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Mucopolysaccharidosis type I (MPS I) is a lysosomal storage disease caused by α-L-iduronidase deficiency, leading to glycosaminoglycan (GAG) accumulation and multi-organ dysfunction, including neurological symptoms. Ferroptosis, an iron-dependent form of cell death, has been implicated in neurotoxicity, but its role in MPS I remains unclear. Recent findings suggested that autophagy-related ferroptosis may contribute to lysosomal disease pathogenesis, with GPER, a key estrogen receptor, potentially playing a role. In this study, we examined the effects of GPER modulation on ferroptosis in MPS I. Fibroblasts from MPS I patients and healthy controls were treated with the GPER antagonist G15. We found increased iron levels and lipid peroxidation in MPS I cells. This suggested ferroptosis dysregulation, which was accompanied by altered levels of ferroptosis marker proteins. G15 treatment modulated these markers in both MPS I and healthy cells, confirming GPER involvement. These findings provide insights into modulation of ferroptosis regulation in MPS I and highlight GPER as a potential therapeutic target.

P-41-074
Investigation of *de novo* variants in the POU neuronal gene family

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Pou3f2 and Pou3f3 proteins play a crucial role in neuronal differentiation. Two gene variants in two patients were identified by exome sequencing, resulting in amino acid changes in the conserved DNA-binding region of the proteins, potentially leading to neuronal developmental disorders. Our research aimed to conduct the functional analysis and the description of the two identified mutations in *POU3F2* and *POU3F3* genes for a potential molecular diagnosis and a better understanding of the pathomechanism. Based on *in silico* analysis, we selected *H2AC6* and *ZEB2* target genes and cloned their promoter regions into pGL3B luciferase vectors. The cloned promoter regions contained one and two putative binding sites for the Pou transcription factors in the *ZEB2* and *H2AC6* 5' regions, respectively. We designed protein-expressing constructs using a pSF-CMV-Puro-NH2-HA vector containing the wild-type genes of *POU3F2* and *POU3F3*. DNA constructs coding the mutant variants of the proteins were created by site-directed mutagenesis. HEK293 cells were used for transient transfection. The efficacy of the transfection and the endogenous levels of the Pou3-proteins were determined by western blot analysis. The impact of the transcription factors was quantified by measuring relative luciferase activity. The results of the luciferase assays were statistically analyzed using t-tests. The functional analysis on HEK293 cell line showed that the mutant variants of Pou3f2 and Pou3f3 proteins could not exert their function to the extent as the wild types of the transcription factors could. This significantly lowered the relative luciferase activity for *POU3F2* with the *ZEB2* promoter ($p = 0.00068$) and for *POU3F3* with the *H2AC6* promoter ($p = 0.017$). The results of our measurements suggest that the mutations change the function of the Pou3f2 and Pou3f3 proteins, and thus, they could cause the neurodevelopmental delay of the two patients.

P-41-075
Transcriptomic analyses of gene expression disturbance in Huntington disease and effects of treatment with genistein

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Neurodegenerative diseases involve progressive neuron loss in the nervous system, often linked to protein aggregation. Huntington's disease (HD) is one such disorder, characterized by movement, psychiatric, and cognitive impairments. HD is caused by an increased number of CAG repeats in the *HTT* gene, leading to mutant huntingtin (mHTT) aggregation. More than 36 CAG repeats result in HD, with severity increasing as repeats grow. Our study examined HEK 293T cell lines with 41, 53, and 84 CAG repeats, as well as a mouse HD model (116 CAG repeats). We treated these models with genistein, a flavonoid that induces autophagy, reducing mHTT levels. Transcriptomic analysis revealed that increasing CAG repeats altered gene

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expression, particularly in autophagy-related genes. Genistein modulated these changes, either normalizing or enhancing gene expression. Protein analysis confirmed autophagy stimulation, potentially reducing mHTT aggregates. Our findings highlight genistein's potential in HD treatment by promoting mHTT degradation through autophagy induction. This research advances our understanding of HD mechanisms and paves the way for targeted therapeutic strategies.

P-41-076
Novel mutations in the Prnp gene affecting prion aggregation discovered using a yeast model

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Amyloids are protein aggregates defined by their fibrillary structure, featuring intermolecular cross-β-sheets. The deposition of amyloid proteins in tissues is linked to a range of socially significant diseases, including Alzheimer's and Parkinson's disease. Among these, infectious prions represent a particularly concerning class of amyloids, as they can be transmitted between organisms causing fatal, untreatable diseases such as scrapie in sheep, and kuru and Creutzfeldt-Jakob disease in humans. The yeast model *Saccharomyces cerevisiae* has been extensively used to study amyloids and prions. Research has demonstrated that when amyloidogenic mammalian proteins are expressed in yeast, they form aggregates that are, in most cases, biophysically and biochemically similar to those found in diseased mammals. While these heterologous aggregates generally lack toxicity to yeast cells, they also do not induce significant phenotypic changes. Here, we used a yeast-based assay [Chandramowlishwaran et al. (2018) J Biol Chem, 293, 3436-3450] for the phenotypic detection of amyloid aggregation of the mouse prion protein (PrP). The assay enables the assessment of the amyloidogenic potential of mammalian proteins by measuring the efficiency of yeast colony growth on selective media. We conducted a large-scale screening to identify mutations that influence the amyloid aggregation of PrP. Through this screening, we identified mutations that effectively inhibit PrP aggregation, as well as novel mutations that may be associated with the onset of hereditary prion diseases. These findings not only enhance our understanding of prion pathology formation but also highlight the utility of yeast as a model system for uncovering molecular insights into protein aggregation and its role in disease. This work opens avenues for further research into therapeutic strategies targeting amyloid-related disorders. The research was supported by St. Petersburg State University Project No 125021902561-6.

P-41-077
Evaluation of the relationship of serum oxidized and soluble low-density lipoprotein (oxLDL and sLOX-1) levels with endothelial dysfunction in dyslipidemic individuals

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Dyslipidemia is one of the most important risk factors for atherosclerotic cardiovascular disease. Oxidized low-density lipoprotein (oxLDL) is formed in the process of atherosclerosis. oxLDL is taken into the cell by scavenger receptors such as lectin-like oxidized low-density lipoprotein receptor-1 (LOX-1) and a foam cell is formed. Endothelial dysfunction results in increased endocan synthesis. This study was conducted to investigate the relationship between serum oxLDL and soluble LOX-1 (sLOX-1) levels and endocan in dyslipidemic individuals with different lipid contents. The study group consisted of dyslipidemic (n = 118) and normolipidemic (n = 39) individuals. The levels of oxLDL and its receptors sLOX-1 and CD36, endocan and vascular cell adhesion molecule-1 (VCAM-1) levels associated with endothelial dysfunction were determined by enzyme-linked immunosorbent assay. In the dyslipidemic group and its subgroups: oxLDL, sLOX-1, CD36, endocan and VCAM-1 parameters showed a significant increase ($p < 0.05$). In dyslipidemic individuals, sLOX-1 showed a higher fold increase compared to CD36. The fold increase in oxLDL and sLOX-1 was used to evaluate the relationship with endothelial damage. In conclusion, the levels of oxLDL and its receptors were significantly increased in dyslipidemic individuals compared to healthy individuals and this change was closely associated with endothelial dysfunction. This relationship was more pronounced in combined hyperlipidemic individuals. *The authors marked with an asterisk equally contributed to the work.

P-41-078
Molecular insights into the coenzyme A-assisted auto-acetylation of Tau protein

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Tau protein plays a key role in neurodegenerative diseases, where its aggregation and misfolding contribute to pathology. Lysine acetylation has been involved in regulating these processes, yet the precise mechanisms remain unclear. Tau is known to undergo auto-acetylation, but how this occurs at the molecular level is not fully understood. Given its intrinsically disordered nature, defining Tau's interactions with potential acetylation cofactors, such as acetyl-coenzyme A (Ac-CoA), remains a challenge. This study investigates whether Tau directly interacts with coenzyme

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A (CoA), a key component of Ac-CoA, and explores its role in auto-acetylation. To address this, we combined experimental and computational approaches. Full-length and truncated Tau variants (wild-type and a Cys-to-Ala mutant with reduced auto-acetylation) were expressed in *E. coli*, purified, and subjected to a pulldown assay using immobilized CoA. This assay provided direct biochemical evidence that CoA binds Tau, suggesting a stable complex relevant to auto-acetylation. To complement these findings, molecular modeling was performed to map CoA binding sites and assess their relevance to auto-acetylation. Structural models of Tau were generated using AlphaFold3, their energy was minimized, and they were analyzed for CoA interactions. The GAFF2 force field was used to parameterize CoA, and Robosample [previously published in: Spiridon L et al. (2020) BBA, 1864(8):129616] was used to explore Tau's conformational space. Clustering analysis identified key structural conformations for docking, revealing preferential CoA binding sites. In conclusion, our study provides the first combined biochemical and computational evidence that Tau directly interacts with CoA, supporting its role in auto-acetylation. These findings contribute to understanding Tau auto-acetylation and its implications in neurodegenerative diseases. *The authors marked with an asterisk equally contributed to the work.

P-41-079
Nebivolol regulates glucose induced proliferation in human coronary artery smooth muscle cells via netrin-1 related pathways

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Chronic hyperglycemia in diabetes induces vascular damage, leading to vascular smooth muscle (VSM) cell proliferation, migration, and intimal hyperplasia that progress to stenosis and myocardial infarction. Netrin-1, a key regulator of cell migration and signaling, contributes to atherosclerosis by promoting VSM proliferation and vascular remodeling. Elevated serum netrin-1 in diabetic patients is linked to vascular complications via activation of MAPK, PKC, SRC, and PI3K pathways, making it a potential target for preventing coronary artery disease (CAD). Nebivolol, a third-generation cardioselective beta-blocker with antiproliferative, anti-inflammatory, and cardioprotective properties, has not been evaluated for its effects on netrin-1 pathways in human coronary artery smooth muscle cells (hPCASMCs) under diabetic conditions. To address this gap, we mainly focused on netrin-1 knockdown and nebivolol treatment in a diabetic CAD model using 30 mM glucose. Glucose-exposed hPCASMCs exhibited increased proliferation, while nebivolol treatment reversed the proliferative effect of glucose. Additionally, glucose treatment upregulated netrin-1 expression, while nebivolol drastically suppressed it. Netrin-1 siRNA treatment effectively silenced the gene, leading to downregulation of its predicted targets, including UNC5B—a dependence receptor involved in apoptosis and migration inhibition—and the pro-survival regulator AKT1, while DCC, another dependence receptor involved in adhesion and migration, remained unchanged. AKT1 expression increased in glucose-treated cells but decreased with nebivolol. In conclusion, these findings highlight netrin-1's critical role in vascular remodeling under hyperglycemia and demonstrate that nebivolol not only modulates netrin-1 signaling but also counteracts hyperglycemia-induced proliferation, offering a potential therapeutic strategy for mitigating diabetes-related CAD progression. *The authors marked with an asterisk equally contributed to the work.

P-41-080
The biomechanical and histopathological changes in female and male SOD1G93A ALS rat aorta

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Respiratory failure is the primary cause of death in amyotrophic lateral sclerosis (ALS) patients, and sudden cardiac death and cardiovascular complications are reported as another common cause of ALS-related deaths. We evaluated histopathological and biomechanical changes in the aorta along with the oxidative stress status of the heart. Altered aortic stiffness independent from aging has been found in the superoxide dismutase 1 (SOD1G93A) mutated rats compared to the control groups via biomechanical testing with a custom-built stretcher device. Aneurysm formation, thickening in elastic fibers, and increased collagenization in connective tissue have been found in male and female SOD1G93A rats compared to the control groups, respectively, via H&E, elastic, trichrome, and AB-PAS staining at the pre-symptomatic and symptomatic stages. Fluctuations in the Na, Mg, Zn, and K concentrations have been found in the aorta of SOD1G93A rats of both genders via inductively coupled plasma mass spectrometry (ICP-MS). The oxidative stress status of the heart was investigated via evaluation of the glucose 6-phosphate dehydrogenase (G6PD), 6-phosphogluconate dehydrogenase (6-PGD), glutathione reductase (GR), and glutathione s-transferase (GST) activities. We have found that G6PD and 6-PGD activity decreased in the cytosolic and mitochondrial fractions of SOD1G93A rat heart tissue compared to the control groups. Accumulation of the human mutant SOD1G93A protein was confirmed via western blot. We have reported adverse biomechanical and histopathological changes occurring in ALS at the pre-symptomatic and symptomatic stages, along with the altered antioxidant status of the heart tissue, for the first time in the literature.

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LB-R-41-01
Intracerebroventricular delivery of ASO in combination with anti-inflammatory and ketogenic diet treatment reversed neuropathology in the Tay-Sachs disease mouse model

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Tay-Sachs disease (TSD) is a rare neurodegenerative lysosomal storage disorder caused by the deficiency of β-hexosaminidase A, leading to the accumulation of GM2 ganglioside in the central nervous system. Multiple therapeutical strategies have been investigated, such as gene therapy for Tay-Sachs disease; however, there is still no cure. TSD mouse model shows chronic neuroinflammation, primarily driven by neurodegeneration-induced astrogliosis and increased macrophage activity. Here, we aimed to investigate the efficacy of a novel therapeutic strategy for the TSD mice model using AAV-mediated anti-sense oligonucleotide (ASO) administration to target the *B4galnt1* gene combined with the ketogenic diet (KD) and propagermanium (PG). Intracerebroventricular injections of AAV ($2x10^{11}$ vg/ul) were performed in 30-day-old *Hexa*-/-*Neu3*-/- mice. KD and PG treatments were initiated for 20 days to reduce neuroinflammation when injected mice were 120 days old. TLC analysis showed that delivery of ASO significantly reduced the accumulated GM2 gangliosides in the *Hexa*-/-*Neu3*-/- mice cortex but not in the cerebellum. Immunofluorescence staining of neuroinflammation-related markers in the cortex and cerebellum showed reduced neuroinflammation in *Hexa*-/-*Neu3*-/- mice. Our study demonstrated that silencing of *B4galnt1* injection prolonged survival in a mouse model of TSD and suppressed active neuroinflammation with combined treatment of KD and PG.

LB-R-41-02
A novel mannose receptor agonist enhances self-renewal and therapeutic potential of MSCs

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Mesenchymal stem cells (MSCs) are widely recognized for their therapeutic value in regenerative medicine, yet *in vitro* expansion without loss of stemness remains a challenge. We previously identified plant-derived bioactive compounds capable of inducing reprogramming via mannose receptor-mediated pathways. In this study, we report a novel mannose receptor agonist that significantly enhances MSC proliferation, self-renewal, and resistance to senescence, while preserving their differentiation potential. The compound increased expression of Sox2, Nanog, Oct4, and Tert, and promoted cell cycle progression via Cyclin E. Mechanistic analysis revealed that its effect is mediated through activation of MKK1/2/3/6, phosphorylation of ERK1/2 and P38, and subsequent CREB activation. *In vivo*, administration of this agonist improved cardiac regeneration in a mouse model of myocardial infarction. These findings suggest that this novel compound may serve as a valuable agent for MSC expansion and rejuvenation in cell-based therapies.

LB-R-41-03
Mouse mucopolysaccharidosis IIIC affects the lysosomes of podocytes, tubular cells and macrophages contributing to renal dysfunction

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Mucopolysaccharidosis IIIC (Sanfilippo Disease) is a lysosomal disorder caused by heparan sulfate accumulation in lysosomes due to a deficiency in heparan sulfate acetyl-CoA: α-glucosaminide N-acetyltransferase (HGSNAT). This enzyme catalyzes the acetylation of heparan sulfate, a glycosaminoglycan found in proteoglycans and basement membranes. Mutations in the HGSNAT gene impair heparan sulfate degradation, causing its buildup in lysosomes. This study uses a CRISPR-Cas9-generated knock-in mouse model expressing the Pro304Leu HGSNAT variant, replicating the human Pro311Leu mutation. The model shows early-onset MPS IIIC with dominant-negative effects and stress on the endoplasmic reticulum and lysosomes. We examined $Hgsnat^{P304L}$ kidney cortices by light and electron microscopy, PAS histochemistry, and immunocytochemistry using lysosomal and macrophage markers. Renal function was assessed by plasma and urine creatinine levels and urinary albumin excretion over 16 hours, using age-matched male and female mice on a standardized diet. Results showed lysosome accumulation in podocytes and parietal cells of Bowman's capsule, but not in mesangial cells. Glomeruli were distorted, and mesangial cell proliferation occurred, characteristic of mesangial proliferative glomerulonephritis. Tubular epithelial cells accumulated pleomorphic lysosomes. Interstitial macrophages were increased in number and showed prominent lysosomal storage. HgsnatP304L mice had reduced glomerular filtration rate, elevated plasma-to-urine creatinine ratio, and increased albumin excretion. Our results indicate that impaired heparan sulfate metabolism disrupts renal homeostasis in the MPS IIIC mouse model, with podocytes and tubular cells contributing to heparan sulfate handling, and interstitial macrophage activation potentially exacerbating glomerular and tubular pathology. (Supported by NSERC and CIHR, Canada) *The authors marked with an asterisk equally contributed to the work.

LB-R-41-04
The role of brain-derived neurotrophic factor in prognostic assessment of hip fracture outcomes in the elderly

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Hip fractures represent a significant health concern among the elderly, often resulting in morbidity and loss of independence. Brain-derived neurotrophic factor (BDNF), a neurotrophin crucial for neuronal survival, synaptic plasticity, and cognitive functions, has garnered attention as a potential biomarker for neurological health and physical recovery. This study explores the molecular mechanisms that influence neurological outcomes in patients hospitalized due to

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osteoporotic hip fractures resulting from low-energy trauma. Patients were categorized based on fracture type as either intertrochanteric (ITF) or femoral neck (COL) fractures. Serum BDNF levels were quantified using the single-molecule array (SIMOA) technique, an ultrasensitive digital immunoassay that allows for the detection of low-abundance biomarkers with exceptional precision and reproducibility. The sensitivity of SIMOA is particularly advantageous for identifying subtle molecular changes associated with neurodegeneration and bone metabolism. When analyzed by gender, BDNF levels varied between groups. In the ITF group, the mean BDNF levels were 14 392.11 ± 4564.97 pg/mL in males and 15 234.03 ± 7184.48 pg/mL in females. In the COL group, the levels were 16 581.25 ± 6769.47 pg/mL in males and 14 837.50 ± 8578.41 pg/mL in females. These results indicate an increase in BDNF levels among male participants in the COL group. Furthermore, when gender was not considered, BDNF levels differed significantly between the ITF and COL groups ($p = 0.032$). These findings suggest that BDNF may serve as a valuable biomarker for predicting recovery and guiding the clinical management of patients following hip fractures. Elevated BDNF levels may facilitate cognitive and functional recovery, providing insight into post-surgical rehabilitation strategies. This study was supported by the Gazi University Scientific Research Projects Unit (Project Code: TGA-20249159).

LB-R-41-05
Role of sphingosine 1-phosphate signaling axis in TGF-β-induced fibrosis in endometriosis

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Endometriosis is a gynecological condition defined by the ectopic presence of endometrial glands and stroma. Although its etiology remains unclear, endometriosis is recognized as a multifactorial disease driven by chronic inflammation, fibrosis and altered cellular metabolism. Transforming growth factor-beta (TGF-β) is a central mediator of fibrosis, promoting extracellular matrix deposition and epithelial-to-mesenchymal transition (EMT). Increasing evidence highlights a critical role for TGF-β in endometriosis, along with dysregulation of the metabolism and signaling of the bioactive sphingolipid sphingosine 1-phosphate (S1P). Given the existence of a functional interplay between TGF-β and S1P signaling in other conditions, we investigated the role of S1P signaling in TGF-β-induced fibrosis in human endometriotic epithelial cells, focusing on the EMT process. Here, we show that TGF-β up-regulates sphingosine kinase (SK)-1, one of the isoforms of the enzyme responsible for S1P synthesis, as well as the specific S1P receptor S1P3 in endometriotic epithelial cells. Notably, pharmacological or short interfering RNA-mediated inhibition of SK1 prevented the induction of EMT markers by TGF-β. Moreover, inhibition of S1P3 strongly attenuated the profibrotic response to the cytokine. Besides increasing the SK1 expression, we showed that TGF-β activates the enzyme, being able to induce SK1 translocation to the membrane. Preliminary results suggest a critical role for calcium and integrin binding protein 1 (CIB1) in TGF-β-induced SK1 activation in endometriotic cells. Taken together, these data provide new compelling information on the mechanism by which TGF-β gives rise to fibrosis in endometriosis, demonstrating that the SK1/S1P3 axis is responsible for transmitting the profibrotic action of TGF-β and identifying S1P signaling as an innovative non-hormonal therapeutic target for endometriosis-associated fibrosis.

LB-R-41-06
Long non-coding RNA PELATON expression levels can be associated with the pathogenesis of coronary artery disease

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Coronary artery disease (CAD) is a pathological condition characterized by the accumulation of obstructive or non-obstructive atherosclerotic plaques in epicardial arteries. Atherosclerosis is an immuno-metabolic disease involving chronic inflammation, oxidative stress, epigenetic and metabolic dysfunction. Macrophages and foam cells are the main producers of reactive oxygen species (ROS) resulting from oxidative stress. Macrophages are in dynamic communication with vascular cells and are responsible for phenotypic changes of vascular smooth muscle cells. Expression of the lncRNA PELATON (plaque enriched lncRNA in atherosclerotic and inflammatory bowel macrophage regulation) has been reported to be localized in necrotic, inflammatory and macrophage-rich regions of atherosclerotic plaques and to be significantly higher in rupture-prone plaques. The aim of our study was to determine the expression levels of lncRNA RNA PELATON in patients undergoing coronary artery bypass grafting (CABG) and healthy controls and to determine the plasma levels of 8 hydroxyguanosine (8-OHG), a marker for oxidative damage of RNA. Sixty-five patients who underwent CABG operation and 70 healthy controls were included in the study. Following total RNA isolation and cDNA synthesis from blood samples, PELATON expression levels were determined by qRT-PCR. Plasma 8-OHG levels were determined by ELISA method. PELATON expression levels were significantly higher in the CABG group when compared with controls ($p < 0.001$). Plasma 8-OHG levels were higher in the CABG group compared to controls, but did not reach statistical significance ($p = 0.185$). Correlation analysis revealed no significant correlation between PELATON expression and serum CRP levels ($p = 0.207$). It is suggested that PELATON transcripts can play a role in the pathogenesis of CABG.

LB-R-41-07
Evaluation of the molecular basis of autoimmune cholestatic liver diseases via serum cytokine release

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Primary biliary cholangitis (PBC) is the most common autoimmune cholestatic liver disease. Patients having PBC have mostly positivity for AMA autoantibodies, but those autoantibodies are neither diagnostic nor prognostic in the disease process of PBC. NK cells damage the intraepithelial

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biliary epithelial cells through cytokine release in the pathogenesis of PBC. Although PBC is defined as an autoimmune disease, unfortunately, immunosuppressive drugs used in other autoimmune diseases cannot be used in its treatment. Therefore, it is thought that the pathogenesis of PBC is different from autoimmune pathogenesis. In order to evaluate this, we aimed to compare the cytokine and TLR levels of PBC with autoinflammatory pathogenesis (celiac disease: CD) and autoimmune pathogenesis (autoimmune hepatitis: AIH) in our study. The serum levels of IL-1, 2, 4, 6, 10, 12, 13, 17, 23, TNF-α, IFN-γ, TGF-β, and TLR 4-6 of all patient groups were analyzed by ELISA and flow cytometry methods, and similarities and differences between groups were evaluated. Statistical analysis was performed using the Kruskal-Wallis and Mann-Whitney U tests. For analysis of categorical data, appropriate chi-square tests or Fisher's exact test were used. Compared to patients with PBC and CD, TNF-α, IFN-γ, TGF-β1, IL-1β, IL-6, IL-10, IL-12p70, IL-17a, IL-23, and TLR 4 levels were found to be elevated in AIH patients. These parameters were the lowest in PBC patients and showed moderate decreases in CD patients. Compared to CD and AIH patients, IL-2 levels were the lowest and IL-13 levels were the highest in PBC patients. Compared to PBC and AIH patients, IL-4 levels were observed to be the highest in CD patients. Compared to the control group, TLR 6 levels showed statistically significant differences among all patient groups. Our study sheds light on future research on the pathogenesis of PBC to discover novel diagnostic and therapeutic markers and potential therapy strategies.

LB-R-41-08
PI3K inhibition modulates migration and fibrotic marker expression induced by THC metabolites in H9c2 cardiomyocytes

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Cannabis-derived metabolites, particularly THC-OH and THC-COOH, have been linked to alterations in cardiac cell behaviour. This study examines whether inhibition of the PI3K pathway via LY294002 modulates the fibrotic and migratory effects induced by these metabolites in H9c2 cardiomyocytes. Scratch wound assays showed that both metabolites (250 ng/mL) enhanced cell migration within 24 hours. Co-treatment with LY294002 (5 μM) significantly reduced migration, restoring levels close to control. Western blot analysis revealed that α-smooth muscle actin (α-SMA) expression, a key marker of fibrotic activation, was increased following metabolite exposure and was markedly reduced with LY294002 co-treatment. These results suggest that PI3K inhibition may mitigate early fibrotic and migratory responses triggered by THC metabolites. The data presented have not been previously published and form part of a broader *in vitro* investigation into the cellular effects of cannabinoid exposure. Additional analyses are ongoing to assess the role of cytoskeletal regulators (e.g., Eb1, Clasp2), extracellular matrix components (e.g., Col1a1), and microarchitectural changes using scanning electron microscopy. These datasets are not shown here and are being prepared for future publication. This study provides insight into PI3K-related signalling mechanisms involved in cannabinoid-induced cellular remodelling and may inform future research on the cardiovascular impact of cannabis metabolites.

Neuroscience

P-42-001
MicroKiss: the beginning of a story about kisspeptin and microglia interaction in sex-specific social development

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The sexual differentiation of the brain, which plays a key role in sex-specific behaviors, is shaped by a complex interplay of genetic, epigenetic, hormonal, and metabolic factors. While these processes are well-established in the context of gonadal development, the mechanisms underlying sex-specific brain differentiation remain unclear. Recent studies have identified kisspeptin (kiss) as a key regulator, playing an important role in early-life hormonal fluctuations, particularly affecting male brain development through the neonatal testosterone surge (NTS). Given that microglia are essential for synaptic pruning and the refinement of social circuits, we hypothesized that the interaction between kiss signaling, and microglia plays a fundamental role in the development of sex-specific behaviors. For the first time, we showed that rodent microglia express the kisspeptin receptor (Kiss1R). Additionally, disruption of the kiss pathway at the neonatal period, at P0 during NGT surge, results in sex-specific differences in microglial morphology in adulthood. Furthermore, we found significant differences on juvenile and adult social behaviour between animals injected at P0 with either, a vehicle or kiss1R antagonist. These findings seem to stablish an association between the influence of kiss1 signalling during the NTS in microglial function and its long-term impacts on social behavior, however, further studies are needed to uncover with precision these mechanisms. In conclusion our research advances the understanding of brain sexual dimorphism and its implications for neuropsychiatric conditions, with potential relevance for individuals with gender dysphoria.

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P-42-002
Compartmentalized cAMP/PKA signaling in retinal ganglion cell survival and axonal outgrowth: The role of mAKAPα and soluble adenylyl cyclase

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Retinal ganglion cells (RGCs), like other neurons in the central nervous system (CNS), die due to the loss of target-derived trophic signals and reduced responsiveness to these signals. Trophic responsiveness can be restored through electrical activity or by increasing intracellular levels of cyclic AMP (cAMP), which activates protein kinase A (PKA). However, the sources of cAMP and the downstream pathways mediating these effects remain poorly understood, limiting therapeutic interventions. A-kinase anchoring proteins (AKAPs) organize cAMP, PKA, and other signaling enzymes into localized multimolecular signalosomes, enabling precise activation of specific PKA pools by individual adenylyl cyclases (ACs). This study investigates the role of cAMP/PKA signaling in RGC survival and outgrowth, focusing on the newly discovered perinuclear cAMP compartment organized by mAKAPα. Using engineered FRET-based biosensors that allow for real-time recording of cAMP/PKA transients with high resolution, we showed a forskolin-induced robust PKA activity at mAKAPα, demonstrating the spatial restriction of cAMP signaling in retinal precursor cells. Immunoblotting of the adult retina showed an interaction between the mAKAPα scaffold and adenylyl cyclases 2 and 5 (AC2, AC5), as well as soluble adenylyl cyclase (sAC, AC10). To examine the role of sAC, we silenced sAC using a recombinant AAV2 and conducted neurite extension assays in primary neurons. Our findings demonstrate that sAC is critical for both baseline and depolarization-induced neurite extension, emphasizing its importance in spatially restricted cAMP signaling. These results identify the perinuclear compartment as a critical site for cAMP/PKA-mediated neurite outgrowth, offering new approaches for the regeneration of injured CNS neurons. Research is supported by the OPUS 22 grant from the National Science Centre (NCN, Poland), grant number 507/6-086-02/507-60-050.

P-42-003
Relationship between inflammation markers and carpal tunnel syndrome

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Carpal tunnel syndrome (CTS) is a disorder that occurs as a result of compression of the medial nerve and causes symptoms such as tightness, weakness or numbness in the hand. CTS is the most common entrapment neuropathy of the upper extremity and affects daily activities, leading to functional limitations. It has been reported that CTS is more common in women and in the 30–50 age group. In this study, the relationship between the systemic immuno-inflammation index (SII), which has been shown as an inflammation marker in various diseases in recent years, and the severity of CTS was investigated. The study included patients and healthy individuals diagnosed with CTS in the electrophysiology laboratory. In this study, 56 patients with CTS and 58 healthy individuals without CTS whose EMG was performed in Lokman Hekim University Neurology Clinic electrophysiology laboratory between January 2023 and December 2023, were included. Among the laboratory markers, C-reactive protein (CRP), hemoglobin, white blood cell (WBC), neutrophil, lymphocyte, monocyte and platelet counts were recorded. NLR = neutrophil count/lymphocyte count, LCR = lymphocyte count/CRP and SII = neutrophil count × platelet count/lymphocyte count were calculated. NLR, LCR and SII index were compared between patients diagnosed as having CTS according to electrophysiologic evaluations and a control group consisting of healthy individuals. $p < 0.05$ were considered statistically significant. We observed that increased CRP, WBC, neutrophil, monocyte, NLR and LCR were risk factors for CTS. In this study, in which the relationship between the NLR, LCR and CTS was evaluated for the first time, inflammation was thought to play a role in the pathophysiology of CTS. It is thought that the results of the study will contribute to the understanding of the inflammatory mechanisms of CTS. Ethics Committee number: 2024174.

P-42-004
Exploring the role of growth hormone secretagogue receptor 1A (GHSR 1A) and γ-melanocyte stimulating hormone (γ-MSH) in obesity regulation

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Obesity is a complex condition developed by genetic and environmental influences. G protein coupled receptors (GPCR) play important roles in obesity development and metabolism. The GPCR growth hormone secretagogue receptor 1A (GHSR 1A) has an important activity that is primarily activated by ghrelin (the hunger hormone) for the regulation appetite, energy balance, and fat storage. It is constitutively active in Gq/11 and Gi coupling, which enables signaling independent of ghrelin binding, and thus regulates mechanisms of hunger, energy expenditure and weight gain mechanisms. The intrinsic activity of GHSR 1A allows it to form homodimers or heterodimers with other GPCRs, influencing neurotransmitter release, appetite control, cell proliferation, and even insulin secretion. In neuropeptide Y (NPY-containing neurons, ghrelin-induced rises in intracellular calcium are dependent on calcium influx through N-type calcium channels, which are activated via the cAMP-PKA pathway following GHSR 1A coupling with Gs. Although existing literature has not documented constitutive Gs-related activity for GHSR 1A, this receptor is thought to be capable of high cAMP-related activity. The literature indicated that the inhibition of the constitutive activity by using inverse agonists like Substance P was helpful in the treatment of obesity and overeating. Furthermore, the gamma melanocortin stimulating hormone (γ-MSH) related cAMP regulation of GHSR 1A was linked to melanocortin receptor 3 (MC3R) dimerization. In this study, we investigated the GHSR 1A activity by using H187 cAMP biosensor with γ-MSH stimulation and showed that GHSR 1A has a high constitutive cAMP activity which may be partially inhibited with γ-MSH. Our findings may broaden the understanding of GHSR 1A in obesity and we suggest that γ-MSH application could be a potential weight gain treatment strategy to rescue GHSR 1A overactivity related dysfunctions. *The authors marked with an asterisk equally contributed to the work.

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P-42-005
Therapeutic potential of intrathecal delivery of human Neu3 sialidase in a Tay-Sachs disease mouse model

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Tay-Sachs disease (TSD) is a severe neurodegenerative disorder due to mutations in the *HEXA* gene encoding the α-subunit of the β-hexosaminidase A enzyme. This enzyme plays a critical role in breaking down GM2 ganglioside. A deficiency in HexA causes excessive accumulation of GM2 and progressive neurodegeneration in individuals with Tay-Sachs disease. Interestingly, the TSD mouse model (*Hexa*-/-) exhibits mild neuropathological symptoms. To better replicate the early-onset Tay-Sachs disease, our group generated *Hexa*-/-*Neu3*-/- mice with a short life of 20 weeks, a model closely mirroring neuropathological and behavioral alterations in humans. While murine sialidase Neu3 promoting GM2 degradation through a "bypass" mechanism has been shown in *Hexa*-/-*Neu3*-/-, the possible contribution of human NEU3 (hNEU3) in GM2 degradation remains unclear. Here, we evaluated the potential of hNeu3-mediated gene therapy by intrathecally administrating AAVrh10-hNeu3 to 8-week-old *Hexa*-/-*Neu3*-/- using molecular biological, immunohistochemical, and behavioral analyses. We found a prolonged life span of *Hexa*-/-*Neu3*-/- up to 28 weeks and improvements in weight and physical appearance. Using immunohistochemical analyses, we showed decreased GM2 accumulation and LAMP1 levels and increasing CNPase levels in 28-week-old *Hexa*-/-*Neu3*-/- mice compared to those of 20-week-old mice in several brain sections. We investigated the higher expression of hNeu3 in 20- and 28-week-old AAVrh10-hNeu3-injected *Hexa*-/-*Neu3*-/- in the cortex and cerebellum. Moreover, rotarod analyses indicated improvement in motor activities of 20-week-old *Hexa*-/-*Neu3*-/- but not at 28 weeks. Our data suggest that if human Neu3 expression is upregulated in Tay-Sachs patients, it might be a potential therapeutic strategy to reduce GM2 ganglioside accumulation. *The authors marked with an asterisk equally contributed to the work.

P-42-006
Arginase 2 maintains polyamine homeostasis and mitochondrial integrirty in the striatum

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Arginase catalyzes conversion of arginine (Arg) to ornithine (Orn) and is involved in several metabolic pathways, like Arg-dependent nitric oxide (NO) synthesis and Orn-dependent polyamine (PAs) synthesis. Arg2 is the major cerebral arginase isoenzyme, but still little is known about its distribution and functions in the brain, although it was recently shown that its loss in the striatum precedes development of pathology in Huntington's disease (HD) models. We found that striatum is the most Arg2-enriched brain structure from among 18 major brain regions analyzed by us. Our recent study (Nalepa M et al. (2024) Neurochem Int 182, 105907) showed that striatal Arg2 is exclusively expressed in the medium spiny neurons (MSNs; a neuronal population specifically affected in HD), suggesting particular importance of Arg2 for these cells. To reveal the role of striatal Arg2, we used $Arg2^{-/-}$ mouse line, where we analyzed striatal metabolic pathways related to this enzyme. Although Arg2 loss resulted in significant accumulation of Arg, unexpectedly it didn't affect Orn content, suggesting existence of a compensatory mechanism to maintain physiological levels of Orn. It didn't however protect the striatum from disturbances in polyamine synthesis pathway, as evidenced by altered putrescine and spermine levels. Importantly, Arg2 loss didn't affect NO content, ruling out the idea that striatal Arg2 could negatively regulate NO synthesis by competing with NO synthase for Arg. Proteomic analysis of $Arg2^{-/-}$ striatum revealed changes in mitochondrial proteins, including some subunits of respiratory complexes. Further analyses of striatal mitochondria revealed ultrastructural changes and disruption in respiratory chain complex assembly due to Arg2 loss. These findings reveal significant role of Arg2 for striatal metabolism and mitochondrial integrity, suggesting that Arg2 loss observed in HD may potentially contribute to specific sensitivity of MSNs to degeneration in this disease.

P-42-007
Unlocking brain rejuvenation: the role of partial reprogramming in astrocyte biology and aging

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The mammalian brain faces significant challenges in regeneration and maintenance, particularly with aging and the onset of neurological disorders. In our study, we leverage reprogramming technologies, focusing on transient expression of the Yamanaka Factors (4F) to rejuvenate neural cells and restore brain homeostasis. Aging disrupts this equilibrium, triggering neuroinflammation, cognitive decline, and epigenetic alterations. We investigate the transformative potential of partial reprogramming (PR) on astrocytes—key regulators of central nervous system homeostasis with pivotal roles in neuroprotection, immune modulation, and age-related processes. Our work delves into the mechanisms of PR by evaluating changes in age-associated epigenetic markers, cellular metabolism, and nuclear envelope integrity within astrocytes. Early results show that PR not only reshapes the epigenetic landscape but also fosters cellular rejuvenation. *In vivo*, these changes translate to measurable improvements in motor function, spatial memory, and recognition memory, highlighting the systemic benefits of this intervention. By integrating molecular insights with functional outcomes, our study illuminates how partial reprogramming could redefine therapeutic strategies for aging and central nervous system disorders, offering a groundbreaking path toward mitigating the effects of brain aging.

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P-42-008
Microwave-assisted gelatin methacryloyl hydrogel (GelMA): a reliable extracellular matrix for brain organoid generation

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Human brain organoids have emerged as a crucial instrument to investigate neurobiology. Though three primary prerequisites [stem cell, extracellular matrix (ECM), growth factor] are adequate for organoid generation, dependence on commercial basement membrane from Engelbreth-Holm-Swarm mouse sarcoma (Matrigel) presents a considerable drawback in global organoid production. The non-human origin of this ECM demonstrates considerable batch-to-batch variability, causing more unreliable experimental outcomes. This study compared Matrigel with microwave-assisted gelatin methacryloyl hydrogel (GelMA) to cultivate embryonic stem cell-derived organoids. The study modified gelatin, a degraded form of collagen, using methacrylation, employing lithium phenyl-2-4-6-trimethylbenzoylphosphinate and UV light to crosslink the hydrogel. Neuroepithelial tissues were incorporated into either Matrigel or GelMA. Tissue development was observed on multiple ECMs over 35 days, with morphological variations, including increases in diameter and budding formation, documented via light microscopy. Scanning electron microscopy, immunofluorescent microscopy, and Raman spectroscopy were performed after maturation. Light microscopy comparisons indicate that GelMA facilitates neural tissue development and expansion as effectively as Matrigel. The findings from IF labeling utilizing SATB2 (superficial layer neuron), Sox2 (neural progenitor) and Tau (axonal expansion) indicate that GelMA promotes neural growth and axonal expansion more effectively than Matrigel. Nonetheless, proportions of cell types inside each ECM varied. Raman spectroscopy revealed that two hydrogels exhibited axonal peaks (□1650 cm^{-1}) and neural differentiation peaks (□811 cm^{-1} and □1450 cm^{-1}) compared to undifferentiated tissue. The findings indicate that GelMA can function as an alternative ECM for development of brain organoids. This work was supported by the Abdullah Gul University Scientific Research Foundation (FDK-2022-164).

P-42-009
Presepsin in CSF of a critically ill patient with cerebral damage

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There is a problem in differentiating sepsis from non-infectious systemic inflammatory response syndrome (SIRS) in many critically ill patients at the early stage which can lead to inappropriate treatment and therefore worse outcomes. The soluble CD14 subtype (sCD14-ST, presepsin) is generated as the body's response to bacterial infection and its level could significantly increase in patients with sepsis and septic shock. Besides sepsis, blood sCD14-ST is predominantly elevated in patients with cardiovascular diseases, liver cirrhosis, or high blood glucose, and its level is increased in patients with osteoarthritis in synovial fluid. Presepsin in cerebrospinal fluid (CSF) is specific for nervous tissue and, therefore should have better diagnostic and prognostic value for patients with the other comorbidities. Our perspective observational study dated from 2024/6 to 2025/4 focuses on critically ill patients with any injury of the central nervous system (trauma, tumor, hemorrhage including subarachnoid or infection) who have inserted drainage system (external ventricular or lumbar drain). The main aim is to determine the role of CSF presepsin in the context of, SIRS, sepsis, early postoperative meningitis, or subarachnoid hemorrhage in patients with disrupted autonomic regulation and malpresenting clinical and biochemical signs of infections. CSF samples are taken every 36 hours (4 samples of each patient), immediately centrifugated, and supernatant is stored at -80°C until ELISA is performed. Presepsin could be a useful CSF marker of central nervous system infection or sepsis with nervous origin in critically ill patients whose diagnosis might be malicious. Therefore, targeted therapy can be administrated sooner and improve the outcome of patients.

P-42-010
Graphene nanoplatelet-based approach to boost neuronal differentiation of human bone mesenchymal stem cells

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Graphene nanoplatelets (GNPs) offer enormous potential in neural tissue engineering with their exceptional electrical conductivity and biocompatibility. This study investigates the effect of GNPs on the neuronal differentiation of human bone marrow mesenchymal stem cells (hBMSCs). Cells were cultured with GNP-coated surfaces at varying concentrations (0.1, 0.2, and 0.4 μg/ml) in the presence of gelatin. Cytotoxicity assays demonstrated that lower concentrations of GNPs maintained high cell viability, while higher concentrations were detrimental over time. Neuronal differentiation was assessed through morphological analysis, RT-qPCR, and immunocytochemistry for key neuronal markers (MAP2, Nestin, and Tuj1) on days 4 and 7. GNPs significantly enhanced the expression of neuronal markers, with the 0.4 μg/ml GNP coating exhibiting the highest MAP2 and Tuj1 expression. Calcium imaging further confirmed increased neuronal activity, supporting the functional maturation of differentiated neurons. These findings highlight the promising role of GNPs in advancing stem cell-based strategies for neurodegenerative disease modeling and therapeutic applications. Acknowledgment: This research was supported by TUBITAK with grant numbers: 121N158 and 22AG004 and EMBO Installation grant: IG-5352-2023.

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P-42-011
The roles of salt inducible kinase-2 (SIK2) on secretion of the hypothalamic proopiomelanocortin (POMC) neurons

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Hypothalamic obesity is a multi-comorbidity disorder. Dysregulation of neuroendocrine signaling, inflammation, and metabolism contributes to its complex pathophysiology. The hypothalamic arcuate nucleus serves as a primary regulator of energy homeostasis via two counteracting neuronal groups, namely Agouti-related protein (AgRP) and Proopiomelanocortin (POMC) expressing neurons secreting neuropeptide, αMSH. Salt inducible kinase-2 (SIK2) has been implicated in regulating endoplasmic reticulum (ER) stress and autophagy under nutritional stress conditions. Intriguingly, systemic SIK2 knockout mice exhibited a reduction in food intake and energy expenditure, accompanied by dysregulated metabolic parameters, suggesting a potential relationship between SIK2 activity in POMCs and the observed phenotype. In our study, we conducted comparative secretomics in SIK2 overexpressing and knockdown POMCs under ER stress conditions. We suggested that under physiological stress conditions, SIK2 may act as a physiologic switch-like module for the secreted inflammatory factors and signaling molecules critical for cellular survival and energy homeostasis [Türküner, MS et al. (2024) Cells 13, 1565]. In recent follow-up studies, we confirmed that SIK2 regulates secretion of significant therapeutic target neuropeptides such as GDF-15, CX3CL1, APP, and αMSH, which supports the phenotype of systemic SIK-KO mice. We also proposed that SIK2 may regulate POMC processing and hence αMSH secretion. According to our preliminary data, αMSH accumulates in the ER in response to pharmacological ER stress induction, consistent with the concomitant decrease in the Golgi. SIK2 appeared to negatively regulate the secretion of αMSH independently of ER stress induction. Consequently, we are planning to test our hypothesis in vivo settings using a diet-induced obese mouse model.

P-42-012
Incensole acetate improved insulin signaling impairment in an experimental Alzheimer's disease model

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Alzheimer's disease (AD) is one of the most prevalent types of dementia worldwide. Main pathobiological markers of the AD are intercellular amyloid beta plaques and intercellular neurofibrillary tangles. Insulin signaling pathway is impaired in AD which cause neuronal and cognitive dysfunction. In the meantime, type 2 diabetes mellitus is the most frequently observed comorbidity of AD. Natural bio-compound incensole acetate (IA) derived from *Boswellia* plant showed neuroprotective effects in *in vitro* and *in vivo* studies. In this study, bone marrow-derived mesenchymal stem cells (BM-MSCs) were differentiated into neurons and by using an amyloid beta (Aβ) peptide experimental AD model was established within these neurons. Next, the treatment dose of IA was determined by MTT [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide] assay. Expression levels of insulin signaling pathway genes at the RNA and the protein levels were determined by qRT-PCR and western blot. Aβ, SIRT1, SIRT3, SIRT6, NFκB and NRF2 expressions were analyzed by western blot while PPARα expression was assessed by immunofluorescent staining. Statistical analysis was conducted using GraphPad Prism Version 5.01 program. One-way analysis of variance (ANOVA) followed by Tukey post hoc test was used to determine the significance between groups. IA treatment decreased the Aβ protein level significantly and caused insulin signaling cascade activation by increasing INSR, IRS1, AKT and AS160 expressions. Furthermore, SIRT1, SIRT3, SIRT6, NRF2 and PPARα expressions were enhanced upon IA treatment, while elevated NFκB level due to amyloid beta toxicity was brought to basal level following IA administration. Consequently, according to our results, incensole acetate attenuated Aβ aggregation, insulin resistance, inflammation and oxidative stress *in vitro* which highlights its potential for being a candidate molecule. Moving forward, additional studies may shed light on other neuroprotective mechanisms of IA in various signaling pathways in the AD models.

P-42-013
Hemorphin regulates pro-inflammatory cytokine levels in experimental Parkinson's disease

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Hemorphins, the hemoglobin-derived endogenous nonclassical opioid peptides, have been shown to diminish inflammation through regulation of calcineurin activity. Our *in vivo* studies demonstrated potential neuroregulatory efficacy of LVV-hemorphin-3 (LVVYPW, LVV-H3) in calcineurin-mediated neurodegeneration in Parkinson's disease (PD). The aim of this study was to evaluate the regulatory effect of LVV-H3 on pro-inflammatory cytokine IL-2 and TNFα levels in the brain, thymus and spleen exploring rotenone-induced experimental PD in albino rats. The cytokine levels were detected by ELISA method. Chronic rotenone injections significantly increased IL-2 levels in the brain (39.5%), thymus (51%) and spleen (46%) compared to controls. The results showed that synthetic LVV-H3 treatment (1 mg/kg, i.p.) significantly reduced IL-2 level by 26%, 27%, and 23% in the brain, thymus and spleen, respectively, compared to rotenone group ($p < 0.05$). Various data were obtained for TNFα. Chronic rotenone injections led to significant decrease of TNFα levels in the brain (28.5%) and spleen (46%), but substantial elevation of TNFα in thymus (54%) compared to control. LVV-H3 was shown to regulate these changes, restoring TNFα level in the brain and spleen up to 36% and 38%, respectively, while reducing TNFα level in thymus (53%) compared to rotenone group ($p < 0.05$). These data indicate that pro-inflammatory cytokines increase in response to the rotenone exposure in the brain and peripheral tissues. The elevated cytokines IL-2 and TNFα from thymus and spleen may cross the disrupted blood–brain barrier in PD and contribute to further

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inflammatory responses in CNS. Although divergent, but LVV-H3 effects on IL-2 and TNFα levels indicate that regulation of these cytokines by hemorphin is essential to alleviate inflammation and diminish neurodegeneration in PD.

P-42-014
Effect of nicotine on the energy metabolism of substantia nigra cells in MPTP-induced Parkinson's disease

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Parkinson's disease (PD) is a progressive neurodegenerative disorder that affects millions worldwide and remains one of the incurable neurodegenerative diseases despite extensive research. The neurotoxin MPTP serves as an effective model for PD by inhibiting the first complex of the mitochondrial respiratory chain. Conversely, nicotine, the principal alkaloid found in tobacco, has been associated with neuroprotective effects against neurodegenerative diseases, including Parkinson's disease, although the underlying molecular mechanisms remain poorly understood. We investigated the effects of nicotine on the energetic metabolism of substantia nigra cells by assessing the activity of glycolytic enzymes, Krebs cycle enzymes, and respiratory chain enzymes, as well as measuring mitochondrial and cytosolic creatine kinase activity, ATP levels, mitochondrial permeability transition pore (mPTP) activity, and the PI3K-AKT-mTOR signaling pathway. Our results indicate that nicotine administration corrected the energetics deficiency induced by MPTP, leading to improved mitochondrial function markers and increased ATP levels. These effects may be attributed to the activation of the PI3K-AKT-mTOR signaling pathway, which is inhibited by MPTP. This study highlights the potential of nicotine as a neuroprotective agent in the context of Parkinson's disease. *The authors marked with an asterisk equally contributed to the work.

P-42-015
Synergistic neuroprotection of GLP-1 and adiponectin signaling in dementia with Lewy bodies (DLB)

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Dementia with Lewy bodies (DLB) is a neurodegenerative disorder characterized by pathological α-synuclein aggregation. While amyloid β (Aβ) has long been associated with Alzheimer's pathology, evidence suggests its role in initiating or exacerbating α-synuclein aggregation, contributing to neuronal dysfunction and apoptosis. Given the links between insulin signaling deficits and DLB progression, glucagon-like peptide-1 receptor agonists (GLP-1 RAs) have been investigated for their neuroprotective potential. Additionally, adiponectin signaling has gained attention for its regulatory role in metabolic and inflammatory pathways. Studies indicate that adiponectin enhances neuronal survival, reduces oxidative stress, and facilitates autophagic clearance of toxic protein aggregates in neurodegenerative diseases, including DLB. This study explores the neuroprotective effects of semaglutide (a GLP-1 RA) and adipoRon (an adiponectin agonist) using cellular and animal models of DLB. In HT-22 hippocampal neuronal cells, α-synuclein overexpression and Aβ exposure induced neurotoxicity. Treatment with semaglutide mitigated these effects by reducing apoptotic markers and limiting α-synuclein aggregation. The combination of semaglutide and adipoRon showed a synergistic effect, further promoting cell survival and enhancing autophagic activity. This neuroprotective synergy was partially mediated through activation of the AMP-activated protein kinase (AMPK) signaling pathway. *In vivo*, semaglutide reduced α-synuclein accumulation in the CA2-CA3 hippocampal region and improved cognitive function in DLB rats, with adipoRon amplifying these benefits. These findings highlight the potential of GLP-1 and adiponectin-based therapies in mitigating neurodegenerative processes. Future research should optimize combinatorial strategies to develop viable, personalized therapeutic interventions for DLB and related neurodegenerative disorders. *The authors marked with an asterisk equally contributed to the work.

P-42-016
Study of the N-glycosylation of the cannabinoid type 1 receptor N terminus

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The cannabinoid type 1 receptor is the most abundant GPCR in the central nervous system. It takes part in the regulation of multiple processes such as memory formation, motor control, or analgesia, making it a potential therapeutic target for treating diseases such as anxiety or multiple sclerosis pain. CB1 is a class A GPCR 472 amino acids long, from which 111 constitute the N terminus. Such a long N terminus makes it difficult to translocate to the endoplasmic reticulum lumen, a necessary step for the receptor to acquire a correct structure. When translocated, the N terminus can undergo several post-translational modifications, such as the canonical N-glycosylation of N77 and N83, important for proper receptor functioning, stabilizing its structure, and promoting traffic toward the plasma membrane. CB1 N terminus translocation takes place *in vivo* without a signal peptide, while it has been described that it is necessary *in vitro*. This study has characterized the N-glycosylation state of CB1 *in vitro* and the role each modified residue has regarding receptor functions. Briefly, it has been validated, as described in the bibliography, the need for a signal peptide *in vitro* to achieve translocation of the N-terminus by immunofluorescence and electrophoretic mobility assays after pharmacological and enzymatic deglycosylation. In addition, it has been determined that CB1 only possesses two N-glycosylation sites occupied *in vitro* by deglycosylation assays on N-glycosylation site mutants. Furthermore, it has been observed that N-glycosylation in N83 is important for signaling via ERK 1/2.

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P-42-017
Growth hormone strongly induces hSMN2 promoter driving construct gene expression in mammalian cells

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Spinal muscular atrophy (SMA) is the second most common neurodegenerative disease caused by the absence or insufficient of the SMN (survival motor neuron) protein. There are two genes responsible for the SMN protein: telomeric *SMN1*, which produces 100% functional protein, and centromeric *SMN2*, which produces only 10% functional protein. Deletion or mutation on *SMN1* gene results in insufficient and abnormal protein production and the disease manifests itself. *SMN2* copy number is known to have an impact on the clinical severity of the disease. In this study, we examined the effect of growth hormone (GH) on *SMN2* promoter activity using reporter in cell culture. Three different sizes of DNA fragments from the promoter region of the *SMN2* gene were used to show how gene expression of the reporter construct responds to GH in this study. The first region is 588 bp, second region is 1.036 bp and the third region is 1.705 bp from transcription site of *SMN2* gene, respectively. These fragments were amplified by PCR with specific *SMN2* primers. *SMN2* promoter PCR products were inserted into the *p*GL3 reporter vector with T4 DNA ligase and the ligation products were transformed into ampicillin plates. Positive colonies containing inserts were identified by direct colony PCR using vector primers. *SMN2* promoter inserts in the plasmids were confirmed by PCR with *SMN2*-specific primers and the plasmids were transfected into CHO cells. After transfection, CHO cells expressing *SMN2* promoter driving luciferase gene were treated with growth hormone for 24 hours and then luciferase activity of CHO cells was measured. All reporter constructs containing *SMN2* promoters responded to GH. GH induced the highest level of gene expression for the reporter construct containing the 1.036 bp *SMN2* promoter. However, GH suppressed gene expression of 1.705 bp *SMN2* promoter vector when compared to the gene expression of reporter plasmid alone with GH. GH could be a candidate for the treatment of SMA disease.

P-42-018
Isomerization of beta-amyloid enhances its transport across the blood–brain barrier

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Alzheimer's disease (AD) is the most common neurodegenerative disease. One of the hallmarks of AD is the accumulation of beta-amyloid peptide (Aβ). Aβ undergoes post-translational modifications, the most abundant of which is the isomerization of the Asp7. This modification makes Aβ to be more toxic and more prone to aggregate. Aβ can enter the brain from circulation by binding to RAGE (receptor for advanced glycation end-products) and trigger the pathology of AD. It is known that 90% of Aβ in blood serum is bound to albumin. However, the transport of isomerized Aβ across the blood–brain barrier (BBB) and the role of albumin in this process have not been previously studied. Here we characterized the transport of unmodified ($Aβ_{42}$) and isomerized Aβ (isoD7-$Aβ_{42}$) in the absence and presence of albumin across the BBB. We used brain endothelial cells seeded on transwell membrane as an *in vitro* model of the BBB. Transport of Aβ isoforms was assessed using ELISA. Various inhibitors were used to characterize Aβ transport mechanisms across the BBB. The Aβ isoforms' interaction with RAGE were determined using microscale thermophoresis. IsoD7-$Aβ_{42}$ was found to cross the BBB better than unmodified $Aβ_{42}$. Transport of $Aβ_{42}$ was dependent on caveolin, and transport of isoD7-$Aβ_{42}$ was dependent on both caveolin- and clathrin-mediated endocytosis. IsoD7-$Aβ_{42}$ bound to RAGE an order of magnitude weaker than $Aβ_{42}$. Albumin enhanced transport of both Aβ peptides across the BBB. Complexes of $Aβ_{42}$ and isoD7-$Aβ_{42}$ with albumin were detected in the medium after passing through the BBB endothelium and in cell lysates. Thus, isomerization of Aβ increases the rate of its transport across the BBB. We first showed an alternative and more effective albumin-dependent transport mechanism of Aβ peptides through the BBB, which may be important for the pathology and treatment of AD. Supported by the Ministry of Science and Higher Education of the Russian Federation (Agreement 075-15-2024-530).

P-42-019
L-Arginine as a potential antidepressant: effects on cognitive function and antioxidant enzyme activity in depressed rats

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Depression is a well-established problem in the modern society. Nowadays, there are several antidepressant treatment strategies and several medications, including serotonin reuptake inhibitors. Therefore, rising evidence suggests that many biologically active substances might also have a positive impact on depression. Our attention was drawn to the amino acid L-arginine (Arg), an essential amino acid participating in various pathways. Experiments were conducted on Wistar male rats (80–100 g), and the depression was induced by 14 days of administration of corticosterone (20 mg/kg). Open-field, forced swim and tail suspension tests evaluated cognitive and emotional parameters. Biochemical markers were determined through spectrophotometric measurements. We observed that the daily supplementation of Arg (150 mg/kg) in CORT-induced depression for 14 days significantly changed specific parameters. Notably, there was an improvement in cognitive functions and the levels of serotonin and dopamine, which were reduced due to depression, in the prefrontal cortex and hippocampus. To investigate the effects of dietary Arg against oxidative stress, we studied quantitative changes in hydrogen peroxide and malondialdehyde. Markedly, depression caused an increase in those markers, which was reliably reduced by Arg intake. Along with that, there was an improvement in depression-induced decline in mitochondrial superoxide dismutase and catalase activities. Kinetical analyses suggest that Arg's positive effect isn't caused by the enzymes' structural changes but by intensifying biosynthesis. In summary, supplementation of Arg had a significant positive effect on depression-related upregulation of oxidative processes. Therefore, the exact molecular mechanisms provoking those changes are still unclear and need further investigation. The designated project has been implemented with the financial support of the Shota Rustaveli Georgian National Science Foundation (Grant #FR-23-7525). *The authors marked with an asterisk equally contributed to the work.

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P-42-020
Characterization of the palmitoylation of cannabinoid CB1 receptor

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Cannabinoids have become a promising therapeutic strategy for many diseases, including inflammatory, neurodegenerative diseases or cancer. However, side effects related to the complex signaling of Cannabinoid Receptor type 1 (CB1R) must be addressed. Cannabinoids bind to CB1R in the central nervous system, modulating the endocannabinoid system. Despite its canonical signalling pathway involving PKA inhibition, CB1R exhibits pleiotropic actions influenced by cellular context and receptor conformation. Palmitoylation is a common post-translational modification for GPCRs that involves the addition of palmitic acid to cysteine residues. In humans, this reaction is catalyzed by 23 different palmitoyl acyl transferases with a conserved DHHC motif in the catalytic domain. Palmitoylation can stabilize the protein in ordered membrane domains, assist their traffic to the plasma membrane, or alter its interactome, changing the signaling and effects of ligands. Thus, in this work we decided to characterize CB1R palmitoylation. Using co-immunoprecipitation, we identified specific CB1R interactors among the 23 DHHC enzymes. DHHCs 1, 3, 5 and 8 were found to be specific interactors. Moreover, the interaction of DHHC3 was limited to the carboxyterminal domain of CB1, where the three putative palmitoylation sites are located. We also studied the effects of different CB1R palmitoylation states on cellular functions using CB1R mutants unable to be palmitoylated at certain residues. These mutants showed differences in the ERK activation pathway and DMR experiments related to the wild-type CB1R. Furthermore, DHHCs 3, 5 and 8 were found to increase the DMR signal of CB1R signaling when co-expressed with the receptor. Our results aim to establish an interaction map that shows what mechanisms are responsible for CB1R palmitoylation and the effects of these modifications in the cellular signalling. This knowledge could help to potentiate cannabinoids beneficial actions and try to avoid harmful ones.

P-42-021
Assessment of depression-like behaviors and depression-related biomarkers in a corticosterone-induced mouse model of depression

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The pathophysiology of depression remains unclear, but hypotheses involving hypothalamic-pituitary-adrenal (HPA) axis dysregulation, impaired neuroplasticity, and immune dysfunction are prominent. This study aimed to investigate depression-like behaviors and their mechanisms by examining neuroplasticity and neuroinflammatory markers in a corticosterone-induced depression model that mimics HPA axis hyperactivity. Male mice received corticosterone (20 mg/kg, subcutaneously) daily for 21 days. Depression-like behaviors were assessed using the sucrose preference test (SPT), forced swim test (FST), and tail suspension test (TST), while anxiety-like behaviors were evaluated through the open field test (OFT). Neuroplasticity markers (BDNF, PSD-95, synapsin 1) and neuroinflammatory markers (HMGB1, S100B, NLRP3, TNF-α, IL-1β) were measured in hippocampal tissue using western blotting. Corticosterone-treated mice exhibited depression-like behaviors, evidenced by increased immobility in the FST ($p = 0.068$) and TST ($p = 0.019$), as well as decreased sucrose intake in the SPT ($p < 0.001$). These mice showed anxiety-like behaviors, characterized by reduced center entries ($p = 0.046$) and prolonged starting latency ($p = 0.043$) in the OFT. Corticosterone reduced neuroplasticity marker expression, including BDNF ($p = 0.036$), PSD-95 ($p = 0.002$), and synapsin 1 ($p = 0.005$), in the hippocampus. Conversely, sterile inflammation-related markers such as HMGB1 ($p = 0.001$), S100B ($p = 0.026$), and NLRP1 ($p = 0.007$) were elevated. Additionally, IL-1β level increased ($p = 0.035$), while TNF-α level showed no significant changes. These results suggest chronic corticosterone induces hippocampal dysfunction by reducing neuroplasticity and enhancing sterile inflammation. These findings support that the corticosterone-induced depression model is a useful tool for investigating antidepressant candidates that target neuroplasticity and neuroinflammatory pathways.

P-42-022
Casticin increases tumor necrosis factor-α-induced protein 8-like 2 expression in lipopolysaccharide-stimulated microglial cells

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Microglia are the myeloid lineage cells that play important roles in homeostasis, inflammatory responses, and tissue repair in the brain. Microglial activation plays a central role in neuroinflammation which is associated with neurodegenerative diseases. Tumor necrosis factor-α-induced protein 8-like 2 (TIPE2), a member of the TNFAIP8 family, maintains immune homeostasis and is an important negative regulator of inflammation. Flavonoids are naturally occurring polyphenolic compounds that are known to have neuroprotective effects. Casticin is a polymethoxyflavone which has been shown to have anticancer, antioxidant, and anti-inflammatory activities. In this study, we aimed to investigate the effect of casticin on TIPE2 expression in the lipopolysaccharide (LPS)-stimulated microglial cells *in vitro*. N9 mouse microglial cells were treated with 0.1, 0.5, and 1 μM of casticin for 24 h, and cell viability was analyzed using WST-1 assay. N9 cells were pretreated with 0.1 and 0.5 μM of casticin for 1 h and then incubated with 1 μg/mL of LPS. TIPE2 mRNA expression was analyzed by qPCR, and protein expression was examined by western blotting. 0.1 and 0.5 μM of casticin had no effect on cell viability, whereas 1 μM of casticin reduced viability. TIPE2 mRNA expression levels decreased at 3,

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6, and 24 h after LPS incubation. 0.5 μM of casticin increased LPS-induced decreases in TIPE2 mRNA levels at 24 h. TIPE2 protein expression levels also decreased at 2, 4, 8, and 24 h after LPS incubation. 0.5 μM of casticin increased LPS-induced decreases in TIPE2 protein levels at 24 h. Our results demonstrated that casticin could modulate inflammation by increasing TIPE2 expression in microglial cells, suggesting its neuroprotective potential in neuroinflammation.

P-42-023
Proteomic analysis of citicoline's impact on mitochondrial and oxidative stress in SH-SY5Y cells

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Neurodegenerative diseases are closely linked to oxidative stress and mitochondrial dysfunction. Citicoline (CDP-choline) is a neuroprotective agent known to support membrane stability, enhance mitochondrial function, and reduce oxidative damage. This study evaluated citicoline's protective effects in SH-SY5Y cells exposed to hydrogen peroxide (H_2O_2) and Antimycin-A. SH-SY5Y cells were treated separately with H_2O_2 (500 μM) and Antimycin-A (10 mM) to induce oxidative and mitochondrial stress, respectively. Cells were also treated with citicoline alone or in combination with each stressor. Optimal dosages were determined using the WST-1 assay, targeting 80% cell viability. LC-MS/MS proteomic analysis with bioinformatics tools was conducted to identify key proteins and pathways impacted by citicoline. Proteomic analysis revealed distinct expression patterns in response to H_2O_2 and Antimycin-A, particularly in pathways linked to mitochondrial function, oxidative stress, and cellular repair. Citicoline treatment partially restored protein expression profiles, suggesting improved cellular homeostasis. Key proteins involved in mitochondrial integrity, antioxidant defense, and cytoskeletal stability showed enhanced expression in citicoline-treated groups. These findings suggest that citicoline protects neuronal cells by modulating protein expression patterns linked to mitochondrial function and oxidative stress regulation. This highlights citicoline's potential as a therapeutic agent for neurodegenerative conditions. Further research is warranted to confirm these protective mechanisms.

P-42-024
The effect of Class IIa HDACs (HDAC4 and HDAC5) on primary hippocampal neuron survival

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Optic nerve injury due to ischemic stroke or other retinopathies may cause permanent blindness due to RGC axon damage and retrograde cell death. In our research, we investigate the hypothesis that class IIa HDAC-mediated repression of the transcription factor MEF2A promotes neurosurvival and neuroprotection in stroke providing new insight into the mechanisms underlying the pathophysiology of ischemic injury. Class IIa HDACs when localized to the nucleus bind MEF2 transcription factors on chromatin and mediate gene repression. Primary neuronal cultures derived from rat e18 embryos were tested for the impact of MEF2A and class IIa HDACs – HDAC4 and HDAC5 – for neurosurvival and axon growth as a model for axon regeneration. Confirming our hypothesis, nuclear localization of HDAC4 and HDAC5 via inhibition of MEF2A-dependent gene expression promoted neuronal survival and axon outgrowth in primary neurons. Inactivation of MEF2A, overexpression of HDAC4 and HDAC5 wild-type and constitutively cytosolic mutants did not show any changes of axonal growth. Nonetheless, constitutively nuclear HDAC4/5 mutants promote survival and neurite extension *in vitro* in the absence of growth-promoting stimuli such as KCl. Additionally, expression of apoptosis and axonal growth-related genes and were changed for constitutively nuclear HDAC4/5 mutants. These results confirmed the crucial role of histone deacetylases in neurosurvival and could be a basis for novel gene therapy with use of HDAC4/5 mutants for retinal ischemic disease and white matter stroke.

P-42-025
Abstract moved to TALKS SpT-05.3-5.

P-42-026
Identification of key biological pathways and genes in multiple sclerosis via integrating domain knowledge into the machine learning model

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Multiple sclerosis (MS) is a complex autoimmune disease affecting the central nervous system. Although genetic and environmental factors, and lifestyle choices play a significant role in its development, the precise underlying mechanisms remain unclear due to its multifactorial nature. In this study, to identify affected molecular pathways and ontology terms in MS, machine learning analyses that incorporate biological domain knowledge are performed on publicly available RNA sequencing data (GSE138614 dataset). To this end, GeNetOntology and PriPath tools utilize Gene Ontology (GO) and KEGG pathways as external knowledge. Model performance was evaluated using Monte Carlo cross-validation across 10 iterations. Key dysregulated GO terms and KEGG pathways in MS patients were identified, demonstrating strong associations with disease progression. The most significant GO terms included adaptive immune response, leukocyte-mediated immunity, blood microparticle, immunoglobulin complex, and antigen binding and signaling receptor binding. The most influential KEGG pathways were hsa04080 (Neuroactive ligand-receptor interaction), hsa04060 (Cytokine-cytokine receptor interaction), hsa04151 (PI3K-Akt signaling pathway), hsa04672 (Intestinal immune

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network for IgA production), and hsa04926 (Relaxin signaling pathway), while *GIP*, *RXFP4*, *CCR4*, *IL32*, and *GABRR1* identified as key genes. The GeNetOntology tool achieved high predictive performance, with an AUC of 1.0 using 55 genes in the GO-BP category; 0.956 using 58.2 genes in the GO-CC category, and 0.95 using 48.3 genes in the GO-MF category. PriPath tool yielded an AUC of 0.88 using 11.1 genes. The machine learning models generated by GeNetOntology and PriPath tools successfully identified biologically relevant KEGG pathways and gene ontology groups associated with MS. These findings enhance the molecular understanding of MS and provide a foundation for future research and biomarker discovery. This study was funded by TUBITAK 124Z081 *The authors marked with an asterisk equally contributed to the work.

P-42-027
Genistein as a natural therapy for Alzheimer's disease: a promising molecule that ameliorates learning and memory deficits in three different mouse models

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Alzheimer's disease (AD) is the most common cause of dementia, characterized by impairment of cognitive processes as a result of progressive neurodegeneration, which is accompanied by the formation of non-physiological forms of proteins capable of aggregation and resistant to the action of proteolytic enzymes and disturbances of signal transduction pathways. According to the American Alzheimer's Society, dementia already affects 55 million people worldwide and by the middle of the 21st century the number of sufferers may exceed 152 million. In Europe, the number of patients with dementia is predicted to exceed 14 million by 2050. Despite over 100 years of research, there is still no effective drug that stops the progression of the disease or reverses the effects of neurodegenerative changes. Therefore, the aim of the present study was to verify the therapeutic potential of genistein in reducing cognitive impairment in AD. The experiments were conducted on mice of both sexes that are genetically different models of AD (5xFAD, THY-Tau22, and 3xTg-AD). Analysis of cognitive processes in genistein-supplemented and untreated animals was carried out using tests that examine different types of memory (Morris water maze, elevated plus-maze test and novel object recognition test) and the microscopic verification of a reduction in the number of amyloid beta/p-tau deposits in key brain structures for memory processes (septum and hippocampus). The obtained results confirm the therapeutic potential of the tested isoflavone, especially in the scope of improving long-term and working memory, as well as by reducing the number of protein aggregates in the brain areas crucial for the proper course of cognitive processes. However, the observed effect is sex-dependent, which indicates that sex hormones or differences in the immune response between males and females may be a factor determining the effectiveness of the tested therapeutic agents.

P-42-028
The role of PKA, ERK5, and RSK anchoring to AKAP6 in RGC survival and axon outgrowth

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cAMP-dependent signaling is a critical regulator of retinal ganglion cell (RGC) survival following injury. This signaling is highly compartmentalized due to the formation of localized, multimolecular complexes mediated by A-kinase anchoring proteins (AKAPs). While the role of cAMP compartmentalization in pro-survival signaling is well recognized, few specific compartments outside of dendritic synaptic regulation have been characterized in terms of molecular composition or function in neuronal survival and regeneration. Here, we demonstrate that a perinuclear signaling compartment, organized by the large scaffold protein AKAP6, facilitates RGC survival and axon outgrowth through cAMP, PKA, ERK5, and RSK signaling. Using novel molecular tools to precisely modulate local cAMP levels—monitored via live-cell FRET imaging—we show that disrupting PKA, ERK5, and RSK anchoring to AKAP6 enhances RGC survival *in vivo* following optic nerve crush injury in mice. Our findings identify a previously uncharacterized neuronal compartment that regulates neuroprotection and axon growth. Targeting this signaling hub may offer a therapeutic strategy for optic nerve degeneration.

P-42-029
Identification of insulin immunoreactivity in myenteric neurons and their nitrergic subpopulation in an acute model of type 1 diabetes

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Type 1 diabetes is an autoimmune condition characterized by the destruction of insulin-producing pancreatic cells. However, research has shown that insulin can originate from other cell types beyond β cells, even in neurons within the brain. Despite the functional connection between the pancreas and the gut, the presence of insulin in enteric neurons has not been previously investigated. Notably, enteric neurons, particularly their nitrergic subpopulation, are highly vulnerable to hyperglycemic damage in a gut segment-specific manner, leading to gastrointestinal motility disorders in diabetic patients. The primary objective of our study was to determine whether insulin immunoreactivity is present in total myenteric neurons or specifically within the nitrergic subpopulation of control rats. Additionally, we examined the impact of acute hyperglycemia on insulin-immunoreactive (IR) neurons along different segments of the intestinal tract. One week after inducing hyperglycemia, whole-mount preparations from various gut segments of both control and streptozotocin-induced diabetic rats were analyzed using triple-labeled fluorescent immunohistochemistry for insulin, neuronal nitric oxide synthase (nNOS), and peripherin. In control rats, insulin-IR neurons were detected in the myenteric

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ganglia across all examined gut segments. The lowest proportion was found in the duodenum (4.2 ± 0.7%), while it nearly doubled in the ileum (7.7 ± 0.9%) and colon (8.8 ± 0.1%) compared to the duodenum. Interestingly, in hyperglycemic rats, the percentage of insulin-IR neurons significantly increased in the ileum (15.6 ± 2.1%) and colon (13.6 ± 1.12%), whereas the duodenum exhibited no significant change (6.2 ± 1.1%). Furthermore, the proportion of insulin-nNOS-IR neurons was highest in the colon, with a notable increase observed under hyperglycemic conditions. In conclusion, our findings confirm the presence of insulin-IR myenteric neurons and reveal intestinal segment-specific changes in response to hyperglycemia in a rat model.

P-42-030
Effect of radiofrequency electromagnetic field exposure on oxidant–antioxidant balance and pain related genes in acetic acid-induced pain in zebrafish

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The rapid development of technology has greatly increased the place of electronic devices such as mobile phones and computers in our lives. Exposure to radiofrequency electromagnetic fields (RF-EMF) has become quite common due to the widespread use of mobile phones. The absorption of RF-EMR emitted from mobile phones by cells directly affects living systems and raises concerns about possible diseases that RF-EMR may cause. RF fields can cause harmful effects on tissues due to their ability to move charged particles such as electrons and ions that penetrate tissues. RF-EMFs can cause many important effects on various cellular components, including cell proliferation, DNA damage, and various mutations. In our study, we applied RF-EMF exposure to adult zebrafish for 60 minutes every day for 30 days. The groups included SHAM, RF-EMF, acetic acid (AA) induced pain, and RF-EMF + AA groups. At the end of 30 days, lipid peroxidation, nitric oxide levels and activities of glutathione S-transferase and superoxide dismutase were measured spectrophotometrically. The expressions of genes in brain related to pain mechanism including *cox-1* were measured using RT-PCR. RF-EMR exposure caused alterations in the brain oxidant–antioxidant status and pain related genes in zebrafish. The relationship between pain mechanisms and oxidative stress in the context of RF-EMF exposure is an area of growing interest in neuroscience and biomedical research. Our study has shown that altered oxidant–antioxidant balance in the brain with RF-EMF exposure can affect the expression of pain-related genes. Our research on the relationship between RF-EMF exposure and pain is ongoing and we believe that our study will pave the way for new studies on the harmful effects of RF-EMF.

P-42-031
Multivariate analysis to identify contributing factors and novel risk biomarkers in Alzheimer's disease

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Alzheimer's disease (AD) is the most common dementia and a major cause of mortality due to the lack of therapies and late diagnosis. Identifying risk factors and predictive biomarkers is therefore essential and the aim of this study. Blood-based transcriptomic biomarkers from peripheral blood mononuclear cells (PBMC) or peripheral blood cells (PBC) offer a minimally invasive approach to study systemic alterations, including those occurring in the brain. We analysed physiological and molecular parameters in cognitively healthy controls (n = 21) and patients with AD (n = 16, cerebrospinal fluid biomarker-supported), aged 55-80 years. Cognitive function was assessed with Mini-Mental State Examination (MMSE), Montreal Cognitive Assessment (MoCA), and Cognitive Reserve Index Questionnaire (CRIq). A partial least squares discriminant analysis (PLS-DA) was performed on 192 variables from: biochemical and hematological analyses, body composition, lifestyle questionnaires, and PBMC/PBC gene expression via RT-qPCR. As expected, cognitive scores were lower in the AD group. PLS-DA significantly distinguished the groups with strong performance (R2Y = 0.91, Q2Y = 0.67). Notably, traditional biochemical markers were not among the top variables based on variable importance in projection (VIP) scores. Instead, gait speed was the strongest discriminator, followed by fat and leg muscle mass, omega-3-rich food intake, and PBMC/PBC expression of *TRIB1*, *DNM1L*, *ABCA1*, and *MFN2*. These genes were upregulated in AD vs control and negatively correlated with cognitive tests, with *DNM1L* and *ABCA1* showing strongest associations. *DNM1L* regulates mitochondrial fission, a process impaired in AD; while *ABCA1* regulates lipid metabolism and cholesterol efflux, crucial for neuronal integrity. Thus, traditional blood biochemical analyses may have limited utility in AD prediction, whereas novel markers linked to physical health and PBMC/PBC gene expression could serve as better indicators of cognitive decline.

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P-42-032
Investigating the divergent roles of frataxin: a controlled approach to understanding enzyme activity and iron-sulfur cluster biogenesis

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Frataxin is a small mitochondrial protein whose limited expression impairs Fe-S cluster biogenesis and promotes iron accumulation, leading to the neurodegenerative Friedreich's ataxia. Frataxin is a regulator of the desulfurase activity in the Fe-S cluster biogenesis machinery both in Eukaryotes and Prokaryotes. In this frame, although the human and the bacterial proteins share high homology in their aminoacidic sequence and tridimensional structure, it has been highlighted a discrepancy by *in vitro* studies. While CyaY slows down the kinetics of Fe-S cluster formation on IscU, human frataxin has emerged as an activator of Fe-S cluster biogenesis. It is difficult to clarify this apparent paradox by studying the process *in vitro* where the isolated proteins are in an artificial environment or *in vivo* under non-controlled conditions. To clarify the issue, we have used a new approach to study the IscS activity in controlled settings—like *in vitro* experiments—while also replicating the complex, crowded and confining environment found within cells. We have started this project testing the bacteria IscS desulfurase activity adding different crowding agents such as dextran, PEG 2000/20000 and cell lysate. The enzymatic activity was measured at different crowding agent concentration at single end point of 20 minutes. This gave us the possibility to select the best crowding agent to measure the kinetics parameters such as K_m and V_{max} of the IscS desulfurase and compering them with those obtained in aqueous solution. We could observe changes particularly within the V_{max}. We believe this approach will provide essential information to understand the difference presented by the frataxin homologs in different organisms but can also give rise to a new experimental set up for studying enzymes in a controlled system but closer to their cellular environment. *The authors marked with an asterisk equally contributed to the work.

P-42-033
T cell contributions to brain homeostasis and neurodegenerative diseases through integrative omics approaches

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This study investigates the intricate interplay between aging, neurodegenerative diseases, and the adaptive immune response within the central nervous system (CNS). Utilizing advanced omics techniques, including imaging based spatial transcriptomic technique of MERFISH and single-cell RNA sequencing (scRNA-seq), we aimed to elucidate the precise mechanisms underlying the contribution of T cells to brain homeostasis and neurodegeneration. The ability of MERFISH to investigate T-cells and their impact within the brain has been demonstrated from previous work from our lab [Androvic et al. (2023) Nat Commun 14, 4115] where T-cell types alongside with interferon-responsive (IR) glial states, which have been implicated separately in CNS pathology [Kaya et al. (2022) Nat Neurosci 25, 1446–1457] were identified with spatial context. Through the establishment and utilization of suitable models of aging or neurodegeneration, coupled with comprehensive molecular profiling, we delineated the spatial distribution and transcriptional states of both brain-resident and circulating immune cells. Our findings reveal key molecular mechanisms governing immune responses within the CNS, shedding light on the regulatory pathways that influence T cell specificity, phenotypes, and activity within the brain. This comprehensive understanding provides crucial insights into the genetic basis of neurodegenerative diseases and paves the way for the development of targeted therapeutic interventions and precision medicine approaches.

P-42-034
Trimethylamine-N-oxide (TMAO) as a marker of neuroinflammation in migraine patients

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Migraine is a primary headache disorder with recurrent moderate to severe pain, often unilateral. In recent years, the effects of intestinal microbiota and its metabolites on neurological diseases have received increasing attention. Trimethylamine-N-oxide (TMAO) is a metabolite synthesized by intestinal flora from nutritional compounds such as phosphatidylcholine, carnitine, and betaine. TMAO has been shown to play a role in various pathophysiological processes, especially cardiovascular and neurological disorders. While a neuroinflammatory role is suggested for TMAO on different neurological backgrounds, its possible relationship with migraine pathogenesis have not yet been fully established. The aim of this study is to investigate the potential involvement of TMAO levels, which we suggest may play a role in the pathophysiology of migraine. Sixty migraine patients (n: 52 females; age: 36 ± 11 years) and 46 age-matched controls (n: 40 females; age: 39 ± 8 years) who were admitted to the Neurology outpatient clinic of Lokman Hekim Akay Hospital between December 2023 and December 2024 were included in the study. Blood samples were centrifuged at 1300 g for 10 minutes, and the supernatants were stored at -80°C until analysis. TMAO levels in serum were measured using liquid chromatography-mass spectrometry (LC-MS/MS). Migraineurs and healthy controls did not differ significantly in age, smoking and body mass index (BMI) ($p > 0.05$). When the control group and the migraine group were compared, it was observed that TMAO, carnitine, betaine and choline levels increased significantly in the migraine group ($p < 0.0001$). This study revealed increased TMAO levels in migraine patients, providing evidence for the possible mechanistic involvement of TMAO in the neuroinflammatory cascade associated with migraine. This study was approved by the Lokman Hekim University Scientific Research Ethics Committee (code number: 2023238). This study was supported by TUBITAK Project number 1919B012332600.

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P-42-035
Evaluation of arginine and methylated derivatives in dementia patients

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Dementia, particularly Alzheimer's disease (AD), is a growing health concern due to the aging population. Arginine, an essential amino acid, plays a key role in protein and creatine synthesis and serves as a precursor to methylated derivatives such as asymmetric dimethylarginine (ADMA), symmetric dimethylarginine (SDMA), and N-monomethyl-L-arginine (L-NMMA). These metabolites are shown to be associated with inflammatory diseases such as cirrhosis, diabetes, atherosclerosis, cardiovascular and kidney diseases. This study aimed to investigate the relationship between arginine levels and methylated arginine metabolites in dementia patients. Forty-six dementia patients (n: 39 females; age: 75 ± 5 years) and 37 age-matched controls (n: 28 females; age: 76 ± 5 years) who were admitted to the Neurology outpatient clinic of Lokman Hekim Akay Hospital between December 2023 and December 2024 were included in the study. Venous blood samples were taken and centrifuged at 1300 g for 10 minutes. Supernatants were transferred to Eppendorf tubes and stored at -80°C until the day of analysis. Serum arginine and methylated arginine products of controls and dementia patients were determined by tandem mass spectrometry. Dementia patients and healthy controls did not differ significantly in age, smoking status, and body mass index ($p > 0.05$). When the control group and the dementia group were compared, ADMA, SDMA, arginine, ornithine citrulline levels were significantly decreased in dementia patients ($p < 0.05$). No significant difference was observed in L-NMMA and homoarginine levels ($p > 0.05$). This study shows that ADMA levels are lower in people with dementia, which may contribute to the pathophysiological processes behind cognitive decline and neurodegeneration due to abnormalities in nitric oxide metabolism and endothelial function. This study was approved by the Lokman Hekim University Scientific Research Ethics Committee (code number: 2023235).

P-42-036
Fluoxetine-induced weight change and the role of 5-HT2C receptor modulation in appetite regulation: a study in C57BL/6 mice

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Selective serotonin reuptake inhibitors (SSRI) are one of the most widely used drugs for psychiatric disorders. With the increasing incidence of psychiatric disorder, the usage rates of this drug group are also on the rise. However, beside their clinical benefits, these drugs also cause a range of side effects. One of these adverse effects is weight gain, a critical risk factor, especially considering the increasing prevalence of obesity. That has negative impacts on multiple organ systems and also leads to a significant increase in healthcare costs. Biological mechanisms underlying weight gain associated with SSRIs have not yet been fully elucidated. Our hypothesis suggests that SSRI-induced weight gain is a consequence of these drugs altering expression levels of serotonin 2C receptor (5-HT2C-R) which results in changes in the levels of hypothalamic neuropeptides that regulate eating behavior and energy mechanisms in the brain. Thus, the sensation of satiety is impaired, preventing the proper regulation of food intake and ultimately causing weight gain. This study examined the most commonly used SSRI, fluoxetine, on weight-gain in C57BL/6 male mice. It was also aimed to antagonize fluoxetine effects on the weight-gain using a selective 5-HT2C-R neutral antagonist which can reverse SSRI-induced desensitization of 5-HT2C-R. To examine the combined effect of both SSRI and the neutral antagonist, behavioral tests were given to mice to test their cognitive abilities. Then, the hypothalamic neuropeptides and 5-HT2C-R expression levels were evaluated with RT-qPCR to understand hypothalamic molecular mechanisms in these animals. The findings showed that fluoxetine treatment influenced body weight, though this effect was not altered by the 5-HT2C-R neutral antagonist. Behavioral evaluations suggested possible cognitive impacts of SSRI therapy, while molecular analyses indicated changes in 5-HT2C-R expression and neuropeptide levels linked to appetite control.

P-42-037
An innovative probiotic, Ln-AGA58, demonstrates immunosuppressive effects in treating multiple sclerosis

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Multiple sclerosis (MS) is an inflammatory and autoimmune demyelinating disease of the central nervous system (CNS) with an unknown etiology that primarily strikes young individuals. Regulating the immune system and suppressing inflammatory pathways have a good effect on recovery from MS; however, the exact etiology of the disease is still unknown. Research suggests that probiotic consumption improves immune and inflammatory responses in MS via modifying the gut microbiome. The present study set out to investigate the impact of a newly identified probiotic from turnip juice, *Liquorilactobacillus nagelii* AGA58 (Ln-AGA58), in a mouse model of MS through examining the roles played by genes involved in MS etiology. First, the doses of the Ln-AGA58 probiotic that could be given to the mice were raised. Then, the animal MS model of experimental allergic encephalomyelitis (EAE) was induced in 8-10-week-old female C57BL/6 mice. Subsequently, the Ln-AGA58 probiotic was given to EAE-induced mice intragastrically for 21 days at 108 CFU/day. At the end of the treatment, RNA was isolated, and cDNA was synthesized by RT-PCR in the brain tissues of control, EAE, and EAE- Ln-AGA58-treated animals. The mRNA levels of H2EB1, CCL5, CXCL10, TNF-α, IL6 and IL17 were

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significantly reduced in Ln-AGA58-treated mouse brains, by 66.8%, 44.8%, 55.9%, 77%, 58% and 85% respectively. Furthermore, A notable decrease in pro-inflammatory cytokines in the serum of EAE mice treated with Ln-AGA58 was detected. These results suggest the potential of the Ln-AGA58 probiotic, which belongs to the genus *Lactobacillus*, to be a valuable probiotic for MS treatment due to its immunomodulatory properties. This work is supported by TUBITAK 221Z131.

P-42-038
Extracellular targets of zinc signaling in glaucoma neurodegeneration

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Retinal degenerative disorders represent an urgent problem of modern ophthalmology, as damage to retinal neurons leads to irreversible and currently incurable loss of vision. The central place among such disorders is held by glaucoma, an optical neuropathy characterized by multifactorial pathogenesis and lack of specific therapy. A breakthrough in understanding glaucoma pathogenesis was the recognition of mobile zinc as a potential mediator of retinal and optic nerve degeneration. This discovery is particularly important in light of the recent identification of the signaling function of zinc in the cytoplasm and extracellular space and the interconnection of zinc-dependent pathways with calcium signaling. In the present work, we addressed glaucoma mechanisms using animal model of the disease, cellular and biochemical studies, and structural approaches based on machine learning and X-ray crystallographic analysis, identifying novel zinc-dependent extracellular targets in this disease. It was shown for the first time that secreted zinc suppresses neurotropic activity in the retina by affecting the processing of the signaling factors involved and their interaction with cell surface receptors. The discovered mechanism may be common to a wide range of neurodegenerative diseases and become a target for various treatment options, including gene therapy. This work was supported by the Russian Science Foundation Grant No. 24-15-00171.

P-42-039
Therapeutic potential of pyrvinium pamoate in multiple sclerosis applying brain organoids

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Multiple sclerosis (MS) is a neurodegenerative autoimmune disorder with no known cure. Pyrivinium pamoate (PP), an antiparasitic drug, is suggested to promote myelination by suppressing WNT via Axin2. This study investigates PP as a potential MS treatment using two- and three-dimensional (2D and 3D) cell culture models. Cytotoxicity and scratch assays were first performed in 2D cultures, followed by RNA isolation, cDNA synthesis, real-time PCR, and immunohistochemistry on vascularized brain organoids derived from human embryonic stem cells to assess cell viability, wound healing, gene expression, and MS-related markers. The effect of PP on wound healing was first evaluated using scratch assays in fibroblasts. Ascorbic acid was used as a positive control. The results showed $38.91 \pm 25.81\%$ and $15.25 \pm 9.34\%$ wound closure at IC_{10} and IC_{50}, respectively, while ascorbic acid resulted in $53.2 \pm 22.13\%$ wound closure. Cytotoxicity assays in human oligodendroglioma cells was performed and IC_{08} was identified as 0.025 μM for cells and 0.058 μM for organoids for subsequent organoid experiments. To model MS-related inflammation, lipopolysaccharide was administered to the organoids, and inflammation induction was validated by the increased expression of Iba1 (≈2.68-fold) and GFAP (≈1.93-fold). PP's effects on myelination were assessed using MBP, showing a significant ≈70.1-fold increase. To evaluate PP's anti-inflammatory effects, real-time PCR analysis was conducted using TNF-α and NF-κB markers. While changes in inflammation markers were observed, they were not statistically significant, preventing a definitive interpretation of PP's anti-inflammatory effects. Our initial findings, increased MBP expression, and enhanced wound closure suggest PP's potential as an MS therapy, warranting further investigation. This study was funded by TUBITAK 124Z081.

P-42-040
Co-culture spheroid model for neurodevelopment and disease modeling

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Neurodevelopmental and neurodegenerative disorders require relevant three dimensional (3D) *in vitro* models to study disease mechanisms. While brain organoids require long period for differentiation and are not costly effective, two-dimensional culture models lack neural network complexity. To address this, we developed a neuronal-glial spheroid model by co-culturing HOG (oligodendroglial precursor) and SH-SY5Y (neuronal-like) cells in a 3D system with neuronal growth factors. This study validates our spheroid system as a scalable and reproducible platform for glial-neuronal interactions, disease modeling, and brain development. Depending on the morphology based light microscopy analysis, Spheroid growth was monitored over 24

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days, starting at 103.33 μm on Day 2 and expanding 9.4-fold to 973.08 μm by Day 26. Growth acceleration became evident after Day 14 ($p < 0.001$), indicating a critical phase of cellular reorganization. qPCR analysis revealed that CNP expression was 20-fold higher in spheroids than in monolayer HOG cells and 2.1-fold higher than in SHSY5Y cells, supporting enhanced oligodendroglial differentiation. Similarly, IFN-γ expression increased 5.5-fold compared to HOG cells and 1.6-fold relative to SHSY5Y cells ($p < 0.05$), suggesting an environment conducive to neuroimmune interactions. To further assess differentiation, immunofluorescence staining was performed. Statistical analysis showed no significant difference in Tau expression between spheroids and organoids ($p > 0.05$), while SATB2 staining intensity was moderately higher in spheroids, suggesting variation in neuronal lineage specification. The physiological organization and gene expression pattern of this model emphasize its applicability to neurodegenerative studies. These results validate our neurospheroid model as a physiologically meaningful alternative for monolayer cultures and organoids. This study was funded by TUBITAK 124Z081.

P-42-041
A novel hippocampus-supramammillary nucleus circuit modulates physical dependence on opioids in mice

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Opioid medications are widely used as potent analgesics to relieve moderate to severe pain. However, these drugs also cause numerous side effects. Among them, long-term opioid users often experience severe withdrawal symptoms upon discontinuation, a phenomenon known as physical dependence. In mouse models, a common behavioral response to opioid withdrawal is withdrawal jumping. However, the neural mechanisms regulating this behavior remain unknown. In this study, we identified a specific hippocampus-supramammillary nucleus circuit using pAAV-mDlx-GFP-Fishell-1hDLx-EGFP. Further investigation with head-mounted microscopy and pAAV-mDlx-GCaMP6f-Fishell-2 revealed that GABAergic interneurons in this circuit are activated during opioid withdrawal-induced behaviors but do not respond to other opioid-related side effects. Additionally, inhibition of this circuit using pAAV-hDlx-GiDREADD-dTomato-Fishell-5 significantly reduced opioid withdrawal-induced jumping, whereas direct activation with pAAV-hDlx-GqDREADD-dTomato-Fishell-4 induced jumping behavior even in the absence of opioid exposure. In summary, improper discontinuation after repeated opioid exposure leads to the activation of GABAergic interneurons in the hippocampus-supramammillary nucleus circuit, which in turn triggers withdrawal jumping.

P-42-042
3′, 4′, 7-Trihydroxyflavone extracted from *Albizzia julibrissin* activates the CREB-BDNF axis and restores memory impairment in Alzheimer's disease mouse models

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Alzheimer's disease is a major neurodegenerative disorder that leads to dementia, yet specific treatments remain elusive. Although *Albizzia julibrissin* has been used in traditional oriental medicine to treat insomnia and disorientation by its anti-inflammatory properties, there are currently no studies in animal models. This study aims to identify potential therapeutic candidates for Alzheimer's disease by examining how 3′,4′,7′trihydroxyflavone (THF), isolated from *Albizzia julibrissin* stem bark, as a potential therapeutic candidate for Alzheimer's disease by examining memory recovery in a scopolamine-induced memory deficit mouse model. THF administration both orally and centrally in scopolamine-induced AD mice led to significant improvements in cognitive performance. Biochemical assays revealed restoration of cholinergic markers (ACh, AChE, ChAT) and an increase in BDNF levels in the hippocampus. Electrophysiological recordings confirmed that THF restored long-term potentiation (LTP) reduced by scopolamine, indicating improved synaptic plasticity. These findings suggest that THF has the potential neuropharmacological agent to protect the brain from memory loss induced by Alzheimer's disease through enhancing cholinergic system activity and activating the CREB-BDNF signaling pathway in the hippocampus.

P-42-043
Temporal dynamics of glutamate receptors and locomotor activity in *Drosophila*

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Rare metabolic diseases impact approximately 350 million people worldwide, with synaptopathies—disorders affecting synaptic function—representing a significant portion. For most synaptopathies, the underlying mechanisms are poorly understood, complicating diagnosis and treatment. Our research group uses *Drosophila* (fruit flies) as a model organism to investigate the molecular mechanisms behind synaptopathies, explicitly focusing on the fourth muscle 1b neuromuscular junctions. Previous studies on synaptopathy models revealed inconsistencies in immunostaining results, which led us to examine the role of time as a factor influencing synaptic properties. Our study used third-instar *Drosophila* larvae to examine how Zeitgeber Time (ZT) affects glutamate receptor (GluR) levels and locomotor behavior. We identified a striking time-dependent pattern through immunostaining, confocal microscopy, and behavioral assays: GluR levels peak between ZT 16–17 but decline sharply at ZT 19–20. This decrease is in tune with locomotor activity, with high locomotor behavior at ZT16-17 that decreases at ZT 19-20 suggesting a direct link between synaptic receptor availability and motor function. These findings highlight the importance of time as a critical variable in both behavioral analysis and immunostaining. Standardizing

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experimental timing could enhance data reproducibility and provide deeper insights into disease mechanisms.

P-42-044
PAX6 knockdown ameliorates cognitive deficits via modulating inflammation in a mouse model of Alzheimer's disease

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Alzheimer's disease (AD) is primarily characterized by abnormal accumulation of β-amyloid plaques and neurofibrillary tangles in the brain. We have previously identified paired box 6 (PAX6), a critical transcription factor for eye and brain development, as an important molecular link between amyloid-β and tau hyperphosphorylation. To explore the potential of PAX6 as a therapeutic target for AD, this study focused on examining its impact on cognitive functions and AD-related pathologies in 5XFAD mice, a transgenic mouse model of AD. We found that PAX6 knockdown rescues cognitive impairments and ameliorates amyloid plaque burden in these mice. We also showed that PAX6 knockdown reduces the mRNA levels of the inflammatory cytokines tumor necrosis factor-α (TNF-α) and interleukin-1β (IL-1β) both *in vivo* and *in vitro*. Further investigation revealed that PAX6 directly binds to the promoter regions of TNF-α and IL-1β genes, regulating their transcription. These findings indicate that the beneficial effects of PAX6 knockdown on cognitive function in AD mice might be at least partially due to the reduction of inflammation. In conclusion, the study suggests that targeting PAX6 could be a promising strategy for AD therapy.

P-42-045
The anti-ferroptosis mechanism underlies oleamide's neuroprotective and antiseizure effects

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Oleamide, a prototype fatty acid amide lipid messenger, exhibits various effects in the brain. However, its therapeutic potential in neurological diseases remains largely unexplored. Previously, we demonstrated that oleamide significantly reduced seizure activity and excitotoxic damage in the striatum of kainic acid (KA)-induced epileptic rats through calpain inhibition. Emerging evidence suggests that ferroptosis plays a role in epilepsy pathogenesis, highlighting it as a promising therapeutic target. In this study, we propose that oleamide exerts antiepileptic and neuroprotective effects via ferroptosis inhibition as a novel intracellular mechanism. We confirmed its anti-ferroptotic effects in both *in vitro* neuronal systems and *in vivo* epileptic mouse models induced by KA or pentylenetetrazole (PTZ). Furthermore, oleamide demonstrated both preventive and therapeutic effects against seizures when administered pre- and post-exposure to KA or PTZ. These findings suggest that oleamide may serve as a potential therapeutic agent with a novel mechanism for treatment-resistant epilepsy.

P-42-046
Neurobehavioral effects of gamma-glutamylcysteine ethyl ester on the kainic acid rat model of status epilepticus

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Epilepsy is a chronic neurological disorder with recurrent seizures leading to cognitive and behavioral comorbidities. Antiepileptic drugs are effective in controlling seizures but these drugs are inadequate in preventing the progression of the disease. Gamma-glutamylcysteine ethyl ester (GCEE) is an antioxidant that crosses the blood–brain barrier, enhancing glutathione (GSH) levels, potentially offering neuroprotective benefits. The objective of this study was to investigate the neuroprotective effects of GCEE on seizure severity, learning and memory, anxiety levels, GSH and *BDNF* alterations in cortex and hippocampus tissues in a rat model of kainic acid (KA)-induced status epilepticus. Male Sprague Dawley rats were divided groups: Control, KA, GCEE, and KA + GCEE. KA (10mg/kg) and GCEE (10 mg/kg) were administered to the rats intraperitoneally (i.p.). Seizure behaviors were assessed with Racine Scale. Learning, memory and anxiety evaluated with behavioral tests. The levels of GSH and *BDNF* mRNA were determined spectrophotometrically and real-time PCR, respectively. KA significantly increased seizure activity, as well as impairing retrieval performance during the probe trial. KA also decreased the levels of GSH and *BDNF* mRNA expression both in hippocampus and cortex when compared to the controls. A single acute dose of 10 mg/kg GCEE significantly reduced seizure severity, enhanced spatial memory retrieval but there was no significant change in anxiety levels when compared to KA-treated animals. GCEE treatment increased GSH levels compared to KA group but not in the cortex. Furthermore, GCEE treatment changed *BDNF* expression levels compared to KA, but not significantly. Consequently, GCEE may provide neuroprotective effects in epilepsy by reducing seizure severity, enhancing memory and preventing neurotrophic loss. This study was supported by the Scientific Research Project Coordination of Ege University, Izmir, Turkey (23149).

P-42-047
The effect of dexamethasone induced-prenatal stress on the hippocampal renin-angiotensin system in male rats

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Prenatal stress (PS) during early life alters the fetal brain and has long-term consequences on brain development, increasing the risk of neurodevelopmental, psychiatric, and neurodegenerative disorders. The renin-angiotensin system (RAS) in the brain controls the function of the cerebrovascular system, neuroinflammation, oxidative stress, and the survival of neurons.

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RAS components have been studied in peripheral tissues for many years to elucidate the mechanisms underlying metabolic diseases predisposed by PS. However, the number of studies investigating the effects of PS on cerebral RAS components is very limited. This study was aimed to examine the effects of PS induced by dexamethasone (Dex) exposure on protein levels of RAS components such as angiotensin 2 receptor type 1 and 2 (AGTR1 and 2) and angiotensin-converting enzyme 1 and 2 (ACE1 and ACE2) in the hippocampus of male rats. Pregnant rats were daily injected intraperitoneally with Dex (0.2 mg/kg) or saline during the third week of gestation. After birth, male rats were killed at 90 days of age (n = 5 for each group). The proteins in hippocampal tissues were quantified using the bicinchoninic acid protein assay kit. The protein levels of AGTR1, AGTR2, ACE1, ACE2, and NEP were measured with ELISA kits according to the manufacturer's guidelines. Statistical comparisons were performed with the Mann-Whitney U test. Prenatal Dex caused a significant increase in AGTR1 and AGTR2 protein levels, while ACE1, ACE2, and NEP protein levels were found to be significantly decreased in the hippocampus compared to the control. Our results emphasize the effect of PS on protein levels of RAS components in male rats and suggest that PS may lead to disruption in the balance of pro-inflammatory and neuroprotective RAS components, leading to long-lasting effects on stress responses, cognition, and emotional regulation. Targeting these pathways may offer new therapeutic approaches for mitigating the effects of PS on brain health.

P-42-048
7,8-dihydroxyflavone reduces NLRP3 inflammasome activation and pyroptosis in microglia

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Microglia, the resident immune cells of the central nervous system (CNS), are essential mediators of innate immunity. The NOD-like receptor family pyrin domain-containing 3 (NLRP3) inflammasome is a multiprotein complex that plays a central role in neuroinflammation. Its hyperactivation contributes to the pathogenesis of neurodegenerative diseases (NDs). 7,8-Dihydroxyflavone (7,8-DHF), a brain-derived neurotrophic factor (BDNF) mimetic and a selective agonist of tropomyosin receptor kinase B (TrkB), has shown promising neuroprotective and immunomodulatory effects. This study aimed to investigate the impact of 7,8-DHF on NLRP3 inflammasome activation, gasdermin D (GSDMD)-mediated pyroptosis, and nuclear factor-kappa B (NF-κB) signaling in microglial cells. Murine N9 microglial cells were stimulated with lipopolysaccharide (LPS) and adenosine triphosphate (ATP), followed by treatment with 7,8-DHF. We assessed NF-κB pathway, inflammasome activation, caspase-1 activity, and levels of interleukin-1 beta (IL-1β) and interleukin-18 (IL-18). Our results showed that 7,8-DHF significantly suppressed NLRP3 inflammasome activation, reduced active caspase-1 levels, and inhibited IL-1β and IL-18 secretion. These effects were associated with inhibition of the IκBα/NF-κB pathway. In conclusion, BDNF mimetic 7,8-DHF attenuates microglial NLRP3 inflammasome activation and GSDMD-mediated pyroptosis by modulating NF-κB signaling, suggesting its potential as a therapeutic candidate for neuroinflammatory conditions.

P-42-049
Modulation of microglial activation and inflammatory gene expression by cyclosporine A-loaded theranostic nanocarriers in an *ex vivo* ischemic stroke model

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Microglial activation plays a key role in neuroinflammation associated with ischemic injury. The oxygen-glucose deprivation (OGD), *ex vivo* model of ischemic stroke, is particularly useful in the study of neuroinflammatory responses. However, the specific dynamics of microglial activation in this model remain insufficiently characterized. We investigated whether OGD alters the expression profile of pro- and anti-inflammatory genes in organotypic hippocampal cultures (OHCs). Furthermore, effects of immunomodulator, cyclosporine A (CsA) in free form and loaded in polycaprolactone (PCL) nanocarriers, on OGD-induced changes in the gene expression profiles were studied. OHCs were obtained from the hippocampi of 6–7-day-old rat pups. On the 7th day *in vitro*, slices were pre-treated for 30 min with varying concentrations of CsA (0.05 and 0.1 μM), PCL or PCL loaded with CsA. OHCs were subjected to the OGD procedure by transferring them into wells containing pre-equilibrated, deoxygenated OGD medium within a hypoxic chamber under controlled atmospheric conditions: 1.5% O_2, 5% CO_2, and 85% N_2. After 40 min of OGD, slices were transferred to a fresh oxygenated culture medium and returned to normoxic conditions: 21% O_2 and 5% CO_2. The expression levels of pro- and anti-inflammatory genes (*Cd40*, *Cd206*, *Arg1*, *Igf-1*) in control and OGD-exposed OHCs were analyzed using RT-PCR. Results showed that OGD increased microglial *Cd40* and *Cd206* expression and decreased *Igf-1* expression in OHCs. CsA, both free and loaded in theranostic nanoparticles effectively modulated these changes. These data enhance our understanding of molecular aspects of microglial activation under ischemic conditions and highlight the potential of theranostic nanocarriers in stroke treatment. Further research using in vivo models of stroke is needed to confirm a potential translational value of the obtained data. This research was supported by grant no. 2020/39/B/NZ7/01913 from the National Science Centre, Poland.

P-42-050
Sex- and brain region-specific effects of chronic mild stress on kynurenine metabolites in rats

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Depression is twice as prevalent in women as in men, but its biological basis remains unclear. The kynurenine pathway, linking inflammation and neurotransmission, has been implicated in stress-related depression. Chronic stress has been reported to influence kynurenine metabolism and alter the balance of its neuroactive metabolites but how these effects vary by sex and brain region is poorly understood. Most studies focus on male rodents, leaving a gap in our understanding of the female stress response. We hypothesized that sex differences in depression might arise from variations in kynurenine metabolites between males and females. To test this, 16 male and 16 female Wistar rats were evenly assigned to control and chronic mild stress (CMS) groups for 8 weeks. Behavioral assessments included sucrose preference, forced swim, open field, elevated plus maze, light-dark box, and social interaction tests. Kynurenine metabolites were measured in plasma, frontal cortex, hippocampus, and striatum via LC-MS/MS. In plasma, both male and female stress groups had elevated kynurenic and quinolinic acid (QUIN) levels. Although no major sex differences were observed in overall metabolite profiles, stressed females showed significant increases in plasma xanthurenic acid (XA) and 3-hydroxykynurenine, with no change in males, suggesting that kynurenine metabolism may contribute to sex differences in depression. QUIN increased exclusively in the hippocampus of both stress groups, whereas XA significantly increased in the frontal cortex and striatum but not in the hippocampus. This region-specific pattern underscores the complex effects of stress on kynurenine metabolism. XA's region-specific alterations indicate a previously unrecognized role in stress-related neurobiological changes, warranting further investigation as a novel marker. Our study is the first to compare CMS-induced kynurenine metabolite shifts across brain regions and sexes, addressing a key gap in depression research.

P-42-051
The effects of prolonged fasting on energy metabolism and mitochondrial functions in Alzheimer's disease model applied HT22 cells

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Mitochondrial dysfunction is recognized as a key factor contributing to neurodegeneration in Alzheimer's disease (AD). While glucose metabolism is impaired in AD, ketone metabolism remains unaffected. Therefore, ketones may offer a potential therapeutic approach for neurodegenerative diseases with impaired glucose metabolism. This study aims to investigate the metabolic effects of prolonged fasting under pathological conditions and to preserve neuronal function. The pathological Alzheimer's disease model was simulated in vitro by applying 10 μM Aβ (1-42) to HT22 cells. A fasting model was simulated using EBSS (Earle's Balanced Salt Solution) medium, and 5 mM beta-hydroxybutyrate (βOHB) was administered. Aβ accumulation in the cells was examined using immunofluorescence techniques and confocal microscopy. Metabolic parameters, including beta-hydroxybutyrate, glucose, lactate, lactate dehydrogenase, and amino acids, were measured from the cell medium and lysates. Mitochondrial function was assessed by measuring citrate synthase enzyme activity. According to results, beta-hydroxybutyrate improved the viability of experimental groups ($P < 0.5$), while Aβ (1-42) exhibited toxic effects on HT22 cells, which were mitigated by βOHB treatment. Higher citrate synthase activity was observed in both the βOHB-control and βOHB-fasting groups. In groups treated with both Aβ (1-42) and βOHB, enzyme activity was increased compared to groups without βOHB ($p < 0.1$). Additionally, cells incubated with βOHB utilized both glucose and ketones for energy metabolism, leading to enhanced mitochondrial activity during ketone utilization. These findings suggest that while the addition of ketones can alleviate the damage caused by amyloid-beta, it does not completely restore neuronal function. The results highlight the potential for developing alternative interventions to preserve neuronal function in various neurodegenerative diseases.

P-42-052
The brain-derived neurotrophic factor mimetic 7,8-dihydroxyflavone mitigates microglial inflammasome activation by enhancing the negative regulatory pathways

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The NOD-like receptor family pyrin domain-containing 3 (NLRP3) inflammasome is a multiprotein complex that is crucial for innate immune system. Aberrant NLRP3 inflammasome activation is engaged in the pathogenesis of neurodegenerative disorders. Understanding the mechanisms that inhibit the inflammasome is crucial for identifying novel therapeutic strategies for these disorders. 7,8-Dihydroxyflavone (7,8-DHF) have become the focus of attention in studies on NDs due to exert its neurotrophic effects in the CNS. Recent studies have shown encouraging outcomes targeting immunomodulator and neuroprotective properties. 7,8-DHF is a specific tropomyosin-related kinase receptor B (TrkB) agonist and bioavailable brain-derived neurotrophic factor (BDNF) mimetic, which has anti-inflammatory properties in the CNS. In the context of this research, we aimed to research the effects of BDNF mimetic 7,8-DHF on negative regulators of NLRP3 inflammasome activation in LPS plus ATP-induced murine N9 microglial cells. Our results showed that BDNF mimetic 7,8-DHF activated selective autophagy and ESCRT-III-dependent plasma membrane repair, both of which play crucial roles in the negative regulation of NLRP3 inflammasome activation. Overall, these observations reveal that BDNF mimetic 7,8-DHF prevents excessive microglial inflammasome activation by promoting ESCRT-III-dependent plasma membrane repair and enhancing selective autophagy.

P-42-053
Role of second-generation antidepressants in traumatic brain injury: focus on TGF-β1 pathway and the gut/brain axis

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Traumatic brain injury (TBI) is a major global health issue that results from long-lasting cognitive impairments in millions of

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patients with high susceptibility to Alzheimer's disease-like neurodegeneration. One of the major aspects of the pathological mechanism of brain injury is the impairment of the neuroprotective and anti-inflammatory pathway of TGF-β1 signaling that is critically responsible for cognition. Recent data suggest that even post-TBI, gut dysbiosis affects homeostasis of TGF-β1 and, subsequently, enhances neuroinflammation related with cognitive decline. Therefore, we proposed to study the actions of second-generation antidepressants, specifically fluoxetine and fluvoxamine, upon TGF-β1 signaling and the gut-brain axis in a validated murine model of TBI. We evaluated the impact of chronic treatment with antidepressants on cognitive function, neuroinflammation, and the gut microbiota findings. Behavioral analyses, histopathological assessments and molecular studies of TGF-β1 signaling were investigated. The results suggest that chronic administration of fluoxetine and fluvoxamine achieves substantial amelioration of cognitive impairment, reduction in neuroinflammation, and normalization of TGF-β1. The treatment also improved the health and homeostasis of the gut microbiota. Therefore, the treatment demonstrated the interconnection between the gut-brain axis based on TGF-β1 signaling to restore cognition and pathophysiology from brain injury. Further work should explore the translational relevance of these findings to human TBI pathology and related therapeutic interventions.

P-42-054
Identifying miRNA networks linking cognitive stimulation to synaptic function in Alzheimer's disease

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Synaptic dysfunction and neurodegeneration are hallmarks of Alzheimer's disease (AD), leading to the progressive cognitive decline characteristic of the disorder. Given the lack of a cure and the limited effectiveness of available therapies, there is an urgent need to identify novel strategies that promote neuroprotection and synaptic plasticity. Environmental enrichment (EE) is a well-established approach that induces cognitive stimulation and enhances synaptic plasticity. Multiple studies have demonstrated that EE acts as a plasticity modulator in various conditions and experimental models. Indeed, EE has been shown to upregulate BDNF, synaptophysin, and PSD95, leading to improved synaptic function. However, the mechanisms linking EE to increased synaptic markers remain largely unknown. This study aims to evaluate how miRNA levels change following cognitive stimulation. Twelve-month-old APP/PS1 transgenic mice will be randomly assigned to either a naïve or an enriched group. The naïve group will be maintained in a standard housing environment, while the enriched group will be exposed to an enriched environment for four weeks, consisting of various interactive toys. After the enrichment period, each mouse's brain will be divided for analysis: one half will be used for full miRNA profiling via RNA sequencing, allowing us to identify miRNAs that change in an activity-dependent manner. The differentially expressed miRNAs will then be analyzed using bioinformatics tools to identify their target genes, with a primary focus on synaptic targets. The remaining half of the brain will be used for protein extraction and validation of bioinformatics predictions. Overall, we anticipate that our findings will uncover novel mechanisms linking cognitive stimulation to enhanced synaptic activity. These insights may help guide innovative therapeutic approaches to mitigate this debilitating disorder.

P-42-055
Investigating GABA transporter 3 (GAT3) dysregulation in Alzheimer's disease

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Alzheimer's disease (AD) is characterized by progressive cognitive decline, which is strongly linked to synaptic dysfunction. Thus, understanding the mechanisms underlying synaptic dysfunction in AD may provide critical insights into disease pathogenesis. Recent evidence suggests that GABA transporter 3 (GAT3), primarily expressed in astrocytes and responsible for GABA reuptake from the synaptic cleft, may contribute to disease progression. Disrupted GAT3 activity can lead to excessive GABA accumulation, impairing synaptic plasticity and memory formation. In AD, a significant reduction in GAT3 immunoreactivity has been observed in the stratum pyramidale of the CA1 and CA3 regions, as well as in the subiculum and entorhinal cortex. Interestingly, following traumatic brain injury, GAT3 has been detected in neurons, despite its usual localization in astrocytes. This suggests a potential pathological redistribution of GAT3 that could affect neuronal function and disrupt neurotransmitter homeostasis. In this study, using a mouse model of AD, we will investigate how GAT3 levels change across different brain regions and at various disease stages to elucidate its potential role in disease progression. Additionally, we will determine whether Aβ accumulation drives neuronal GAT3 expression. Our findings will offer new insights into GAT3 dynamics in AD and its viability as a therapeutic target.

P-42-056
A pharmacoresistant *in vitro* blood–brain barrier model for screening potential antiepileptic drugs

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The blood–brain barrier (BBB) is a specialized cellular structure that regulates substance transport between the bloodstream and the central nervous system (CNS). However, in pathological conditions such as pharmacoresistant epilepsy, BBB cells often overexpress efflux transporters and increase drug-metabolizing enzyme activity, limiting drug entry into the CNS and reducing antiepileptic drug efficacy. Current *in vitro* BBB models fail to replicate these pathological changes. Therefore, we aim to develop a pharmacoresistant *in vitro* BBB model to assess potential antiepileptic drug transport. We establish this model as a transwell system by co-culturing human cerebral endothelial

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cells, astrocytes, and pericytes. BBB integrity is evaluated through long-term measurements of the transendothelial electrical resistance (TEER), while a fluorescein permeability assay at the end of the cultivation confirms functional barrier properties. Our results indicate that best barrier formation occurs in a contact co-culture system, with endothelial cells seeded apically on the inserts and astrocytes and pericytes basolaterally. Inserts are pre-coated with collagen and the application of fibronectin (apically) and poly-L-lysine (basolaterally) enhances barrier stability. Additionally, insert size significantly affects barrier integrity, with 12-well inserts yielding much higher TEER values than 24-well inserts. To induce pharmacoresistance, endothelial cells are exposed to sub-toxic concentrations of selected antiepileptic drugs over prolonged cultivation, which is expected to upregulate efflux pumps and drug-metabolizing enzymes. The pharmacoresistant *in vitro* BBB model will serve as a platform to screen antiepileptic compounds that either evade efflux transport or inhibit efflux pumps, improving drug delivery to the CNS. This project is supported by the Technology Agency of the Czech Republic, No. TN02000109, and by the Academy of Sciences of the Czech Republic, grant RVO 61388963.

P-42-057
Investigation of the effects of Spastin protein on myelination

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Hereditary Spastic Paraplegia 4 (HSP-SPG4) is a rare neurodegenerative disease caused by mutations of the *SPAST* gene encoding spastin. Clinically, most patients with HSP-SPG4 exhibit progressive spasticity in lower extremities, known as a pure phenotype; however, some patients exhibit complex phenotypes, including cognitive impairment and demyelination. Genetic studies have revealed SPG4 variants in some multiple sclerosis (MS) patients, indicating a possible connection between HSP and MS. Axonal transport defects, a hallmark of both diseases, are associated with the alteration of tubulin modifications. We recently showed that the mutant M1-C448Y spastin caused a decrease in microtubule acetylation, while the activity of histone deacetylate 6 (HDAC6) is correspondingly increased (Piermarini et al.2022, Human Molecular Genetics, 1844–1859). Although evidence suggests a relation with intracellular traffic disruption and aberrant myelination, the correlation between demyelination and axonal transport degeneration remains poorly understood. We aimed to investigate effects of the M1-C448Y mutation, which impairs microtubule severing and leads to aberrant HDAC6 activity, on axonal myelination in cortical neuron-oligodendrocyte co-culture system. Immunocytochemical analysis demonstrated that the M1-C448Y significantly reduced the myelination index. Additionally, using a cuprizone-induced demyelination mouse model, we observed a significant decrease in spastin protein levels by western blot, correlated with demyelination. Moreover, given that spastin has been associated with axonal regeneration, we posited that spastin contributes to axonal myelination. To address this, a cuprizone-induced cell culture demyelination model was used to show spastin's protection effect against cuprizone-induced demyelination. These findings indicate that spastin plays a role in axonal myelination, suggesting a promising target for developing therapeutics for demyelinating diseases.

P-42-058
Missense mutations as risk factors for Alzheimer's disease from a functional perspective

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Neurodegenerative disorders, such as Alzheimer's disease (AD), represent a major challenge for public health around the globe. AD is characterized by abnormal buildup of amyloid-β in the brain and constitutes the most common form of dementia. A family history of dementia is associated with a higher risk for AD, suggesting a genetic background to the disease. This study aims to provide a functional analysis of missense mutations in genes associated with autosomal-dominant AD – *APP*, *PSEN1* and *PSEN2* – in terms of their potential effects on pathogenicity, protein stability and structure using predictive *in silico* analysis tools. Single nucleotide variations associated with AD risk were retrieved from ALZFORUM, which is a repository of AD-related gene variants. Protein stability and pathogenicity scores were predicted using MUpro and FATHMM-XF, respectively. Structural alterations were determined via HOPE server. Predicted expression profiles in different brain regions were retrieved from the Human Protein Atlas. We found a total of 586 point mutations in *APP*, *PSEN1* and *PSEN2* genes, among which we selected 20 previously uncharacterized mutations for further analyses. We showed that the majority of these single nucleotide variations resulted in decreased protein stability and were found to be pathogenic. Furthermore, our results indicated that the amino acid changes introduced by these mutations caused differences in size, charge and hydrophobicity, which potentially resulted in structural alterations within the protein and affected their contacts with other proteins and ligands. We also showed that *APP*, *PSEN1* and *PSEN2* are predicted to be expressed in different regions of the brain. In conclusion, this study provides a comprehensive analysis of missense mutations as risk factors for AD regarding their potential damaging effects which might contribute to the pathogenesis of AD.

P-42-059
Periostin expression in the cerebral cortex of genetic absence epilepsy rats: a novel insight into epilepsy pathophysiology

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Epilepsy is a chronic brain disease with an urgent need for alternative treatments. Periostin, a newly discovered protein, has an unclear physiological role. This study investigates periostin

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expression in the cerebral cortex of "absence epileptic" rats. Cerebral cortex was obtained from genetic absence epilepsy rats from Strasbourg (GAERS) (n = 6), a genetic animal model of generalized absence epilepsy, and normal Wistar rats (n = 6). Total RNA was extracted, and cDNA was synthesized. Periostin protein was analyzed by western blot and immunohistochemistry with specific anti-periostin antibodies. *Postn* Ct values were normalized to *Actb* and analyzed with SPSS.17. The Shapiro-Wilk test showed normal distribution in the Wistar group ($p > 0.05$), while the GAERS group did not ($p < 0.05$). The Mann-Whitney U test found no significant difference in the *Postn*/*Actb* ratio in mRNA levels ($p > 0.05$). No statistically significant difference in periostin mRNA levels was found between the GAERS and control groups ($p > 0.05$), although qPCR results indicated a slight decrease in the GAERS group. Immunohistochemistry findings similarly showed a slightly lower periostin expression in the GAERS cortex compared to the control group, but this difference was not statistically significant. Western blot analysis also revealed reduced periostin protein levels in the GAERS cortex. Although this reduction was not statistically significant, it aligned with the immunohistochemistry results. This is the first study to investigate the relationship between periostin and epilepsy, providing a foundation for future research in this field. Further studies are needed to better understand the underlying molecular mechanisms of epilepsy. *The authors marked with an asterisk equally contributed to the work.

P-42-060
Evaluation of potential IL-17A inhibitors selected by drug repurposing study in cuprizone mouse model

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Multiple sclerosis (MS) is an inflammatory and demyelinating disease of the central nervous system characterized by the loss of the myelin sheath, oligodendrocyte degeneration, and axonal destruction which results in neurological dysfunction by triggering an inflammatory cascade. IL-17A, a pro-inflammatory cytokine, plays a crucial role in the progression of MS by stimulating microglia and astrocytes, contributing significantly to the inflammatory cascade. The study aimed to target the structural IL-17A/IL-17RA interaction to identify effective therapeutic strategies through drug repurposing approaches. The structures of IL-17A and IL-17RA were mapped using the Protein Data Bank (PDB), and ligand-receptor proteins were prepared for simulations. *In silico* screening identified twelve FDA-approved drugs as potential IL-17A inhibitors based on binding site compatibility, affinity scores, MM/GBSA scores, therapeutic activity, and interactions with critical amino acids. *In vitro* study contained reporter assay to determine the inhibitory effects of candidate drugs on IL-17R-overexpressing reporter HEK cells by monitoring NF-κB and AP-1 activation. Nine of the drugs exhibited the inhibition activity for ligand-receptor interactions. The IC_{50} values ranged between 0.7 and 80 μM. *In vivo* studies were conducted by using the cuprizone-induced demyelination model. Four selected candidate drugs were administered following cuprizone-induced demyelination. Peripheral inflammation in mice was analyzed using flow cytometry and cytokine panels, while recovery of demyelination in the brain was assessed using LFB, MBP, GFAP and NeuN markers. Immune analysis demonstrated that drugs modulated inflammation against cuprizone-intoxication. Furthermore, histological evaluation revealed a recovery in demyelination following drug treatment. In conclusion, the repurposed candidate molecules can modulate immune responses and enhance remyelination, providing promising therapeutic potential for MS. *The authors marked with an asterisk equally contributed to the work.

P-42-061
Molecular impact of maternal high-fat consumption in offspring's cortex and hippocampus

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The intrauterine and early post-natal developmental periods are critical windows during which an altered maternal environment, such as high-fat diet (HFD) consumption, increases the risk of neurodevelopmental disorders by altering structures of several brain regions. However, the precise mechanisms underlying the negative effects of maternal HFD remain unclear. Therefore, we hypothesise that maternal HFD consumption during gestation/lactation or exclusively during the preimplantation period – without inducing obesity – affects the offspring's cortex and hippocampus. To investigate this, female MF1 mice were assigned to one of three groups after conception: Embryonic-HFD (EHFD: HFD up to embryonic day 3.5; n = 8); HFD (n = 9) and NFD (control; n = 9) groups, consuming HFD and chow diet, respectively, throughout pregnancy and lactation. mRNA expression levels of genes related to mitochondrial biogenesis (*Tfam*, *Nrf-1*, *Pgc1-alpha*); oxidative stress and mitochondrial function (*Nrf2*, *Sox2*, *Cycs*); cholesterol metabolism (*ApoE*, *Hmgcs1*, *Lpl*, *Fdps*) and epigenetic regulation (*Hdac1*, *Dnmt1*) were evaluated in the cortex and hippocampus of adult offspring using qPCR. One male and one female offspring per mother were analysed using the hierarchical linear models. Expression analysis of selected genes showed no differences in either the hippocampus or cortex (all $p > 0.05$), except for *Pgc1-alpha*, the master regulator of mitochondrial biogenesis, which were significantly higher in the cortex of adult female offspring (EHFD vs NFD, $p = 0.012$; HFD vs NFD, $p = 0.037$). These findings suggest a region- and sex-specific effect of maternal HFD exposure on the transcriptional regulation of genes involved in mitochondrial function in the offspring brain.

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The upregulation of *PGC-1alpha* might indicate an adaptive response to the HFD exposure, potentially influencing mitochondrial biogenesis in the cortex. Further research is needed to elucidate these effects and their implications for brain health.

P-42-062
The role of Sep2 and Sep5 in synaptic architecture and motor function in *Drosophila*

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Septins, a family of GTP-binding proteins, play an essential role in cytoskeletal organization and synaptic function. However, their contribution to neuromuscular coordination and movement remains poorly understood. This study focuses on the impact of Sep2 and Sep5 loss-of-function mutations in Sep2 in *Drosophila* larvae, specifically examining their effects on synaptic structure and locomotion. In *Drosophila*, septins are essential for membrane interactions during cytokinesis and cell adhesion. Mutations in *Sep2* and *Sep5* lead to significant alterations in neuromuscular junctions (NMJs), including an increased bouton count and disorganization of presynaptic components. The mislocalization of key synaptic proteins disrupts vesicular regulation and cytoskeletal stability, resulting in disorganized active zones. Additionally, these mutants exhibit defects in endocytosis and vesicle recycling as evidenced by reduced dye uptake at NMJs. These findings underline the essential roles of Sep2 and Sep5 in maintaining synaptic architecture and vesicle turnover. Behavioral evaluations reveal that double mutants experience notable locomotor impairments, including reduced velocity and abnormal postures, indicative of disrupted neuromuscular function. Furthermore, septin mutants display abnormal myonuclear organization, characterized by clustered nuclei of varying sizes and shapes. Overall, this study highlights the critical roles of Sep2 and Sep5 in ensuring synaptic integrity, coordinated movement, and NMJ stability through precise protein localization and vesicle recycling. These insights enhance our understanding of how septins support the cytoskeleton and sustain synaptic integrity, shedding light on their importance in neuromuscular coordination. Ultimately, this research identifies Sep2 and Sep5 as key regulators of synaptic function and motor activity, contributing to the larger discussion on the role of septin roles in neurodevelopmental and neurodegenerative disorders.

P-42-063
Overexpression of SVCT2 restores dendritic and axonal arborization in a human *in vitro* model of amyotrophic lateral sclerosis (ALS)

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Amyotrophic lateral sclerosis (ALS) is a multifactorial and fatal neurodegenerative disease, characterized by the selective degeneration and death of motor neurons (MNs). Vitamin C (VC) deficiencies have been linked to an increased risk of ALS, and several studies have highlighted the role of the sodium-dependent vitamin C transporter 2 (SVCT2) and VC in neuronal maturation. However, the impact of SVCT2 expression on human MNs with ALS-like defects remains unexplored. In this study, human induced pluripotent stem cell (iPSC)-derived cultures were differentiated over 16 days, followed by the overexpression of either the wild-type (SOD1-wt) or mutated (SOD1-G93A) forms of superoxide dismutase 1 (SOD1). The precursors were then matured for an additional 20 days to induce human motoneurons (MNs). Electrophysiological recordings, immunofluorescence analysis, and RT-PCR were employed to confirm key functions and phenotypic features. The effects of lentiviral overexpression of SVCT2 in ALS MNs were assessed through confocal microscopy of synaptic and morphological proteins, along with quantitative analysis of neuronal arborization. Additionally, qRT-PCR was conducted to evaluate synaptic gene expression. The *in vitro*-generated MNs exhibited both excitatory and inhibitory functional activity, and expressed βIII tubulin, choline acetyltransferase (CHAT), and HB9. Overexpression of SVCT2 in ALS MNs restored dendritic and axonal networks by 64% and 105%, respectively, and reduced disease-associated hyperexcitability by decreasing PSD95 expression. Lentiviral overexpression of SVCT2 successfully restored the neuronal morphology damaged by SOD1-G93A expression, potentially stabilizing the excitatory synapses of these neurons. Grant Fondecyt Regular #1221147 (FN).

P-42-064
NABi as a therapeutic β-sheet blocker for inhibiting SOD1G93A aggregation and reducing cytotoxicity: targeting shared pathways in amyotrophic lateral sclerosis and Alzheimer disease

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Neurodegenerative diseases, such as Alzheimer disease (AD) and amyotrophic lateral sclerosis (ALS), are characterized by the accumulation of toxic protein aggregates that lead to neuron dysfunction and death. In ALS, mutations in Cu/Zn superoxide dismutase (SOD1), particularly the G93A mutation, drive the formation of pathogenic aggregates that lead to motor neuron degeneration. Similarly, AD is characterized by aggregates of amyloid-β (Aβ) peptides into insoluble plaques, which are central to AD pathology and result in cognitive decline and neuron loss. Our study investigated the therapeutic potential of natural Aβ binder and Aβ-aggregation inhibitor (NABi), a known potent inhibitor of Ab aggregation derived from the 90 N-terminal amino acids of SOD1. ALS and AD share common mechanisms involving protein aggregation; however, the potential of NABi to specifically target and mitigate aggregation and associated cytotoxicity in ALS remains unexplored. We have demonstrated that NABi binds with high affinity to SOD1G93A, stabilizing the mutant protein in its monomeric form. This interaction prevents cytotoxic aggregate formation and reduced cell death, indicating that NABi mitigates SOD1G93A-induced cytotoxicity by maintaining the mutant protein in a soluble, nontoxic, monomeric state. Our findings highlight NABi as a promising

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therapeutic candidate for ALS and AD, both of which are characterized by β-sheet-rich amyloid fibrils. By targeting shared aggregation pathways, NABi shows potential to slow disease progression and reduce neurotoxicity in ALS and AD, providing valuable insights and a targeted molecular approach for addressing these protein aggregation-related neurodegenerative diseases.

P-42-065
Responses of interleukin 6 (IL-6), neurotrophin 3 (NT-3) and acute phase proteins (APP) in patients with ischemic stroke (IS)

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Acute cerebral ischemia triggers an inflammatory reaction in the injured brain and could also induce systemic acute phase response by the release of pro-inflammatory cytokines. IL-6 is released into cerebrospinal fluid (CSF) and blood, acting as an autocrine and paracrine factor by IL-6R and sIL-6R (trans-signaling) and induces synthesis of acute phase proteins (APPs) in the liver. Neurotrophin 3 (NT-3) is produced by glial cells as an adaptative response to hypoxia. In the present work, we investigated the responses of IL-6, NT-3, α1antitrypsin (AAT) and transferrin (TF) in serum and CSF in patients with ischemic stroke (IS) on 7 day after onset. 18 patients (58-83 years) were investigated after IS confirmed by computed tomography. Quantification of stroke-related disorders was performed using the National Institutes of Health Stroke Scale (NIHSS) score on days 1, 2 and 7 from onset. Serum and CSF samples were analyzed using Coulter ELISA kits (NT-3, IL-6) and radial immunodiffusion (classical serum test; optimized protocol for CSF by Toparceanu F., 2005 (AAT, TR). The Pearson correlation coefficient (r) was calculated. Serum concentrations correlated positively with CSF concentrations for AAT (100%) and for TR (50%). They were associated with negative correlations between IL-6 and NT-3 levels in the serum and CFS ($r = -0.61$, $p = 0.05$; $r = -0.75$, $p = 0.05$). NIHSS scores on day 7 were < 14. Our results suggest that responses of AAT and TR in serum and CSF, characterized by a positive correlation on 7 days after IS onset, in presence of a negative correlation in serum and CSF between IL-6 and NT-3, is associated with patient survival. NIHSS scores < 14 confirmed a good recovery. The detection of the investigated parameters in CSF may be due to increased blood-brain barrier permeability, without excluding a limited local inflammatory response.

LB-R-42-01
Abstract withdrawn.

LB-R-42-02
Autophagic signatures in peripheral blood mononuclear cells (PBMCs) as novel biomarkers for early Parkinson's disease detection

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Parkinson's disease (PD) is a progressive neurodegenerative disorder characterized by motor impairments and the accumulation of misfolded α-synuclein. Dysregulation of the autophagy-lysosomal pathway (ALP), responsible for degrading misfolded proteins, has been implicated in PD pathogenesis. However, current diagnostic approaches rely heavily on motor symptoms, which occur due to substantial neurodegeneration, limiting early detection and intervention. This study investigated the potential of ALP-associated proteins in peripheral blood mononuclear cells (PBMCs) as diagnostic biomarkers for early-stage PD. Quantitative analysis revealed a significant reduction in optineurin (OPTN) levels in PBMCs from PD patients, and the expression levels of various ALP-associated proteins were tightly correlated, suggesting a coordinated dysregulation of the pathway. Correlation analyses revealed associations between ALP-associated features and clinical characteristics, such as age at onset and motor impairment. Furthermore, the study identified multiple positive correlations among ALP-associated proteins and functional readouts, highlighting the interconnectivity within the pathway. Notably, a PBMC biomarker model incorporating lysosomal-associated membrane protein 1 (LAMP1) and OPTN exhibited high diagnostic accuracy (86%) in distinguishing PD patients from controls. These findings highlight the potential of ALP-associated protein signatures in PBMCs as novel diagnostic biomarkers for early detection and intervention in PD, offering insights into the systemic manifestations of the disease.

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LB-R-42-03
Identifying the cellular alterations in the ventral tegmental area and substantia nigra in Alzheimer's and Parkinson's disease

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Neuropsychiatric symptoms (NPS), including apathy, anxiety, and depression, affect up to 98% of Alzheimer's disease (AD) patients, often emerging years before memory loss and cognitive decline. These early behavioural changes suggest the involvement of neural circuits beyond traditional circuits involved in memory, particularly the dopaminergic (DAergic) system. The ventral tegmental area (VTA), a key DAergic hub in the midbrain, plays a critical role in motivation, reward, and emotion, projecting to the hippocampus, prefrontal cortex, amygdala, and nucleus accumbens – regions involved in both memory and reward evaluation. The VTA shows early disruptions in AD pathogenesis, with neuroimaging studies showing reduced VTA volume and connectivity, and mouse models revealing early DAergic degeneration preceding amyloid-beta (Ab) plaque formation, correlating with hippocampal atrophy and cognitive deficits. In Parkinson's disease (PD), DAergic neuron loss originates in the substantia nigra, leading to progressive motor symptoms. However, the cellular diversity between these two midbrain regions, and their selective vulnerability across AD and PD, remains poorly understood. To address this, we aim to map the cellular landscape of the human VTA and SN in AD and PD. This work will generate a high-resolution, cell-type-specific atlas of regional and disease-specific susceptibility, providing new insights into DAergic pathology and its potential role in NPS.

Nutrition

P-43-001
Chios mastic gum and inflammatory pathways in IBD: clinical and *in vitro* insights

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Inflammatory bowel disease (IBD) comprises a group of chronic conditions characterized by intestinal inflammation, with alternating periods of remission and exacerbation. Chios mastic gum (CM), the resin of *Pistacia lentiscus* var. Chia, has shown promising anti-inflammatory properties and may serve as a nutraceutical aid in prolonging remission [1]. This double-blind, randomized, placebo-controlled trial investigated the clinical and psychological outcomes of Selpic (CM with Supermastic®) in IBD patients in remission. Fecal calprotectin (CAL), zonulin (ZO) (as markers of inflammation and intestinal permeability, respectively), and psychological outcomes were assessed before and after supplementation. To elucidate the anti-inflammatory mechanisms of Selpic, we evaluated four inflammatory signaling pathways *in vitro* using LPS-stimulated THP-1 macrophages treated with Selpic or Supermastic® at three concentrations [2]. We analyzed cytokines, endocannabinoids (ECs), eicosanoids (Es), and reactive oxygen species. Clinically, Selpic supplementation significantly reduced fecal ZO compared to placebo, while CAL showed a non-statistically significant trend. No statistically significant effect was observed on psychological outcomes. *In vitro*, Selpic- but not Supermastic® – induced a dose-dependent and statistically significant decrease in IL-6, IL-2, IL-17, TNF-α, MCP-1, and GCSF levels [2]. ECs and Es quantification showed that both Selpic and Supermastic® significantly decreased PEA and SEA, while increasing 2-arachidonoylglycerol, arachidonic acid, and eicosapentaenoic acid. Even if both formulations reduced prostaglandin (PGE2, PGD2) and thromboxane (TBX) levels, these changes were not statistically significant. Although, these are preliminary findings, they suggest that Selpic exerts a beneficial effect on key inflammatory signaling pathways involved in IBD.
References: 1. Ottria R et al. (2023) Int J Mol Sci. 24, 12038. 2. Papalois A et al. (2012) J Med Food 15, 974–983.

P-43-002
Antioxidative activity of extracts from rocket and spinach biofortified using selenium nanoparticles

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Selenium nanoparticles (SeNPs) present a novel selenium formulation that can be used for plant biofortification with the aim of increasing the content of Se and antioxidants. In this work, rocket and spinach were biofortified with different forms of selenium – selenate, SeNPs stabilized with polyvinilpirolidone (PVP SeNPs), polysorbate 20 (PS SeNPs), and humic acid (HA SeNPs). The plants were sampled, dried by lyophilization and the extraction of antioxidants was performed. The extracts were characterized using Trolox equivalent antioxidant capacity and Folin-Ciocalteu assays. Antioxidative activity of the extracts was investigated in vitro on HepG2 and Caco-2 cells. The cells were incubated with rocket or spinach extracts in two concentration levels selected based on preliminary biocompatibility studies (0.5 and 0.05 mg/mL) for 24 h. Oxidative stress was then induced by adding prooxidant tert-butyl hydroperoxide (t-BOOH, 100 μM) and cell viability was measured by performing MTT assay. Extracts obtained from rocket and spinach biofortified using HA SeNPs had the strongest antiradical (Trolox equivalents) and total reductive capacity (gallic acid equivalents). *In vitro* antioxidative activity assessed by measuring cell viability was shown in HepG2 cells in the case of rocket biofortified using PS SeNPs and spinach biofortified using PS SeNPs and HA SeNPs. Caco-2 cells were more responsive to treatment with rocket extracts compared to spinach, in terms of their protective activity against t-BOOH. All tested rocket extracts had protective effects, while in the case of spinach, only extract biofortified with PS SeNPs and control extract exhibited significant effect, under lower concentration level. This research demonstrated that SeNPs could be utilized for the development of novel functional foods with improved antioxidative

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characteristics. It also showed that different plant species can exhibit distinct response to biofortification with SeNPs.

P-43-003
The active compounds and anti-inflammatory, antioxidant, antibacterial properties of *Anthriscus cerefolium*

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Anthriscus cerefolium is widespread in Ararat, Syunik, Artsakh, Tavush and other Armenian regions. Due to its aromatic taste and smell, the plant is used in cuisine of many countries. In Armenia, the leaves are used as a spice and vegetable to prepare pie, soups, salads and meat preparations. Although this herb is used in traditional medicine and as food, it is poorly investigated from the scientific point of view. The aim of this work was to study the chemical composition of water and ethanol extracts of fresh and dried *Anthriscus cerefolium* and investigate the antioxidant, antibacterial properties, as well as the extracts influence on the activity of adenosine deaminase (ADA). The qualitative analysis of water and ethanol extracts of fresh and dried plant revealed the abundance of flavonoids, coumarins, tannins, while cardiac glycosides, saponins, anthracene derivatives and anthocyanins were absent. Coumarins were more abundant in water preparations of fresh and dried *Anthriscus cerefolium*, but tannins were more abundant in ethanol extracts. Alkaloids were only found in water extracts. The IC_{50} value for ADA activity inhibition from concentration of both extracts was 0.5 ± 0.23 mg/ml and 0.4 ± 0.27 mg/ml, respectively. According to obtained results, both extracts of *Anthriscus cerefolium* demonstrate mild inhibitory effect and could be selected as anti-inflammatory agent with less side effects. The antioxidant power of both extracts of dried *Anthriscus cerefolium* with ABTS radical scavenging capacity registered IC_{50} values 1.4 ± 0.03 mg/ml and 0.413 ± 0.01 mg/ml, respectively. The ethanol extract possesses 3.5 times higher antioxidant action. The fresh plant mass 5-10 times stimulated *Lactobacillus rhamnosus* bacteria growth. The fresh mass demonstrated antimicrobial activity towards *E. coli* and *Staphylococcus aureus* by diffusion method. We can conclude that *Anthriscus cerefolium* is a source of anti-inflammatory, antioxidant, antibacterial and probiotic agent. *The authors marked with an asterisk equally contributed to the work.

P-43-004
Olive leaf extract as a natural preservative and nutritional enhancer in fresh cheese: Antioxidant and antimicrobial benefits

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Fresh cheeses are highly susceptible to microbial spoilage due to their high moisture content and lack of heat treatment. As consumer preference for natural preservatives increases, plant-derived extracts, like olive leaf extract (OLE), offer a promising solution for extending shelf life and improving product quality. OLE, rich in bioactive compounds such as phenols and flavonoids, has proven antimicrobial properties and health benefits. This study aimed to assess the potential of OLE as a natural preservative and nutritional enhancer in fresh cheese made from cow's milk. The extract was rich in polyphenols (47.47 ± 0.261 mgGAEg^{-1}) and flavonoids (0.26 ± 0.003 mgQEg^{-1}), with an antioxidant capacity of 20.23 ± 0.31 µmolTEg-1. OLE was added at 2% and 5% (v/v) concentrations during cheese production, along with starter culture, microbial rennet, and protective culture. The results showed that 2% OLE led to the highest sensory acceptability and extended shelf life. Additionally, the extract improved the cheese's nutritional content, increasing protein, fat, and saturated fatty acids, while reducing moisture and salt content. Lower concentrations of OLE were more effective at inhibiting *E. coli* and yeasts/molds, keeping them within acceptable limits for longer. OLE offers a promising natural preservative and nutritional enhancer, though further optimization is needed for long-term preservation. This study was supported by Erasmus EDU – 2021 –PEX – COVE – AgriNext (n°101056023), by European Union – Next Generation EU project SIROFONIJA (Class: 602-01/23-01/392, Reg. no.: 2133-61-01-23-11), and performed using the facilities and equipment funded within the European Regional Development Fund project Atrium of Knowledge (KK.01.1.1.02.0005) and European Structural and Investment Fund project SIRENA (KK.01.1.1.04.0096).

P-43-005
The CaMKKβ-AMPK-SIRT1 axis is targeted by vicenin-2 in lipopolysaccharide-exposed THP-1 cells

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Flavonoids found in fruits and vegetables are recognized as molecules able to reduce the inflammatory cascade through their numerous mechanisms of action. The aim of our study was to investigate the molecular mechanisms underlying the anti-inflammatory effects of vicenin-2 explored in lipopolysaccharide (LPS)-exposed THP-1 cells. In our *in vitro* model, vicenin-2 counteracted the increase of tumor necrosis factor (TNF)-α, interleukin (IL)-1β, and IL-6 levels, as well as the overexpression of both inducible nitric oxide synthase (iNOS) and cyclooxygenase (COX)-2 triggered by the LPS stress. Afterwards, we concentrated on SIRT1 since its significance in the inflammatory process is widely recognized. Our results showed that LPS was able to decrease SIRT1expression, whereas vicenin-2 restored the levels of this enzyme up to those of control cells. These effects were also detected in terms of acetylated p53, a known SIRT1 substrate. Interestingly, we found that vicenin-2 does not directly activate SIRT1. Thus, we explored the possible role of AMP-activated protein kinase (AMPK), a SIRT1 upstream activator. The blockage of AMPK by dorsomorphin hindered the protective effects elicited by vicenin-2 on both SIRT1 expression and activity, suggesting the involvement of AMPK. Consequently, this caused a downstream loss of the anti-inflammatory effect of vicenin-2, unable to decrease both the

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activation of nuclear factor (NF)-κB and the production of cytokines induced by LPS. Lastly, docking simulation hinted that vicenin-2 might act as an activator of Ca^{2+}/calmodulin-dependent protein kinase kinase β (CaMKKβ), one of the regulators of AMPK. Our results suggest that the anti-inflammatory effects of vicenin-2 may arise from the interaction with the CaMKKβ-AMPK-SIRT1 axis.

P-43-006
Molecular properties of whole wheat pasta: role of semolina quality and processing

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Whole wheat pasta is not only a source of proteins and carbohydrates but provides consumers with a staple food intrinsically rich in fiber and phenolics. Not many studies tackled the complexity of the protein pattern in whole wheat semolina and their interaction with bioactives before and after processing. This study applies a multidisciplinary approach to investigate at the molecular level the relationship between product structure and biological functionality. Pasta was produced in a pilot plant from whole wheat semolina with different protein contents (12% and 15%) using either low (55°C)- and high (85°C)-temperature drying diagrams. The antioxidant capacity and the profile of both free and bound phenolic compounds in the raw materials were similar, mainly formed by flavonoids. The starch gelatinization kinetics is slightly different in raw materials, likely due to a different water distribution among phases. Proteins in the two raw materials share a similar macromolecular organization, if not for a very different distribution of hydrophobic patches on the protein surface. The drying diagram had a significant impact on the aggregation state of proteins and antioxidant compounds. High-temperature drying promoted thermal coagulation of proteins by covalent (disulfide) cross-linking. High-temperature drying also slightly affected phenolic composition and deeply increased the antioxidant capacities. In order to ascertain the impact of the drying diagram on the bioavailability of phenolics and their anti-inflammatory activity, the digestate from *in vitro* digestion is being utilized to study various responses in a cultured intestinal cell model. These experiments are currently underway. This study is part of the Italian PRIN 2020 project (Grant Number 2022SCYHWK).

P-43-007
Role of long-chain fatty acid receptors in the respiratory systems

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As the aging population rapidly grows worldwide, aging-related conditions increasingly impact healthspan and lifespan. Changes in the mechanical properties and immune function of the respiratory systems with advancing age induce physiological respiratory dysfunction and high susceptibility to various infectious diseases in the elderly states. Recently, several orphan GPCRs (G-protein coupled receptors) have been identified as receptors for long-chain fatty acids. GPCRs are seven-transmembrane proteins belonging to a large protein family of receptors that detect extracellular molecules and then activate intracellular signal transduction pathways and ultimately trigger cellular responses. While the expression of GPCRs in the respiratory systems has been clarified, the role of GPCRs in the respiratory systems remains unclear. In this study, we aimed to clarify the effect of GPCRs on respiratory homeostasis, focusing on their function in the respiratory systems during aging and inflammatory models. Here, we found that GPCRs for long-chain fatty acids deficiency caused the increase in inflammatory responses in lung epithelial cells. Moreover, GPCRs for long-chain fatty acids deficiency during aging were observed in the excess mucin production in the lung. These results suggested that GPCRs may have been involved in the regulation of inflammatory responses in the respiratory systems. Further analysis will suggest that targeting GPCRs for long-chain fatty acids may be a novel therapeutic strategy for the prevention and treatment of respiratory aging-related diseases, contributing to healthy life expectancy in the super-aging society.

P-43-008
Phytosterols and policosanols from sorghum extracts modulate NPC1L1 expression: a new frontier against hypercolestererolemia

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Hypercholesterolemia represents the main risk factor in the pathogenesis of cardiovascular diseases, currently the leading cause of death worldwide. Along with pharmacological therapies the employment of nutraceuticals may represent an efficient strategy to reduce plasma cholesterol levels, and there is emerging particular interest in sorghum phytocompounds. The aim of this study is to evaluate the effects of phytosterols and polycosanols extracted from sorghum in modulating the expression of NPC1L1, a transporter responsible for the uptake of 70% of exogenous cholesterol in enterocytes. Here, an *in vitro* intestinal barrier model was developed consisting of Caco-2 and HT-29 cells, which in 14 days of co-culture differentiate into enterocytes and mucin-producing cells, respectively. Monolayer integrity was assessed by measuring trans-epithelial electrical resistance (TEER) before treating the cells for 24 hours with increasing doses of different fractions obtained from sorghum extract: (1) enriched in phytosterols (FAS), (2) enriched in polycosanols (FAP), and (3) without either (FNA). None of the treatments influences the cell viability and the barrier integrity with respect to control. Protein levels of NPC1L1 and the phosphorylated form of its transcription factor STAT-3 were then assessed. Results showed that the FAS fraction significantly reduced NPC1L1 protein levels by 40% similarly to the treatment with Ezetimibe, the drug currently used for NPC1L1 inhibition. Furthermore, unlike Ezetimibe, FAS results in a reduction in phosphorylation levels of STAT-3, suggesting a different mode of action between FAS and the drug that likely

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goes through regulation of NPC1L1 transcription. Finally, preliminary experiment on cholesterol transport in the different conditions are underway. The final goal should be the intake of supplements and/or functional foods enriched in sorghum extracts to support the ongoing therapies in the management of hypercholesterolemia. *The authors marked with an asterisk equally contributed to the work.

P-43-009
Endogenous MCFAs regulate glucose and lipid metabolism via GPR84

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Lipids, along with carbohydrates and proteins, are one of the essential macronutrients and serve as an important energy source. However, with the Westernization of diets, the excessive consumption of fats leads to metabolic disorders, such as obesity and diabetes. Long-chain fatty acids, such as palmitoleate, are recognized as risk factors for these disorders due to their lipotoxicity. In contrast, medium-chain fatty acids (MCFAs) have been demonstrated to offer metabolic benefits; however, the molecular mechanisms underlying these effects remain unclear. Among MCFAs, C10:0 and C12:0 are recognized as ligands for G protein–coupled receptor 84 (GPR84). Therefore, in this study, we investigated whether MCFAs-mediated GPR84 activation affects glucose and lipid metabolism using a high-fat diet (HFD)-induced obese *Gpr84*-deficient mouse model. Under HFD conditions, which increased endogenous MCFA production, *Gpr84*-deficient mice remained lean but exhibited hyperglycemia and hyperlipidemia compared to wild-type mice. As part of this mechanism, *Gpr84*-deficient mice not only exhibited impaired insulin secretion resulting from glucagon-like peptide-1 (GLP-1) secretion deficiency but also demonstrated exacerbated insulin resistance. Additionally, medium-chain triglyceride (C10:0) intake suppressed obesity, improved plasma glucose and lipid levels, and increased plasma GLP-1 levels in wild-type mice; however, these effects were partially diminished in *Gpr84*-deficient mice. Our results indicate that the long-term MCFA-mediated GPR84 activation improves glucose and lipid homeostasis. These findings provide valuable insights into the potential of targeting GPR84 as a therapeutic approach for treating metabolic disorders like obesity and type 2 diabetes. Previously published in: Nishida A, *et al*. (2024) *FASEB Bioadv*. 6: 526-538.

P-43-010
Anti-obesity effects of exopolysaccharides produced by sucrose-preferring gut microbes

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Excessive intake of high-fat and high-sugar diets disrupts energy balance, in particular, increased sucrose consumption has been associated with rising health issues, including obesity and diabetes. The gut microbiota plays a crucial role in host health by producing metabolites from dietary carbohydrates through bacterial glycometabolism; however, the mechanisms remain unclear. Gut microbes produce short-chain fatty acids (SCFAs) from indigestible polysaccharides. These SCFAs not only serve as energy sources for the host but also act as signaling molecules via G protein-coupled receptors (GPR41 and GPR43), influencing host metabolism and energy balance. Furthermore, some bacteria can produce exopolysaccharides (EPS), a type of indigestible polysaccharides, from sugars. In this study, we identified *Streptococcus salivarius*, as a high-EPS-producing commensal bacterium. On high-fat fed mice, *S. salivarius*-producing EPS (SsEPS) supplementation contributed to anti-obesity effects by promoting SCFAs production with changes in the gut microbiota. These results demonstrate that gut microbes prevent host obesity caused by excessive sucrose intake, through the EPS–SCFA–carbohydrate metabolism axis. Our findings reveal a key mechanism by which host-commensal interaction regulate energy homeostasis and suggest EPS as a novel strategy for preventing obesity and related diseases. Previously published in: Shimizu H, *et al*. (2025) *Nat Commun*. 16: 1145.

P-43-011
Diet-sensitive gut microbial metabolites define nicotine-induced metabolic alterations

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The nutrient environment in the body provides fundamental information for understanding energy metabolism. The gut microbiota is increasingly recognized as a crucial determinant of physiological functions in response to the host's nutritional condition. The dynamics of nicotine exposure, the major bioactive component of tobacco, can restructure the gut milieu and induce shifts in the commensal microbial community, including changes in microbial composition and metabolite signatures. Indeed, gut microbiota-derived metabolites that exhibit distinct changes in response to smoking and smoking cessation have been elucidated, with emerging evidence suggesting their intricate involvement in host metabolic regulation and the pathophysiology of metabolic disorders. However, the precise mechanisms linking host nutritional status, dietary microbial metabolites and metabolic balance under nicotine exposure remain largely unexplored. Our findings reveal the complex crosstalk between gut microbiota, their metabolites and host metabolic regulation under nicotine exposure, which is intricately modulated by nutritional contexts. We demonstrated that intraperitoneal nicotine administration influences body weight regulation and metabolic phenotypes, independently of caloric intake reduction. Our findings suggest that nicotine-induced weight loss is mediated by changes in the gut microbiota, particularly *Lactobacillus* spp. and their linoleic acid-derived metabolites, modulating metabolism under high-fat diet conditions. Overall, our study highlights the intricate interplay between nutritional condition, gut microbiota, and metabolic homeostasis, particularly related to smoking and smoking

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cessation, providing new insights into host–microbe interactions in metabolic regulation. Reference: Ohue-Kitano R et al. (2024) Sci Rep 14, 1056.

P-43-012
Safety and tolerability of oral glutamine supplementation in older adults: a randomized placebo-controlled study

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In this randomized, double-blind, placebo-controlled study, we assessed the effects of effervescent glutamine (EGln) in older adults recruited from three daycare centers. Participants received a daily dose of 12.4 g of oral effervescent glutamine (EGln group) or maltodextrin (placebo group) for 60 days. Fifteen patients in each group completed the study. The mean ages were 77.0 ± 9.1 years in the EGln group and 79.0 ± 6.9 years in the placebo group. Evaluations included aminogram, hemogram, plasma levels of glucose, prealbumin, albumin, urea, creatinine, uric acid, C-reactive protein, vitamin D, calcium, sodium, potassium, plasma activities of aspartate aminotransferase and alanine aminotransferase, inflammatory markers, and growth factors. Additionally, the potential effects of EGln supplementation on body mass index, right-hand grip strength, left-hand grip strength, right and left calf circumferences, 4-m gait speed, overall gait speed, and muscle mass were measured. The results showed that oral EGln did not cause significant changes in the evaluated parameters, and no adverse effects were reported. These findings indicate that the glutamine dosage used in this study was safe and well-tolerated. This research contributes to the broader understanding of glutamine supplementation, emphasizing its safety and supporting its potential as a viable intervention to maintain health in aging individuals.

P-43-013
Curcumin's anti-adipogenic effects on plasticizing agent perfluorodecanoic acid: *in vitro* study

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Adipogenesis is defined as the process of differentiation of preadipocytes to mature adipocytes. Inhibition of adipocyte differentiation can be used as a potential therapeutic strategy against adipogenesis and, thus, obesity. We aimed to investigate the adipogenesis process in widely used 3T3-L1 preadipocytes differentiated to adipocytes and to suppress the induced lipid accumulation with perfluorodecanoic acid (PFDA) during this differentiation process with curcumin (Cu.). PFDA, a plasticizing agent used in food containers to which humans may be exposed, has been observed to induce adipogenesis. Curcumin is known to have anti-adipogenic and anti-inflammatory properties. Cells were cultured with curcumin and PFDA separately or in combination. WST1 assay was used to determine cell viability. OilRedO staining was used to evaluate differentiation and intracellular lipid accumulation. Spectrophotometric measurement was performed for lipid quantification. Gene expression profiles of adipogenic genes PPARγ and C/EBPα were analyzed with RT-PCR. According to our WST1 results, high concentrations of curcumin and PFDA were observed to cause cell toxicity. According to the results of the OilRedO staining, Cu. doses that can suppress lipid accumulation on 3T3-L1 adipocytes were observed as 20, 25, 30, 35 and 40 μM, while PFDA doses that caused lipid increase were observed as 50, 75, and 100 μM. According to the gene expression experiment results, the Cu. doses that can suppress lipid accumulation on 3T3-L1 adipocytes were observed as 20 and 30 μM, while the PFDA doses that caused lipid increase were selected as 50 μM. However, it was observed that 30 μM curcumin dose was more effective than 20 μM. When used as a combination, 30 μM Cu. + 50 μM PFDA showed more significant suppression than 20 μM Cu. + 50 μM PFDA doses. In our preliminary results of RT-PCR, anti-adipogenic effects of curcumin were detected, and PFDA has the potential to induce adipogenesis alone and in combination. *The authors marked with an asterisk equally contributed to the work.

P-43-014
Exploring the anti-adipogenic effects of boron compounds by analyzing PPARγ and C/EBPα transcription factors

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Obesity is a global public health problem characterized by abnormal or excessive fat accumulation that may impair health. Preadipocytes transform into adipocytes by interacting with several transcription factors, such as PPARγ and C/EBPα and they are essential targets for obesity-related therapeutic approaches. Nutritional and medicinal substances like boric acid (BA) and calcium fructoborate (CaFB) may critically contribute to regulating adipogenesis and, thus, to treating obesity. CaFB is found in vegetables such as celery and broccoli, as well fruits such as grapes and plums. This study aimed to investigate the anti-adipogenic potentials of BA and CaFB by analyzing the gene expression levels of PPARγ and C/EBPα. 3T3-L1 preadipocyte cells were used as an *in vitro* culture model. Differentiation of preadipocytes to adipocytes was determined morphologically by OilRedO staining. The concentration of BA and CaFB was determined after finding IC_{50} doses by WST1 assay. RT-PCR analyses were performed for the expression of PPARγ and C/EBPα genes from mRNA after administration of 2 and 5 mM CaFB and 100 and 250 μg/mL BA to differentiate 3T3-L1 cells. ELISA assay was used for the measurement of PPARγ and C/EBPα protein expression. The expression of PPARγ and C/EBPα genes and protein levels were suppressed in boron-treated cells compared to the control. As far as we know, these results are the first to demonstrate the effects of CaFB on the mRNA and protein expression of PPARγ and C/EBPα in mouse adipocytes *in vitro*. CaFB administered at 5 mM was more efficient than BA for all-day intervals. This study demonstrated the anti-obesity effects of boron compounds by inhibiting lipid accumulation. Natural boron compounds may be alternatives in the fight against obesity without side effects. In this study, the beneficial impact of CaFB on the regulation of adipogenesis is

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reported, and the mechanisms underlying the anti-obesity effects of boron were provided. *The authors marked with an asterisk equally contributed to the work.

P-43-015
Establishing population-based indirect reference intervals through data mining

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Reference intervals (RIs) are crucial for the accurate interpretation of laboratory test results. The Clinical Laboratory Standards Institute (CLSI) recommends that each laboratory establish its own reference intervals based on the population it serves. Data mining techniques have made indirect methods more advantageous. The International Federation of Clinical Chemistry (IFCC) also supports the use of indirect reference interval. However, since the Endocrine Society recently withdrew its endorsement of specific thresholds of vitamin D [25(OH)D] for the general population, there is no universal consensus among the scientific community regarding optimal cutoff levels. Therefore, establishing RI for 25(OH)D has great importance. In this study 25(OH)D reference intervals were calculated using refineR and data collection preprocessing, model selection, and analysis processes were examined in detail. The derived reference intervals aim to provide a stronger foundation for clinical interpretation. This study analyzed 25(OH)D results that were routinely requested in our laboratory between January 1, 2022 and January 1, 2025. Test results were obtained from the Laboratory Information System. After filtering results for 24036 individuals were obtained. The median 25(OH)D concentration was 12.9 ng/mL (IQR: 8.91-18.8). According to the 2011 Endocrine Society criteria deficiency (≤20 ng/mL) was observed in 78.9%, insufficiency (21-29 ng/mL) in 16%, and sufficiency (≥30 ng/mL) in 5%. RI was calculated based on gender, age group, test request unit, and season. The manufacturer's reference interval was 7.4-44 ng/mL. Reference intervals determined using refineR (Unit: ng/mL): Women: 4.55-31.1, Men: 4.7-31.7, 18-40 years: 4.41-28, 41-65 years: 4.7-31.3, 66-80 years: 7.26-37.3, Winter: 4.62- 26.2, Summer: 4.47-32.9, Autumn:5.45-34.7, Spring:4.58-24.8. This study shows that the reference interval provided by the manufacturer may not fully reflect the actual distribution of the population.

P-43-016
Proof of principle study on the effect of bovine exosomes on *Escherichia coli* proliferation rates

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Exosomes, nanoscale extracellular vesicles secreted by various cell types, play pivotal roles in intercellular communication by transporting biomolecules such as proteins, lipids, and RNAs. Recent studies highlight the impact of exosomes on gut microbiota composition and function, influencing host health and disease states. Dietary microRNAs (miRNAs), encapsulated within these exosomes, have been observed to resist digestive processes [1]. Previous *in vitro* studies demonstrate that bovine miRNA bta-miR-154c withstands digestion, indicating a potential regulatory role in the gut microbiota. This study investigates the effect of bovine exosomes, derived from edible beef parts, on *Escherichia coli*, a facultative anaerobic bacterium integral to the human microbiome. *E. coli* cultures are exposed to synthesized bta-miR-154c mimics and bovine exosomes to assess their impact on bacterial proliferation compared to controls, evaluating the miRNA's regulatory effect. Additionally, the uptake of bta-miR-154c by *E. coli* is explored using a GFP-expressing strain and Cy3-conjugated miRNA mimics. Confocal microscopy and flow cytometry are used to investigate the internalization of miRNAs by *E. coli*. The physicochemical characterization of bovine exosomes is also performed using dynamic light scattering (DLS) determine their size distribution. Preliminary findings from similar studies indicate miRNA uptake, although further research is required to elucidate the specifics of intracellular localization and its functional consequences. These findings provide insights into the role of ingested miRNAs or exosomes in regulating the gut microbiota, advancing our understanding of their potential impact on human health and disease.
[1] Pieri et al, A bovine miRNA, bta-miR-154c, withstands in vitro human digestion but does not affect cell viability of colorectal human cell lines after transfection, FEBS Open Bio, 2022 12, 925-936.

LB-R-43-01
Wheat arabinoxylans and health

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Wheat arabinoxylans (AX), the primary non-starch polysaccharides in wheat grain, are gaining recognition for their functional and health-promoting properties. These dietary fibers, concentrated in the bran and aleurone layers, exhibit considerable structural diversity, which impacts their solubility, fermentability, and physiological effects. My research has focused on the structural characterization of AX, their functional properties in wheat-based foods, and their transformation during processing, particularly in breadmaking. Key findings from our work reveal that AX structure – specifically the degree of substitution and molecular weight – directly influences water retention, viscosity, and interactions with proteins and enzymes during food processing. These characteristics also determine the fermentability of AX by gut microbiota, leading to beneficial short-chain fatty acid production. Soluble AX fractions, in particular, have demonstrated potential prebiotic activity and may contribute to improved gut health, glycemic control, and satiety. We have also shown that processing methods, such as thermal treatment and enzymatic modification, can alter AX structure and enhance their bioaccessibility and functionality. These findings support the development of strategies to optimize AX content and structure in finished products. Furthermore, our studies demonstrate how AX interact with gluten and other wheat proteins, influencing dough rheology and final product quality, especially in whole grain applications. With increasing consumer demand for fiber-enriched and functional foods, AX offer a compelling opportunity to add value to wheat through health-focused innovation. Continued interdisciplinary research – from plant breeding to human clinical studies – is needed to fully

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harness the potential of wheat AX in promoting metabolic and digestive health while maintaining product quality and sensory appeal.

LB-R-43-02
Coffee extract as a potential therapeutic agent for hepatic steatosis: a cellular and molecular approach

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Hepatic steatosis, characterized by excessive lipid accumulation in hepatocytes, is a growing global health concern with limited effective therapeutic options. This study investigates the potential therapeutic effects of coffee extract on hepatic steatosis by examining its influence on lipid metabolism, oxidative stress, and inflammation in a cell culture model. A hepatic steatosis model was established by treating HepG2 cells with oleic acid (250 μM) and palmitic acid (125 μM) for 24 hours. Following lipid induction, the cells were treated with coffee extract at concentrations ranging from 50 to 1000 μg/mL for an additional 24 hours. The antioxidant properties of the extract, including total antioxidant content (TAC), free-radical-scavenging activity, and total phenol and flavonoid content, were analyzed photometrically at concentrations between 10–10000 μg/mL. Cell viability was measured using the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay, while intracellular reactive oxygen species (iROS) and lipid accumulation were assessed using H2DCFDA and Oil Red O staining, respectively. Clinical biochemical parameters were evaluated using commercial assay kits, and the expression of peroxisome proliferator-activated receptor alpha (PPAR-α) was analyzed via western blotting to investigate molecular-level effects. The results demonstrated that coffee extract significantly enhanced antioxidant capacity and phenolic content in a dose-dependent manner. It also reduced iROS levels and lipid accumulation while promoting cell viability without cytotoxic effects. Moreover, coffee extract treatment led to increased PPAR-α protein expression, suggesting a potential role in regulating lipid metabolism. These findings highlight the therapeutic promise of coffee extract in mitigating hepatic steatosis and suggest its potential application in developing novel dietary or pharmacological strategies for the prevention and treatment of this metabolic disorder.

LB-R-43-03
Effect of green coffee bean extract on oxidant/antioxidant status of kidney tissues of rats exposed to high-fat high-cholesterol diet

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Chlorogenic acid (CA) is a polyphenolic compound and its antioxidant potential is well-known. CA is the most abundant polyphenolic compound found in green coffee beans. The aim of this study was to investigate the effects of high-fat high-cholesterol (HFHC) diet alone and HFHC diet plus green coffee bean extract (GCBE) or CA on oxidant/antioxidant status of rat kidney tissues. Thirty-two male Wistar rats were divided into four groups (I-Control, II- HFHC, III- HFHC + GCBE, IV- HFHC + CA) with 8 rats in each group. The groups other than 'control' received HFHC diet for ten weeks. GCBE (20 mg/kg, standardized to 50% chlorogenic acid) and CA (50 mg/kg) were given daily by oral gavage to animals in group III and IV, respectively. At the end of the ten weeks duration (after 12 hours of fasting), all animals were sacrificed and kidney tissues were removed. Activities of the antioxidant enzymes namely; catalase (CAT), glutathione peroxidase (GPX), superoxide dismutase (SOD) and, as an indicator of oxidation, levels of malondialdehyde (MDA) were assayed in kidney tissues. HFHC diet alone decreased CAT (statistically not significant) and GPX ($p < 0.0001$) enzyme activities. Administration of GCBE (statistically not significant) and CA ($p < 0.04$) together with HFHC reversed this effect of HFHC on CAT, but did not have the same effect on GPX. HFHC had no effect on SOD either alone or in combination with GCBE or CA. Administration of HFHC alone ($p < 0.013$) or in combination with GCBE (statistically not significant) or CA ($p < 0.009$) decreased tissue MDA levels compared to the control group. HFHC diet partially suppressed enzymatic antioxidant defense in rat kidney tissue, whereas GCBE and CA (polyphenol) supplementation partially protected the kidney tissue against this effect. The decrease in MDA levels in the groups receiving HFHC diet was considered to be due to chronic exposure of the lipid structures in the tissue to oxidant effect.

Oxidative stress, oxidants and antioxidants

P-44-001
Correlation of erythrocyte reduced/oxidized glutathione, thiol-disulphide homeostasis and albumin/LDH ratio as oxidative stress markers in acute ischemic stroke

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The correlation between erythrocyte reduced/oxidized glutathione, serum thiols and LDH/albumin ratio as biochemical parameters that may be diagnostic indicators of oxidative stress observed after damaged brain tissue in cases diagnosed with acute ischemic stroke (AIS) was investigated. A total of 103 patients, 47 AIS patients and 56 healthy volunteers, were included in the study. Erythrocyte oxidized/reduced glutathione, thiol/disulfide homeostasis (total thiol-native thiol-disulfide changes) tests were performed in serum samples of 47 patients diagnosed with AIS with brain computed tomography and Brain Diffusion MRI. The results were compared with the Control Group and statistical analysis was performed. When the AIS and Control Groups were compared, oxidized glutathione (GSSG) and reduced glutathione (GSH) were found to be lower, while total glutathione (GSH + GSSG) was found to be significantly higher than the Control Group ($p < 0.001$). Total thiol ($p < 0.001$), native thiol level ($p = 0.01$) and disulfide levels ($p < 0.001$) were statistically significantly lower than the Control Group. Cut-off values for serum Albumin/LDH ratio and intracellular

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glutathione, native thiol/total thiols were calculated and found to be significant. In acute ischemic stroke, erythrocyte glutathione levels and serum thiols decrease in favor of oxidative stress, while albumin/LDH ratio increases.

P-44-002
Preclinical evaluation of waste-derived pomegranate extract as potential preventing and therapeutic for benign prostatic hyperplasia

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Benign prostatic hyperplasia (BPH) represents a noncancerous enlargement of the prostate gland resulting from hyperproliferation of the stromal and epithelium region. BPH affects more than 50% of men in the age group around 60 years and up to 90% between 70 and 80 years worldwide. Biological mechanisms involving angiogenesis, inflammation and oxidative stress sustain and promote cellular proliferation in BPH, which may lead to carcinogenesis. Current standard therapy approved for BPH treatment involves drugs which entail considerable side effects. Thus, alternative therapies including the use of phytotherapics and dietary supplements have gained increasing interest, in order to counteract BPH development and prevent pathological consequences. In this context, agricultural-waste-derived extracts can represent a valid and sustainable source of bioactive molecules, reducing waste disposal costs or rather exploit byproducts. Indeed, such extracts are rich in bioactive compounds endowed with health-promoting activity. Our work focused on the evaluation of the beneficial effects and chemo-preventive activity of a pomegranate waste extract (PWE) in preclinical models of BPH. In particular, *in vitro* observations confirmed antioxidant activity of PWE and its ability to reduce both proliferation and migration rates of BPH-1 cells, together with inflammatory cytokines (e.g. IL-6 and PGE2) levels and pro-angiogenic factors (VEGF-ADMA) release. Additionally, testosterone-treated rat model was adopted to evaluate potential PWE preventing effect through the analysis of inflammation and oxidative stress related markers (e.g. CD31, VEGF, NRF2). Moreover, the same analyses were conducted on rat periprostatic adipose tissue in order to investigate potential correlations within the very early stages of BPH development. Overall, PWE has demonstrated to be a valid candidate as an alternative therapeutic option for counteracting BPH progression.

P-44-003
Protective and restorative effects of ferulic acid on oxidative stress-induced damage in skin cells

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Ferulic acid (FA), a natural hydroxycinnamic acid in plant cell walls, is well-known for its antioxidant and free radical scavenging properties. This study evaluates FA's protective and restorative effects against oxidative stress-induced damage in HaCaT cells, a widely used human keratinocyte model. Oxidative stress caused by reactive oxygen species (ROS) is a major contributor to skin aging, inflammation, and various dermatological disorders. FA has gained attention as a therapeutic candidate for mitigating such damage and enhancing cellular resilience. To investigate its effects, HaCaT cells were seeded at 3×10 cells per well in 96-well plates, allowed to adhere for 12 hours, and exposed to 1 mM hydrogen peroxide (H_2O_2) for 2 hours to induce oxidative stress. Following this, cells were treated with FA at concentrations of 0.02, 0.05, 0.2, or 2.0 mM for 1 hour. Cell viability was assessed via the MTT assay. Results showed that H_2O_2 significantly reduced cell viability, with an IC value of 0.82 ± 0.5 mM, demonstrating the vulnerability of HaCaT cells to oxidative injury. Post-treatment with FA significantly improved cell survival in a dose-dependent manner. Importantly, FA exhibited no cytotoxic effects at the tested concentrations over 24 hours, underlining its safety and biocompatibility. Following H_2O_2 exposure, cell viability decreased to $51.3 \pm 1.82\%$, but FA treatment restored viability significantly, highlighting its restorative potential. These findings emphasize FA's potent antioxidant and cytoprotective properties, particularly as a post-injury intervention. FA's effectiveness supports its application in managing oxidative stress-related skin conditions, such as photoaging and inflammation, and its potential inclusion in dermatological therapies and skincare products.

P-44-004
Oxidative stress management in cardiovascular surgery

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Cardiac surgical procedures, particularly those involving cardiopulmonary bypass (CPB), pose significant oxidative stress, affecting both operative success and postoperative recovery. This pilot study investigates the dynamics of antioxidant enzymes and molecules, including superoxide dismutase (SOD), glutathione (GSH), glutathione peroxidase (GPx), and glutathione reductase (GR), in patients undergoing cardiac surgery. The primary objective is to explore the relationship between oxidative stress, antioxidant responses, and patient health, particularly concerning the duration of CPB. Thirty-five patients undergoing elective coronary artery bypass graft (CABG) surgery were divided into two groups based on CPB duration: less than or greater than 60 minutes. Blood samples were collected one hour before and one hour after surgery, and biochemical parameters were analyzed using established methods. Results indicated dynamic changes in antioxidant parameters: preoperative SOD activity was higher than postoperative levels and control values, while GPx and GSH concentrations decreased significantly postoperatively. GR activity showed an initial elevation preoperatively but fell below control levels post-surgery. These findings underscore the pronounced impact of surgical stress on antioxidant systems and the importance of monitoring oxidative status. The study provides critical insights into the biochemical changes associated with CPB duration, suggesting that antioxidant insufficiency

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post-surgery may influence recovery. Further research into additional antioxidant enzymes could refine strategies to mitigate oxidative damage, improve patient outcomes, and enhance care protocols in cardiac surgery. This work was supported by the project APVV-22-0357 (Non-invasive ncRNA-based diagnostics).

P-44-005
Serum levels of methylated arginine metabolites in ischemic stroke

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L-Arginine is a substrate for nitric oxide synthase, essential for vascular function and disease. Its derivatives, asymmetric dimethylarginine (ADMA) and symmetric dimethylarginine (SDMA), are markers of endothelial dysfunction linked to vascular disorders. ADMA is well-established as a cerebrovascular biomarker, while SDMA is emerging as significant in ischemic stroke. This study aimed to assess the serum levels of these metabolites in ischemic stroke patients to explore their roles in vascular dysfunction. The study included 90 ischemic stroke patients and 30 healthy controls. Serum levels of methyl arginine metabolites were measured using the AB SCIEX API 3200 LC-MS/MS method. Statistical analysis was performed using IBM SPSS Statistics 26.0. Serum levels of ADMA and HomoArginine were significantly higher in ischemic stroke patients than in controls: [0.13 (0.06-0.66) vs. 0.108 (0.044-0.18) ng/ml, $p = 0.001$], and [1.4 (0.38-6.58) vs. 0.927 (0.60-1.51) ng/ml, $p = 0.002$], respectively. Positive correlations were found between ADMA and SDMA, Ornithine [$r^2 = 0.510$, $p = 0.000$], and Ornithine and Arginine [$r^2 = 0.371$, $p = 0.000$]. This study shows elevated serum levels of ADMA and HomoArginine in ischemic stroke patients, suggesting their potential as biomarkers for endothelial dysfunction and cerebrovascular risk. These findings support the disruption of the L-arginine-NO pathway in ischemic stroke and highlight their diagnostic value.

P-44-006
Coenzyme Q10 enhances the efficacy of simvastatin in protecting cardiac cells from dyslipidemia-induced oxidative damage

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Dyslipidemia plays a crucial role in cardiovascular complications in individuals with type 2 diabetes (T2D). While statins are effective at reducing cholesterol and improving cardiovascular outcomes, long-term statin use depletes coenzyme Q_{10} (CoQ_{10}), a vital antioxidant and component of the mitochondrial membrane. This depletion may contribute to increased oxidative stress and myocardial damage, particularly in individuals with T2D. Objectives This study aimed to evaluate whether CoQ_{10} supplementation could enhance the therapeutic efficacy of simvastatin in protecting heart cells from oxidative stress-induced damage. H9c2 cardiomyoblasts were pre-treated with CoQ_{10} (2.5 μg/ml) and simvastatin (2.5 μM) for 24 hours before exposure to palmitic acid (0.25 mM), which induces dyslipidemia-related damage. Various markers of oxidative stress and cellular damage were measured, including mitochondrial function, reactive oxygen species (ROS) production, intracellular antioxidants, and apoptotic markers. Cholesterol content and lipid peroxidation were also assessed. CoQ_{10} supplementation effectively protected heart cells from dyslipidemia-induced oxidative damage. CoQ_{10} enhanced the ability of simvastatin to reduce cholesterol and lipid peroxidation, improve mitochondrial respiration, and decrease oxidative stress and cellular damage caused by palmitic acid exposure. CoQ_{10} reduced ROS levels and promoted mRNA expression of Nrf2, a key antioxidant response element. Notably, CoQ_{10} supplementation also lowered mRNA expression of lanosterol synthase, suggesting it may help regulate cholesterol production without reducing endogenous CoQ_{10} biosynthesis. These findings suggest that CoQ_{10} supplementation could provide additional cardioprotective benefits by improving simvastatin efficacy in reducing dyslipidemia-related oxidative damage in heart cells. However, more studies are needed to confirm these results and optimize treatment protocols.

P-44-007
Cardioprotective effect of endogenous glutathione stimulation in aged heart

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Aging is accompanied by mitochondrial dysfunction, oxidative stress and reduced resistance of myocardium to ischemia. The vast majority of studies suggest that cardioprotective mechanisms have seemed to be reduced with aging. Previously, we have found that pretreatment of 6-month-old rats with propargylglycine (PAG) and L-cysteine (P + L) provided cardioprotection via induction of glutathione. The aim of the present study was to test the effect of P + L in old animals. 24-month-old Wistar male rats were treated with PAG (11 mg/kg, 10 min) and L-cysteine (121 mg/kg, 30 min). Langendorf isolated heart model was used to evaluate myocardial resistance to ischemia-reperfusion. Heart homogenates were examined for MDA, H2O2, NOS activity, NOx metabolites (NO_{2-} + NO_{3-}), oxidized (GSSG) and reduced (GSH) glutathione. Cardiac mitochondria were isolated to study membrane potential, respiratory rate and H^+ leak. For statistics, Mann-Whitney test was used. P + L reduced oxidative stress in heart homogenates: GSH was increased by 51.4%, whereas GSSG, H_2O_2, MDA and were reduced by 59.5%, 22% and 48% respectively ($P < 0.01$ for all). Inducible NOS activity was reduced by 52.5% and Ca^{2+}-dependent NOS activity was restored by 2,2 times ($P < 0.01$). Together with reduced NOx metabolites by 25% ($P < 0.05$) these data suggest NOS recoupling in aged heart. Cardiac

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mitochondria membrane potential was higher (178.2 ± 1.1 vs 172.8 ± 1.3 mV in untreated) and respiratory rate was lower (253 ± 14 vs 287 mol O_2/min/mg protein in untreated). Together with decreased kinetics of proton leak, these data indicate improved coupling of oxidative phosphorylation in aged heart mitochondria. Notably, restoration of cardiodynamic parameters of isolated heart after ischemia was significantly improved in P + L group vs untreated. Thus, we showed for the first time that induction of glutathione with PAG + L-cysteine is a potent tool to improve mitochondria function and increase resistance of aged heart. The study was supported by NRFU grant 2021.01/0210.

P-44-008
Upregulation of 3-mercaptopyruvate sulfur transferase improves the function of the aorta in 24-month-old rats

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Aging is associated with increased vascular stiffness and decreased efficacy of vasorelaxant factors, such as hydrogen sulfide (H_2S) and nitric oxide (NO) production, and ATP-sensitive potassium (K_{ATP}) channels. We hypothesized that modulation of the activity of H_2S-synthesizing enzymes with propargylglycine (PG, 11 mg/kg) and L-cysteine (L-cys, 121 mg/kg) may restore vasorelaxation of aortic rings in 24-month-old Wistar rats. Our data showed that free radical processes and lipid peroxidation were increased in the aortic tissue of old rats. mRNA levels of the H_2S-synthesizing enzymes 3-mercaptopyruvate sulfur transferase (3-MST) and cystathionine γ-lyase (CSE) were reduced (2.2 and 4.2-fold, respectively), which correlated with a decreased H_2S level (3.2-fold) in aortic tissue. mRNA levels of endothelial NO synthase (eNOS), an important regulator in vascular function, were also significantly reduced (2.8-fold) in the aorta of old rats compared with adult rats. Endothelium-dependent relaxation of isolated aortic rings in old rats to acetylcholine in concentrations of 0.1, 1, and 10 μmol/L was significantly decreased by 2.3, 1.8-, and 1.7-fold, respectively, compared to control. Treatment with PG + L-cys restored the mRNA level of 3-MST in the aorta of old rats and increased the production of H_2S (by 2.3-fold). PG + L-cys restored constitutive NO production (eNOS expression) and relaxation responses of isolated aortic rings of old rats to control. We also found an increase in the mRNA expression levels of the pore-forming subunit Kir6.1 and the regulatory subunit SUR2 of vascular-type K_{ATP} channels (2.5 and 2.7-fold, respectively), which was accompanied by a significant increase (by 70%) in the relaxation responses of isolated aortic rings to the K_{ATP} channel opener flocalin. Thus, the improvement in aortic relaxation of old rats to modulators of H_2S synthesis is mediated by 3-MST, eNOS, and K_{ATP} channels. This study was supported by NRFU grant 2021.01/0210.

P-44-009
Pyridoxal-5-phosphate decreases cyclophilin D expression and sensitivity of mPTP to Ca^{2+} in cardiac mitochondria in 24-month-old rats

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Cyclophilin D (CypD) is a key regulator of the multiprotein non-specific Ca^{2+}-induced mitochondrial permeability transition pore (mPTP). Upregulation of mPTP occurs in aging, affecting Ca^{2+} transients in the cell, ATP synthesis, and the mitochondrial metabolic function in general. Gas transmitter hydrogen sulfide (H_2S) was shown to have significant effects on mitochondrial function, however, H_2S production is reduced with aging and considered a reason for age-associated arterial hypertension, heart failure, and endothelial dysfunction. Our study aimed to investigate the effect of pyridoxal-5-phosphate (H_2S-synthesizing enzymes cofactor (PLP)) on gene expression of Ppif, which encodes CypD, and sensitivity of mPTP to Ca^{2+} in old rat heart mitochondria. Wistar male rats were divided into 3 groups: adult (6-month-old), old (24-month-old), and PLP-treated old. PLP was administered per os in a dose of 0.7 mg per kg daily for 2 weeks. We have found a significant increase in H_2S content in the heart tissue of PLP-treated old rats compared to untreated ones. PCR analysis showed that the expression of the P*pif* gene was increased by 2.1 times in the heart tissues of old rats compared to adults, whereas PLP treatment reduced the Ppif mRNA level. Study of cardiac mitochondria swelling showed that treatment with PLP inhibited Ca^{2+}-induced opening of mPTP, thus decreasing sensitivity of mitochondria to Ca^{2+} and/or increasing Ca^{2+}-storing capacity of mitochondria. Simultaneously, the inhibition of the mitochondrial H_2S-synthesizing enzymes 3-MST and CSE by O-carboxymethyl hydroxylamine reduced the beneficial effects of PLP on mPTP opening. This indicates the H_2S-dependent mechanism of PLP treatment. Summarizing, our data showed that (1) H_2S is an important regulator of mitochondrial membrane permeability in aging; (2) PLP may possess mitoprotective effect through restoration of age-related H_2S deficiency and downregulation of CypD. The study was supported by NRFU grant 2021.01/0210.

P-44-010
Ecdysterone attenuates oxidative/nitrosative stress induced by synergistic activation of inducible NO synthase and xanthine oxidase in a rat model of type I diabetes

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Oxidative/nitrosative stress, dysregulation of constitutive NO synthase (cNOS), and the imbalance between ROS and RNS are the hallmarks of diabetes mellitus, which remains one of the leading causes of mortality worldwide. This calls for new approaches to treating the disease. The steroid hormone ecdysterone has shown beneficial therapeutic effects in several disease models. We aimed to investigate the effect of ecdysterone on cNOS activity, ROS and RNS production, and lipid

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peroxidation in heart tissue and mitochondria in a rat model of type I diabetes. The diabetes resulted in a dramatic suppression of cNOS activity, accompanied by a reciprocal 3-fold and 6-fold up-regulation of iNOS in mitochondria and heart tissue, respectively. This was consistent with a sharp increase in nitrate in mitochondria and nitrosothiols in heart tissue, and it was accompanied by an increase in superoxide production and diene conjugate content, indicating strong lipid oxidation. Instead, in mitochondria, iNOS activity was correlated with superoxide production, while in heart tissue, superoxide production was correlated with a dramatic upregulation of xanthine oxidase, which was also correlated with an increase in nitrate reductase activity. After ecdysterone treatment, there was a strong suppression of iNOS and a twofold increase in mtNOS activity in the mitochondria, but in the heart tissue, cNOS was only restored to half of the control. This resulted in a reduction of NO3- but a full restoration of NO2- production. Half inhibition of xanthine oxidase resulted in the corresponding reduction of ROS production in heart tissue, accompanied by strong suppression of lipid peroxidation. We concluded that up-regulation of iNOS in heart mitochondria, and strong lipid peroxidation, and possible synergistic actions of iNOS and xanthine oxidase in heart tissue, were the main factors of the oxidative/nitrosative stress and were abolished by ecdysterone treatment in a rat model of type I diabetes.

P-44-011
L-Arginine modulates lipid profiles, oxidative stress, and PON1 activity in diet-induced hypercholesterolemia

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L-Arginine, a semi-essential amino acid, exhibits antioxidant properties, modulating oxidative stress and lipid metabolism. However, its impact on lipid profiles and PON1 activity in hypercholesterolemic conditions remains underexplored [1]. This study aims to evaluate the potential of L-arginine in mitigating oxidative stress and improving lipid profiles and PON1 activity in a hypercholesterolemic rat model. Thirty-six adult male Wistar albino rats were randomly assigned to four equal groups: control (C), L-arginine (LA), hypercholesterolemia (HC), and hypercholesterolemia + L-arginine (HC + LA). Hypercholesterolemia was induced with a 3% cholesterol diet for eight weeks, while L-arginine was supplemented via 3% L-arginine in drinking water. PON1 activity, MDA levels, ATI, and the PON1/HDL ratio in the HC group was found to be elevated. L-Arginine supplementation in the HC + LA group led to a reduction in these markers compared to the HC group. Additionally, the LA group exhibited a significant increase in serum TG, VLDL, and PON1 levels compared to controls. Notably, a strong negative correlation was observed between PON1 and MDA levels, highlighting the potential antioxidant role of L-arginine in hypercholesterolemic conditions. Chronic oral L-arginine administration reduces ATI, serum LDL, and oxidative stress in hypercholesterolemia without affecting PON1 and HDL levels. Histopathological findings indicate that L-Arginine mitigates vascular structural alterations, suggesting a protective role against atherosclerosis. These results support its potential as a therapeutic agent, warranting further studies to elucidate underlying mechanisms and clinical relevance.

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P-44-012
Cysteinamide as a competitive glycation inhibitor to prevent the deterioration of serum components from glucose-derived reactions in diabetes-mimicking conditions

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In patients with diabetes, persistent hyperglycemia causes chemical changes in serum components, increasing the incidence of various complications such as skin wounds. This study explores small molecule nutrients to protect human dermal fibroblasts (HDFs) from the high glucose-derived harmful effects. Various cell culture media with different concentrations of glucose and various test compounds were heat-treated to mimic long-term chemical changes. There was no difference in the content of glycation products between the unheated low-glucose (LG, 5.5 mM) and high-glucose (HG, 50 mM) media or their effects on cell viability. However, the heated HG media had a higher content of glycation products and decreased cell viability compared to the heated LG media. Of the 20 free amino acids, 20 amidated amino acids, and various antioxidants derived from cysteine (Cys) or ascorbic acid (AA) added to the media followed by heat treatment, only cysteinamide (C-NH_2) enhanced the viability and wound healing of HDFs cultured in the heat-treated HG media. C-NH_2 mitigated the glycation of serum proteins and other components while generating its glycation products. This study identified C-NH_2 as a competitive glycation inhibitor that prevents the deterioration of serum components in diabetes-mimicking conditions. Additionally, N-acetyl cysteinamide (NAC-NH_2) showed similar effects to C-NH_2, but well-known glycation inhibitors, aminoguanidine (AGD) and pyridoxamine (PYM), did not. Therefore, C-NH_2 and NAC-NH_2 have distinct properties that can help maintain healthy body fluids in patients with diabetes, enhancing cell survival and promoting wound healing in the skin.

P-44-013
Quinuclidine derivatives trigger oxidative stress and mitochondrial dysfunction

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Quinuclidine-based derivatives have been recognized for their therapeutic potential, especially for designing drugs that target neurological disorders. This study explores the effects of derivates with different alkyl chain length (C8–C16) on quinuclidine core, specifically oximes (QNOH) and alcohols (QOH), on oxidative

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stress biomarkers to assess their cytotoxic effects. Our findings indicate that only C12-substituted derivatives induce reactive oxygen species (ROS) production, lipid peroxidation, and glutathione (GSH) depletion. Cytosolic superoxide dismutase (Cu/Zn-SOD) activity increased, while mitochondrial (Mn-SOD) decreased, correlating with reduced mitochondrial membrane potential. Since C12 chain length was identified as the threshold for oxidative damage, we further examined its impact on key signaling pathways. While no significant changes were observed in Akt or p38MAPK, NF-κB was dephosphorylated, suggesting insufficient antioxidant response and subsequent oxidative damage leading to cell death. STAT3 and AMPK, known regulators of cellular stress and metabolism, showed altered activity, linking mitochondrial dysfunction to impaired adaptive responses. Given that AMPK activation is often protective under oxidative stress, its involvement suggests a potential failure to counteract metabolic stress, while STAT3 alterations may contribute to dysregulated cell survival pathways. These results highlight the role of chain length in oxidative damage and suggest that C12 derivatives disrupt redox homeostasis and stress response signaling, contributing to cell death.

P-44-014
Mitigating bisphenol A-induced oxidative stress in serum: the protective effect of probiotics

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This study investigates the differential impacts of bisphenol A (BPA) exposure and *Lactobacillus rhamnosus* GG (LGG) on blood serum oxidative stress. BPA, a chemical found in a wide range of consumer products, has raised significant concerns due to its potential negative impact on human health. At the same time, probiotics, particularly LGG, have garnered attention for their promising therapeutic potential, including their ability to counteract oxidative stress. In this study, 24 Wistar Albino male rats were divided into control, BPA, and BPA + LGG groups. 50 mg/kg BPA was administered to BPA and BPA + LGG groups for six weeks. 1×10^9 CFU LGG was administered to the BPA + LGG group for six weeks. At the end of the sixth week, blood serum samples were obtained. In blood samples, total oxidant status (TOS), total antioxidant status (TAS) and oxidative stress index (OSI) were measured. Results revealed significant alterations in blood serum oxidative stress levels between the groups and increased oxidative stress in the blood serum of the BPA group. Conversely, LGG administration demonstrated a potential mitigating effect, with improvements observed in blood parameters and a reduction in serum oxidative stress. Comparative analysis of oxidative stress in blood serum samples reveals that LGG exhibits a potential protective role against the harmful effects of BPA. In conclusion, the protective role of probiotics against endocrine disruptors such as BPA can be highlighted as a therapeutic strategy to reduce oxidative damage and support the development of preventive approaches.

P-44-015
Initial results of oxidative and carbonyl stress markers in patients undergoing cardiac surgery on extracorporeal circulation

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Cardiac surgeries impose a considerable strain on the body and are frequently associated with a range of complications that can impact both the surgical outcome and the patient's recovery process. Our aim was to investigate the changes in selected oxidative and carbonyl stress markers in the blood of patients who have undergone coronary artery bypass grafting (CABG) with extracorporeal circulation (ECC) on different durations: less < 60 min, between 60 and 90 min and >90 min. Samples were collected one hour before surgery and one hour after surgery. The analysis focused on several markers, including superoxide dismutase, glutathione peroxidase (GPx), glutathione reductase, reduced glutathione (GSH), glutathione-S-transferase (GST), methylglyoxal, and glyoxalase 1. Although the sample size is currently small, the results are intriguing. Notably, patients receive antioxidant supplements prior to the procedure, which appear to be effective in combating superoxide radicals. However, the control of peroxides formation seems to be less efficient. In each of the three patient groups, GPx activity was significantly lower following ECC. Preliminary results suggest the formation of the dicarbonyl compound methylglyoxal. As observed in the metabolism of methylglyoxal, the increased activity of glyoxalase 1 seems to exert a protective effect on glutathione, as evidenced by the stable concentrations of unreduced GSH following ECC. However, the increased GST activities, particularly in the groups with ECC durations of < 60 min and >90 min, suggest that conjugation with glutathione-modified carbonyl adducts may occur, based on the measured parameters of carbonyl stress. The study currently involves a small sample size. However, if the results are consistently confirmed, minimizing the formation of lipid peroxides and dicarbonyl compounds will become a key objective in suppressing the inflammatory response and improving overall patient outcomes. *The authors marked with an asterisk equally contributed to the work.

P-44-016
The effect of radiotherapy on the level of vitamin C in patients with prostate cancer

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Among men in Poland, the highest incidence rate of 22% is recorded for prostate cancer. Patients have several treatment options. One of the most common treatments is radiotherapy, which causes so-called oxidative stress at the level of cancer cells

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and the entire organism. The aim of the study was to find out whether radiotherapy affects the intracellular content of ascorbic acid (AA) and its oxidized form – dehydroascorbate acid (DHA). Additionally, is there a correlation between intracellular and blood plasma vitamin C content? We have examined patients before and after radiotherapy. The material consisted of leukocytes and blood plasma from patients with prostate cancer, divided into groups supplemented and unsupplemented with vitamin C. The used methods: for determination of AA concentration in the blood plasma – UPLC/UV, for intracellular content of AA and DHA – 2D-UPLC-MS/MS. Based on this study, it was found that after radiotherapy, the supplemented group had higher levels of intracellular AA and DHA than the unsupplemented group. Moreover, we were unable to demonstrate a correlation between blood plasma and intracellular vitamin C. However, it is possible that blood plasma and the cell's interior interact with each other in the response to oxidative stress. In unsupplemented patients, radiotherapy causes a decrease in intracellular vitamin C to maintain its physiological concentration in the blood plasma (no differences after radiotherapy in the blood plasma). In contrast, supplemented patients after radiotherapy have a high concentration of ascorbate in the blood plasma to stabilize its cellular pool (no differences after radiotherapy in the intracellular content). This study was supported by the National Science Centre, Poland (grant No. 2017/27/B/NZ7/01487) and of the University Center of Excellence "Towards Personalized Medicine" operating under Excellence Initiative – Research University. *The authors marked with an asterisk equally contributed to the work.

P-44-017
Role of oxidative stress in cellular function and NLRP3 inflammasome activation in a renal cell model 2D and 3D

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Extracellular histones are released during the innate immune response, tissue damage, and cellular stress, and they have been directly related with organ failure in sepsis and septic shock. Extracellular histones can activate the immune system and trigger inflammatory responses, and induce tissue damage and oxidative stress. Histone-induced oxidative stress is characterized by an imbalance between the production of reactive oxygen species (ROS) and cellular antioxidant defense mechanisms. Cellular damage caused by oxidative stress is mediated by extracellular histones activates intracellular signaling pathways that lead to the activation of the NLRP3 inflammasome, which in turn promotes the release of proinflammatory cytokines (IL-1α, IL-1β, IL-18). Notably, elevated ROS levels serve as a crucial signal for inflammasome activation, initiating a cascade that results in inflammasome assembly and activation. The aim of this study is to elucidate how extracellular histones induce cellular dysfunction and death in three renal cellular models, as well as how extracellular histones produce oxidative stress and, thus NLRP3 inflammasome activation. To investigate the type of cell death, inflammation, and antioxidant response induced by extracellular histones, three renal cell lines—HEK293, RPTEC (proximal tubular epithelial cells), and glomerular endothelial cells—were utilized. Cell death was analyzed via flow cytometry, while gene and protein expression were assessed using RT-qPCR and western blot, respectively. Our study demonstrates that extracellular histones induce oxidative stress, which subsequently activates the NLRP3 inflammasome and pyroptosis. Finally, to explore the regulation of redox switches involved in the NLRP3 inflammasome activation and pyroptosis, we found that glutathione ethyl ester (GSH-EE) effectively reduced extracellular histone-mediated pyroptosis, decreasing both cell death and the expression of inflammatory cytokines released during pyroptosis.

P-44-018
Design and optimisation of cardiac models for the study of sepsis through the analysis of histone-associated cytotoxicity

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Understanding the pathophysiological mechanisms mediated by extracellular histones in the pathophysiology of sepsis is essential to identify new biomarkers and therapeutic targets. Sepsis causes multi-organ damage in which multiple tissues, such as the heart, are damaged. Therefore, the cytotoxicity of extracellular histones was evaluated in human cardiomyocyte models derived from induced pluripotent stem cells (iPSCs) and in a murine cardiomyocyte model (HL-1). The cardiac model was characterized by detection of tissue-specific proteins using confocal microscopy and flow cytometry. Flow cytometric analysis of annexin V/propidium iodide staining revealed a dose-dependent decrease in cell viability and increased apoptosis upon exposure to high concentrations of extracellular histones in both human cardiomyocyte and murine models. Additionally, qPCR analysis to evaluate the expression of genes encoding key antioxidant enzymes (CAT, SOD1, SOD2, GPX1, GPX4) and western blot assessment of their protein expression demonstrated significant alterations in CuZnSOD, MnSOD, Glutathione Peroxidase 1 and 4, and Catalase levels in cardiomyocytes. These findings suggest a dysregulation of antioxidant defenses, which may contribute to oxidative stress-related damage in response to elevated histone concentrations. In conclusion, our findings demonstrate that extracellular histones decrease cell viability and induce apoptosis in cardiomyocytes in a dose-dependent manner. The dysregulated expression of antioxidant enzymes suggests that oxidative stress plays a crucial role in cardiomyocyte damage, highlighting the

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potential involvement of extracellular histones in the pathogenesis of conditions such as sepsis.

P-44-019
Knotweed polyphenols reduce stress response in a cell model of neurodegeneration

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Neurodegenerative diseases, including amyotrophic lateral sclerosis (ALS) and frontotemporal dementia (FTD), represent a significant health burden worldwide. These diseases are characterized by progressive neuronal degeneration leading to motor dysfunction, cognitive decline and ultimately death. One critical aspect of neurodegenerative disease pathology involves the dysregulation of stress granules, cytoplasmic aggregates of RNA and proteins that form in response to cellular stress. They act as a protective mechanism that helps cells to recover from stress. However, aberrant stress granule dynamics have been linked to the progression of ALS, highlighting the need for therapeutic strategies to regulate them. Polyphenols, a diverse class of plant-derived compounds, exhibit antioxidant, anti-inflammatory, and neuroprotective properties, making them promising candidates for therapeutic interventions in neurodegenerative diseases. While previous studies focused on individual polyphenols, we investigated the effects of plant extracts from three knotweed species—*Reynoutria sachalinensis*, *Reynoutria japonica*, and *Reynoutria* × *bohemica*—on stress granule formation and neuroprotection. Using a neuroblastoma cell model expressing the stress granule protein G3BP1 fused to mScarlet, we tested the antioxidant activity of these extracts. None of the extracts exhibited cytotoxicity, and all showed potential neuroprotective effects by reducing stress granule formation under induced oxidative stress. These results suggest that polyphenol-rich knotweed extracts could regulate stress granule dynamics and have neuroprotective benefits, potentially contributing to the development of extract-based therapeutic interventions in ALS and related neurodegenerative diseases. Further studies are needed to elucidate the molecular mechanisms underlying these protective effects.

P-44-020
Evaluation of oxidative stress markers in lung cancer patients

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The aim of the study is to determine the oxidative stress markers in lung cancer patients and to investigate the correlation with the clinical prognostic factors. In this study, we investigated total antioxidant status (TAS), total oxidant status (TOS), oxidative stress index (OSI) and thiol/disulfide homeostasis levels. Forty lung cancer patients who applied to our hospital's Oncology clinic and 40 healthy volunteers who applied to the laboratory department for check-up were included in the study. The treatment protocols, histological grade and the stage of patients with lung cancer were known. Using the serum samples of the patient and control groups, oxidative stress markers were analyzed by colorimetric methods and evaluated together with clinical findings. The measurements of TAS, total thiol, native thiol were notably lower in the lung cancer group compared to the controls ($p = 0.001$, $p = 0.022$, $p = 0.004$). The measurements of TOS, OSI, disulfide, disulfide/total thiol, disulfide/native thiol were significantly higher in the lung cancer group compared the controls ($p = 0.003$, $p = 0.001$, $p = 0.003$, $p = 0.001$, $p = 0.001$). Oxidative stress markers did not show significant differences in the patient group according to histological type. ($p > 0.05$). TAS, TOS, OSI, total thiol, native thiol and disulfide measurements didn't not show statistically significant differences according to the stages ($p > 0.05$). Disulfide/total thiol and disulfide/native thiol measurements of the cases in the stage 4 group were found to be statistically significantly higher than those in the stage 3 group ($p = 0.047$). This study is one of the few study that investigate oxidative stress markers in lung cancer with prognostic factors. It was found that oxidant markers were high and antioxidant markers were low in lung cancer patients. In conclusion poor prognosis in lung cancer has a relationship between higher oxidative stress markers.

P-44-021
When oxidative damage goes undetected: TiO nanoparticles' stealth assault in dynamic intestinal models

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The intestinal epithelium serves as a key interface between the body and the external environment, maintaining selective barrier properties while enabling nutrient absorption. Standard *in vitro* models often lack physiological features, such as mechanical forces, which influence epithelial differentiation. We developed a Caco-2/HT29-MTX-E12 co-culture with mechanical stimulation (0.17 Pa) to better replicate the intestinal milieu [previously published in: Santoni et al. (2025) Front Mol Biosci 11, 1529027]. Dynamic culture accelerated epithelial differentiation, achieving functional properties indicative of a mature intestinal barrier by day 14 instead of day 21. Confocal microscopy revealed enhanced formation of fluid-filled domes under dynamic conditions, correlating with differentiation markers and indicating improved epithelial maturation compared to static culture. The co-culture was then challenged with *in vitro* digested skimmed milk containing TiO_2. We observed lipid peroxidation, elevated ROS production, and depleted antioxidant defences (GSH/GSSG), without membrane damage as shown by unchanged LDH release in the cell culture media. We analysed the cell media metabolite profile by NMR analysis. In the treated cells, significant alterations in cell membrane components, like

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choline-containing phospholipids, were found. These changes indicate peroxidation of polyunsaturated fatty acid chains, which can lead to accumulation of lipid hydroperoxides, membrane destabilization, and ultimately ferroptotic cell death. To confirm this mechanism involved in TiO_2-induced oxidative damage, NADPH oxidase activity is currently under study. This physiologically relevant intestinal model provides a robust microphysiological system for studying nanoparticle-epithelium interactions and advancing food safety assessments.
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P-44-022
Oxidative stress in diabetic patient-derived platelets and the role of natural antioxidants

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The activation of platelets (PLT) plays a key role in atherothrombosis and it has been reported that low-dose aspirin is less effective in type 2 diabetes mellitus (T2DM) patients. In fact, by inhibiting the biosynthesis of thromboxane (TX) A2, aspirin induces the permanent inactivation of PLT cyclooxygenase (COX)-1. Our main hypothesis is that diabetic patients showing poor aspirin (ASA) response have an abnormal PLT phenotype, mechanistically linked to oxidative stress, leading to accelerated PLT turnover and escape from ASA given once daily. Several studies revealed potent anti-inflammatory, antioxidant, and antiapoptotic effects of heme oxygenase (HO) in response to various stressors and particularly a strong association between HO-1 and cardiovascular risk was demonstrated. Indeed, evaluation of HO-1, glutathione (GSH) and lipid peroxides (LOOH) levels in PLT isolated from patients showed a marked oxidative stress feature. Additionally, *in vitro* results confirmed the possible coadjutant role of natural antioxidants in clinical use; in particular, resveratrol and quercetin were able to counteract lipid peroxidation induced by GPX4 inhibitor ML162 on DAMI cells. Resveratrol showed a higher cytoprotective capacity compared to quercetin; thus, its mechanism was further investigated in order to evaluate the modulation of heme oxygenase levels and glutathione (GSH) synthesis. ELISA assay and enzymatic activity showed an increase of heme oxygenase system following ML162 treatment suggesting a compensatory cell response to oxidative condition. Nevertheless, resveratrol treatment reduced ROS levels and restored HO-1 control levels. Overall, we confirmed that inhibition of oxidative stress may be a supporting therapeutic option in counteracting ASA resistance and platelet turnover in diabetic patients.

P-44-023
Evaluation of morpho- and metabolic modifications of red blood cells following exposure to bioactive compounds obtained by *Morus alba* leaves

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Morus alba is a member of the Moraceae family, known for biological properties and the sweetness of its fruits. The leaves and shoots of this plant are rich of bioactive compounds which give different applications and activities including hypoglycemic, hypotensive, and diuretic properties. The study focuses on the biochemical impact of leaf extract on red blood cells (RBCs). The antioxidant properties of the extract (25-50-100 μg/ml) were evaluated with several assays including chelating capacity of iron, ferric reducing power (FRAP), 2,2-diphenyl-1-picryl-drazil (DPPH), elimination of the superoxide anion and hydroxyl radical. The shape and integrity of cell membrane in response to different stresses and protective activity against RBC lysis was showed at concentrations of 50-100 μg/ml. These concentrations of the extract do not alter the oxidative state of hemoglobin and protect RBCs from the action of 2,2-azobis (2-amidinopropane) dihydrochloride (AAPH), as shown by the inhibited oxidation of intracellular and membrane sulfhydryl groups and reduced lipid peroxidation of the erythrocyte. Evaluation of cell viability confirms the antioxidant properties of the extract on RBCs, showing no caspase 3 activation, and the lack of apoptosis triggering. However, 100 μg/ml of extract reduces the kinetic flux trough the AE1 anion exchanger, inhibits PTP1B phosphatase activity and increase the ATP intracellular values suggesting the induction of RBC metabolic alterations. Further studies are currently in progress to improve and better define the impact of *Morus alba* extract on RBC metabolism. *The authors marked with an asterisk equally contributed to the work.

P-44-024
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P-44-025
Oxidative stress and antioxidant balance in pediatric hyperlipidemia

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The incidence of hyperlipidemia in childhood is increasing due to modern lifestyle and eating habits. In addition to genetic predisposition, high-fat diets, physical inactivity and obesity accelerate this process. The prevalence of hyperlipidemia in childhood may trigger oxidative stress mechanisms and predispose to various metabolic disorders. In this study, oxidative stress and antioxidant status were evaluated in children diagnosed with hyperlipidemia. This study was conducted with pediatric patients diagnosed with hyperlipidemia (n = 41) and

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age-sex-matched healthy controls (n = 49) who applied to Ankara Etlik City Hospital Pediatric Metabolic Diseases outpatient clinic between August 2024 and February 2025. The blood samples were centrifuged at 1300 g for 10 minutes. The supernatant portions were transferred to Eppendorf tubes and stored at -80°C until the time of the study. In this study, total antioxidant level (TAS) and total oxidative stress status (TOS) were measured using a commercial kit and OSI value was calculated. In addition, malondialdehyde (MDA) levels were measured to evaluate lipid peroxidation. Glutathione (GSH) levels, one of the important antioxidants, were also determined spectrophotometrically. $p < 0.05$ was considered significant for the results. Hyperlipidemia patients and healthy controls did not differ significantly in age and sex ($p > 0.05$). When the control group and the hyperlipidemia group were compared, it was seen that TOS, OSI and MDA levels increased significantly ($p < 0.05$). There was no difference in TAS, GSH levels ($p > 0.05$). These results suggest that oxidative stress is significantly increased in hyperlipidemia, but the endogenous antioxidant defense system is unchanged, thus a potential imbalance may contribute to disease progression. This study was approved by the Ankara Etlik City Hospital Scientific Research Evaluation and Ethics Committee (Code number: AEŞH-BADEK-2024-761). This study was supported by TUBITAK Project number 1919B012302886.

P-44-026
The role of thiotaurine in hydrogen sulfide metabolism: production, storage, therapeutic potential

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Reactive sulfur species (RSS) produced by oxidation of hydrogen sulfide (H_2S) are collectively named sulfane sulfur-containing molecules. Sulfane sulfur is a highly reactive sulfur form with the unique ability to attach reversibly to other sulfur atoms. Due to its capacity to react with protein thiols to generates persulfides, sulfane sulfur plays regulatory roles in biological systems. The transsulfuration pathway is the main source of sulfane sulfur-containing compounds. However, beyond the transsulfuration path, H_2S oxidation pathways can generate a series of RSS involved in various pathophysiological processes. Interestingly, thiotaurine is a cysteine-derived metabolite bearing a reactive sulfane sulfur. Thiotaurine is produced metabolically by transsulfuration, and it has been investigated for its role in modulating inflammation by influencing human neutrophil functional responses. As a result, thiotaurine with its peculiar sulfane sulfur moiety, can play a role in H_2S-mediated cell signaling mechanisms. In this study, the generation of thiotaurine from hypotaurine and H_2S by horseradish peroxidase was observed. Heme-dependent sulfide oxidation pathways were revealed to be significant sources of RSS. Furthermore, the formation of hypotaurine and the release of H_2S from thiotaurine were investigated using ascorbate and glutathione. Finally, we propose thiotaurine as a safe, non-toxic storage form of H_2S and a key intermediate in the biochemical routes of transport, storage and release of sulfide. Given these properties, thiotaurine could be considered as H_2S/sulfane sulfur donor with potential therapeutical application. The health benefits of sulfur-based therapy, such as sulfur water springs, garlic, and cruciferous vegetables, has been recognized since ancient times. Notably, the common denominator in these remedies lies in their ability to supply H_2S/sulfane sulfur, further supporting the therapeutic potential of sulfane sulfur compounds, such as thiotaurine.

P-44-027
Pinitol improves diabetic foot ulcers in streptozotocin-induced diabetes rats through upregulation of Nrf2/HO-1 signaling

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Diabetic foot ulcers represent a severe complication of diabetes, frequently resulting in amputation and high mortality rates. There is currently no treatment for diabetic foot ulcers other than antibiotics and dressings. In this study, we evaluated the wound healing effects of an antidiabetic agent, pinitol, in lipopolysaccharide (LPS)-damaged human dermal fibroblasts (HDFs) and streptozotocin (STZ)-induced diabetic rat models with a wound on its foot. Our findings indicated that pinitol enhanced cell migration, proliferation, and wound healing by activating Nrf2, thereby mitigating oxidative stress and inflammatory responses at the wound site. Additionally, pinitol recovered mitochondrial energy metabolism, decreased matrix metalloproteinase (MMP) activity, and increased collagen deposition. Furthermore, pinitol facilitated angiogenesis, which contributed to improved wound healing. Taken together, these findings suggest that pinitol can be a promising therapeutic agent for diabetic foot ulcers treatment.

P-44-028
Protective effect of quercetin against lithium-induced oxidative stress in rat kidney tissue

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This study investigated the protective effects of quercetin, a flavonoid with potent antioxidant properties, against lithium carbonate-induced kidney damage. Forty male Wistar rats were randomly divided into four groups: control (Group 1), lithium carbonate (LC) (Group 2), quercetin (Q) (Group 3), and lithium carbonate + quercetin (LC + Q) (Group 4). Lithium carbonate was administered orally at a dose of 45 mg/kg/day, and quercetin at 50 mg/kg/day for 35 days. Serum biochemical analyses and oxidative stress markers in kidney tissue were evaluated, and histopathological examinations were performed to assess structural changes. Statistical analyses were conducted using one-way ANOVA followed by Tukey post-hoc tests. In the LC group, serum creatinine and BUN levels increased by 13.79% and 16.78%, respectively, compared to the control group, with a statistically significant increase in BUN (Group 1-2; $p = 0.019$).

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In the Q group, BUN levels decreased by 15% compared to the LC group (Group 2-3; $p = 0.021$). Kidney tissue SOD levels decreased by 11.48% in the LC group compared to the control group (Group 1-2; $p = 0.045$), while an 18% increase was observed in the Q group compared to the LC group (Group 2-3; $p = 0.005$). Kidney tissue GSH-Px levels showed an 80.41% decrease in the LC group compared to controls (Group 1-2; $p < 0.001$). GSH-Px levels increased by 103% in the LC + Q group compared to the LC group (Group 2-4; $p = 0.022$) and by 168.4% in the Q group compared to the LC group (Group 2-3; $p = 0.014$). Histopathological evaluations revealed marked tubular degeneration, interstitial inflammation, and glomerular structural deterioration in the LC group, whereas these findings were minimal in the LC + Q group, with preserved glomerular structures. In conclusion, quercetin reduced lithium-induced oxidative stress and kidney damage, indicating its potential as a protective agent against lithium-associated nephrotoxicity.

P-44-029
In vitro and *in silico* analysis of the antioxidant profiles of polyphenolic compounds: hesperidin, salicylic acid, and 5-fluorosalicylic acid

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The aim of this study is to evaluate the antioxidant properties of phenolic compounds hesperidin, salicylic acid and 5-fluoro salicylic acid by *in vitro* and *in silico* analyses. 5-Fluoro salicylic acid, one of the selected compounds, is an analogue of salicylic acid. In *in vitro* analyses, the reducing capacity and radical scavenging activities (DPPH and hydroxyl radical scavenging activity) of the compounds were measured and the binding affinities of these compounds to some of the enzymes in the antioxidant system were analyzed by *in silico* modeling. COX-1 and COX-2 indirectly contribute to oxidative stress by generating reactive oxygen species (ROS). *In silico* results showed hesperidin as the most effective COX-1 and COX-2 inhibitor. Superoxide dismutase (SOD), a crucial antioxidant enzyme, must increase under oxidative stress to maintain cellular functions. Hesperidin exhibited the highest SOD activation, while salicylic acid and 5-fluoro salicylic acid also enhanced SOD activity without significant differences between them. Catalase (CAT), which decomposes hydrogen peroxide into water and oxygen, was activated by salicylic acid and 5-fluoro salicylic acid, but hesperidin, due to its larger structure, could not bind to its active site. *In vitro* evaluations performed in parallel with *in silico* analyses also determined that hesperidin was the best radical scavenger. While phenolic compounds are known antioxidants, their mechanisms remain partially unexplored. This study highlights hesperidin's superior inhibitory and activating effects on COX-1, COX-2, and SOD, whereas salicylic acid and 5-fluoro salicylic acid mainly activated SOD and CAT. Overall, hesperidin demonstrated the strongest antioxidant potential, suggesting its relevance for targeted antioxidant therapies in oxidative stress-related diseases.

P-44-030
Transcriptional activation of endogenous glutathione peroxidases by CRISPR/dCas9 technology to improve the antioxidant protection of enterocytes *in vitro* and *in vivo*

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Oxidative stress (OxS) triggered by an imbalance between reactive oxygen species (ROS) production and their removal by the antioxidant system is a critical risk factor for the inception and evolution of cardiometabolic diseases (CMD). The small intestine (SI) has a crucial role in nutrient absorption and plays a barrier function which is impaired in CMD. The first line of defense against ROS is represented by the antioxidant enzymes. We aimed to activate the endogenous glutathione peroxidases (GPx1/2) by the CRISPR/(d)Cas9 system in cultured enterocytes (Ent) and in apolipoprotein E deficient ($apoE^{-/-}$) male mice. To this purpose, human Ent (Caco-2) were transfected with GPx1/2 CRISPR activation plasmids, and their expression and activity were measured in cells lysates. GPx1/2 functionality was evaluated by measuring the effect of transfected Ent conditioned medium (CM) on TNFα-activated endothelial cells (EC) as ROS level, NADPH oxidase subunits and cell adhesion molecules expression, and monocyte adhesion. *In vivo*, the activation plasmids for GPx1/2 + jetPEI transfection reagent were injected by tail vein in $apoE^{-/-}$ mice fed with high fat diet. Animals were euthanized after four weeks, and blood, SI and aorta were collected. Blood glutathione (GSH) levels, serum antioxidant paraoxonase 1 (PON1) activity, GPx1/2 expression in SI were quantified, and atheroma evaluated. Results showed that *in vitro* GPx1/2 transcriptional activation determines their upregulation in Ent, and CM reduced OxS and inflammation in TNFα-activated EC. *In vivo* GPx1/2 transcriptional activation increased their expression in SI, GSH levels and PON1 activity in blood, and diminished atheroma extent. In conclusion, the successful transcriptional activation of the endogenous GPx1/2 by the CRISPR/dCas9 system might be a promising approach for an enhanced anti-oxidant protection which may induce a better anti-atherogenic defense. Work supported by PNRR-III-C9-2022-I8, CF-197/2022 (760059/23.05.2023).

P-44-031
Resveratrol protects against high glucose-induced damage in cultured human retinal epithelial cells

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Prolonged exposure to characteristic hyperglycemia in diabetes is responsible for changes at the retinal level, including those in the retinal pigmented epithelium (RPE). To simulate hyperglycemia in D407 RPE cells, high glucose concentrations (30 mM and 50 mM) were used for 24 hours. Herein, we investigated the protective effects of resveratrol (RV) toward D407 RPE cells exposed to glucose-induced stress. First, the level of the

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internalized RV in D407 cells was determined by HPLC. Next, the cellular viability and the potential toxicity of glucose and/or RV on D407 cells were evaluated by using WST-1 and LDH assays. The quantification of vascular endothelial growth factor (VEGF) and interleukin-6 (IL-6) levels was established using an enzyme-linked immunosorbent assay (ELISA). The viability of D407 cells was unaffected, but the level of LDH increased compared to that of untreated cells. RV stimulates the activity of the enzyme glutathione reductase under both normal and high glucose conditions. According to HPLC analysis, the high-glucose stress increased the cellular uptake of RV. The expression of VEGF and IL-6 was reduced after RV administration. All these findings shed light on the potential protective effects of RV on RPE cells in the fight against DR.

P-44-032
Multi-omics elucidate the health improvement after hydroxytyrosol supplementation in mice

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Phenolic compounds of plant origin have emerged as promising tools for preventing cardiometabolic chronic diseases. Hydroxytyrosol (HT), found in extra virgin olive oil, exhibits antioxidant and anti-inflammatory properties. However, prior animal and human studies have administered HT in conjunction with other bioactive compounds, limiting the ability to directly associate it with specific molecular mechanisms and health benefits. Omics technologies can elucidate the bioactive mechanisms of action, highlighting their biological significance. This study aimed to evaluate the molecular changes induced by HT consumption in a mouse model. Athymic nude mice were allocated into two experimental groups: controls ($n = 5$) and HT ($n = 5$). The HT group received 50 mg/kg/day of HT via oral gavage. Blood samples were collected after 2 months of treatment to integrate three omics analyses (transcriptomics, metabolomics, and proteomics). PLSDA analysis identified 15 altered metabolites following HT treatment, including increased levels of xanthine, hypoxanthine, xanthosine, and glutathione, all with known antioxidant properties. Additionally, 25 proteins associated with anti-inflammatory activities, such as Haptoglobin (HPT) ($p = 5.3 \times 10$), LYAM2 ($p = 0.001$), A1AG2 ($p = 0.002$), and Ceruloplasmin (CERU) ($p = 0.003$), were overexpressed, elucidating the complexity of HT treatment. Enrichment analysis of transcriptomic data indicated that 242 differentially expressed genes (DEGs) related to wound healing, blood coagulation, and hemostasis were primarily associated with HT treatment. These findings suggest that regular HT intake may influence metabolic reprogramming in platelets and various signaling pathways, potentially contributing to the prevention of cardiovascular diseases and diabetes mellitus. This study supports the application of foodomics to elucidate how bioactive compounds, such as HT, impact the metabolome, proteome, and gene expression.

P-44-033
Investigation of the protective effect of silibinin on methotrexate-associated ovarian damage

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Methotrexate (MTX), which has been used in the treatment of cancer, rheumatoid arthritis and psoriasis since the 1950s, is known to cause side effects that reduce quality of life due to its cytotoxic function, despite its role in suppressing tumor proliferation. Silibinin (SLB), which is among the natural products used in the treatment of different diseases for centuries, is known for its antioxidant and anti-inflammatory properties. Despite the evidence that SLB reduces the toxicity of chemotherapeutic agents such as cisplatin and doxorubicin in kidney, liver and heart tissues, studies showing the efficacy of SLB against chemotherapeutic agent toxicity are limited. In the study, 250-300 g Wistar albino female rats were divided into 5 groups with 6 rats in each group: Control, MTX, MTX + SLB (1-2 mg/kg), SLB (2 mg/kg). MTX was administered intraperitoneally at a dose of 20 mg/kg on the first day, followed by three consecutive SLB injections in the treatment and per se groups. On the 5th day, ovarian tissues removed from decapitated rats were biochemically analyzed for malondialdehyde (MDA), total oxidant levels (TOS), oxidative stress index (OSI) and total antioxidant (TAS) levels in order to evaluate MTX-induced oxidative damage and antioxidant effect of SLB. In the study, the decreases in MDA, TOS and OSI levels in MTX + SLB (1-2mg/kg) groups compared to the control group were statistically significant ($p < 0.05$). In addition, the decreases in TAS levels in MTX group compared to the control group were significantly increased in SLB-treated groups ($p < 0.001$). The results showed that SLB exerts a therapeutic effect against MTX-induced ovarian damage by reducing oxidative stress levels and increasing antioxidant levels. Although SLB seems to be a therapeutic agent for the amelioration of MTX-induced ovotoxicity, molecular studies should continue to be investigated to evaluate the positive effects of SLB in ovotoxicity and its possible clinical use.

P-44-034
Effects of caloric restriction or caloric restriction plus resveratrol short-term treatment on mitochondrial biogenesis and quality in aged rat soleus skeletal muscle

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Resveratrol (RSV) is a natural polyphenol with antioxidant and anti-aging properties. Results by our group have shown that its short-term administration in aged rat impinges on retrograde nuclear communication blunting mitochondrial biogenesis, crucial in safeguarding skeletal muscle functionality [Previously

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published in: Di Lorenzo R et al. (2024) Exp Gerontol 194, 112485]. RSV is a caloric restriction (CR) mimetic that ameliorates skeletal muscle metabolism in aging. This work aimed at evaluating the effects of six weeks of CR alone or coupled with RSV (50 mg/kg/day) (CR + R) on mitochondrial biogenesis, quality and dynamics in Sol samples from 27 months old male rats. In CR and CR + R rats, no changes were found in protein levels of SIRT1 and TFAM and a decrease, more marked in the last group, was identified in PGC-1α and PRXIII compared with controls. Both groups also showed an increase in SIRT3 and SOD2, especially in CR rats, likely indicating an improved antioxidant activity elicited by CR. The mtDNA relative content decreased in CR rats, while the frequency of the oxidized base OH8dG and DRP1 protein amount increased in all treated groups. Taken as a whole, these results indicate that neither CR nor CR + R was able to counteract the age-related oxidative stress in the aged Sol skeletal muscle. One explanation for this could reside in the lack of mitochondrial biogenesis activation by CR, and even more by CR + R. Due to the different fiber type-specific responses to CR and CR + R a deeper investigation is needed to consider which dietary strategies may help counteracting age-related skeletal muscle decline.

P-44-035
Investigation of the effects of sodium fluoride and gabapentin on an experimental epilepsy model created in zebrafish

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Our study aimed to examine the effects of sodium fluoride and gabapentin in an experimental epilepsy model in zebrafish. After the embryos obtained from zebrafish were grown under appropriate conditions for 48 hours, they were divided into 5 groups: Control group, pentylenetetrazol (PTZ) group, PTZ + Sodium fluoride (NaF) group, PTZ + Gabapentin (GABP) group, PTZ + NaF + GABP group. NaF and Gabapentin exposure was made 15 minutes after the freshly prepared PTZ application every day. Lipid peroxidation (LPO) and nitric oxide (NO) levels, as well as superoxide dismutase (SOD) and catalase (KAT) activity were examined oxidative stress from the obtained lysates. In addition, in order to evaluate seizure development in zebrafish embryos, the average speeds, exploration rates and distance traveled of the embryos were examined. Compared to the control group, SOD and CAT activities decreased in all exposure groups, while MDA and NO levels increased ($p < 0.0001$). Compared to the PTZ group, MDA and NO levels significantly decreased in the PTZ + NaF, PTZ + GABP, PTZ + NaF + GABP groups, while SOD and CAT activities significantly increased ($p < 0.0001$). Average speeds, Exploration rates and Distance traveled significantly increased in all exposure groups compared to the control group ($p < 0.0001$). Compared to the PTZ group, average speeds, exploration rates and distance traveled were significantly reduced in the PTZ + NaF, PTZ + GABP, PTZ + NaF + GABP groups ($p < 0.0001$). According to our findings, GABP is an antiepileptic agent that can be used to minimize neuronal damage caused by NaF.

P-44-036
Protective effects of kefir against oxidative damage induced by deep-fried palm oil consumption in mice: impacts on blood lipids, lipid peroxidation, and spermatological parameters

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The widespread use of palm oil in the fast-food industry raises concerns about its potential health risks, including oxidative stress and reproductive health impairment. This study aimed to investigate the effects of deep-fried palm oil consumption on blood lipids, lipid peroxidation, and spermatological parameters in mice while evaluating the protective efficacy of kefir. Twenty-four BALB/c mice were randomly assigned to three groups ($n = 8$ per group): Control, which received a standard pellet diet (2.48% fat); Palm, which was fed a diet enriched with deep-fried palm oil (15.6% fat); and Palm + Kfr, which received the same palm oil-enriched diet along with kefir supplementation (0.5%). After a 9-week experimental period, the mice were euthanized, and serum levels of total cholesterol, triglycerides, malondialdehyde (MDA), and testosterone were analysed. Additionally, MDA levels and Johnsen scores were assessed in testicular tissues and sperm suspensions. The findings revealed that palm oil supplementation significantly increased lipid peroxidation, as indicated by elevated MDA levels in both plasma and testicular tissues ($p < 0.05$). Moreover, total cholesterol levels ($p < 0.01$), abnormal sperm percentage ($p < 0.01$), and epididymal fat accumulation ($p < 0.05$) were significantly higher in the Palm group compared to the control group, while the Johnsen score was significantly reduced ($p < 0.01$). However, these detrimental changes were notably reversed in the Palm + Kfr group. Notably, sperm motility was significantly enhanced in the Palm + Kfr group compared to both the Ctrl ($p < 0.01$) and Palm groups ($p < 0.001$). The findings suggest that kefir exhibits antioxidant properties that may mitigate the adverse effects of deep-fried palm oil consumption. This study highlights the importance of dietary interventions in counteracting oxidative stress-induced reproductive damage. Further research is warranted to explore the long-term implications and potential applications in human health.

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P-44-037
Natural antioxidants ovothiols: from organismal function to human health benefits

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Ovothiols are sulfur-containing natural products, characterized by unique chemical properties suggestive of different cellular functions. They may act as cytoprotectants against oxidative stress and play key roles in marine organisms' defence mechanisms, adaptation, and communication. The enzymes responsible for ovothiol biosynthesis are similar to those involved in the production of ergothioneine, recently recognized as a longevity vitamin, which shares with ovothiols similar structural and antioxidant properties. In this work, we have identified ovothiol-like molecules in corals and microalgae, like diatoms. Moreover, we have characterized the antioxidant and photoprotective properties of ovothiol derivatives, whose chemical structure is inspired by these natural products. Our results show that different species of corals like the octocoral *Eunicella singularis*, the Hexacorallia *Astroides calycularis* and *Anemonia viridis* are an extraordinary example of chemical diversity in ovothiol A, B, and C [previously published in Zuccarotto A et al. (2025) Free Radic Biol Med. 227:117-128]. On the other hand, the diatom *Phaedodactylum tricornutum* produces exclusively ovothiol B. The screening of the biological activities of ovothiols derivatives shows that these products display redox and UV-protective properties on human dermal fibroblasts. In detail, they make redox exchange with glutathione and reduce both cytosolic and mitochondrial reactive oxygen species induced by UV exposure in human dermal fibroblasts. Overall, our results shed light on novel antioxidant and photoprotective molecules in corals and microalgae, which likely use them to counteract oxidative stress induced by environmental stressors. On the other hand, the structural and biological properties of synthetic chemical derivatives suggest their potential as dietary antioxidant supplements or UV screening compounds, resembling the action of the most known small thiol, ergothioneine.

P-44-038
Investigation of antioxidant and antidiabetic activities of different extracts of *Vitis labrusca* fruit peels

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Diabetes is a chronic disease. Natural products represent a significant source for the identification of active substances that can be used in the management of diabetes. The present study investigates the antioxidant properties and antidiabetic activities of *Vitis labrusca*, a plant whose purple-coloured fruits naturally grow in the Black Sea region. Ethanol and DMSO extracts of *Vitis labrusca* fruit peels were prepared by maceration. Total polyphenol content of the extracts was determined by Folin-Ciocalteu method, total flavonoid content by aluminium chloride colorimetric method, and antioxidant capacity by iron reducing power and DPPH (2,2-diphenyl-1-picrylhydrazyl) methods. In the antidiabetic activity method, IC_{50} values of alpha amylase and alpha glucosidase enzyme inhibition were measured in mg/ml. According to the data obtained, the polyphenol content of ethanol and DMSO extracts were 7.534 ± 0.232 and 18.998 ± 0.232 mg gallic acid equivalent/g extract, respectively, total flavonoid content was 0.791 ± 0.061 and 1.442 ± 0.061 mg quercetin equivalent/g extract, 23.587 ± 1.27 and 40.979 ± 0.445 mg trolox equivalent/g extract, DPPH IC_{50} values were $196.36 \pm 2{,}033$ and 55.91 ± 0.45 μg/ml. Antidiabetic activity IC_{50} values were 9.62 ± 0.18 and 9.086 ± 0.20 mg/ml for alpha amylase enzyme inhibition and 17.637 ± 0.33 and 17.01 ± 0.17 mg/ml for alpha glucosidase enzyme inhibition. In the data obtained, it was determined that the antioxidant activities and antidiabetic activities of ethanol and DMSO extracts of *Vitis labrusca* fruit peels were different and the highest value belonged to the DMSO extract in all analyses. It is predicted that the differences between the results are due to the difference in the solvents used to obtain the extracts and these results are thought to be due to the difference in the polarity of the solvents. *The authors marked with an asterisk equally contributed to the work.

P-44-039
Development of curcumin-based nanoparticles as an antioxidant and anti-inflammatory formulation for topical application

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The skin acts as a protective barrier being constantly exposed to oxidative stressors, increasing cellular reactive oxygen species (ROS) and causing cell dysfunction. Curcumin, a polyphenol from *Curcuma longa* is known for its antioxidant, anti-inflammatory, and anticancer properties, and may mitigate oxidative stress and inflammation. However, its poor bioavailability, low solubility, and instability limit its application. Solid lipid nanoparticles (SLN) offer a promising solution delivery system enabling its controlled release. This work aimed to evaluate the antioxidant and anti-inflammatory activity of curcumin and develop SLN for its encapsulation and dermal application. Free radical scavenging assays with biological relevance (hydroxyl radical ($^{\bullet}OH$), superoxide $O2^{\bullet-}$, and nitric oxide ($NO^{\bullet}$)), using colorimetric methods, confirmed the antioxidant of curcumin. The safety profile of curcumin was assessed, in skin (HaCaT) and RAW 264.7 (macrophage) cell models, and concentrations ≤ 25 μM were non-toxic. Curcumin protected skin cells against *tert*-butyl hydroperoxide (TBHP)-induced oxidative damage, as cells pre-exposed to curcumin and then treated with TBHP showed 45% higher cell viability compared to cells treated with TBHP alone. Anti-inflammatory activity, evaluated in LPS-stimulated macrophages, showed that 25 μM curcumin reduced NO release by 50%. To improve curcumin bioavailability and delivery, a SLN formulation containing 0.1% curcumin was developed. SLN-blank and SLN-curcumin (0.1%) exhibited similar characteristics (< 100 nm, PDI

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< 0.3, ZP > -20mV). Encapsulation efficiency was 99%, with 60% curcumin release in 24h. These findings confirm curcumin's antioxidant and anti-inflammatory activities and effective SLN encapsulation, ensuring controlled release, and suitability for topical application. Funding: FCT, and NORTE 2030, under the projects https://doi.org/10.54499/UIDB/04033/2020 (CITAB), and grant https://doi.org/10.54499/2021.04528.BD (TEC).

P-44-040
Investigation of PON1 activity in obese individuals

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Obesity is a disease that results from excessive accumulation of body fat mass due to excessive energy intake. Studies have emphasized that oxidative stress may play an important role in the development of metabolic diseases such as obesity-related cardiovascular diseases. PON1 is an enzyme that contributes to the antioxidant properties of high-density lipoprotein (HDL). In this study, we aimed to evaluate how paraoxonase 1 (PON1) activity changes in normal-weight, light-weight and obese individuals. A total of 180 people between the ages of 25–59 who applied to the Internal Medicine outpatient clinic of Başakşehir Çam and Sakura Hospital were included in the study group. Patients were divided into three groups according to their body mass index (BMI): normal weight (n = 60), light weight (n = 60), and obese (n = 60). Serum PON1 activity was measured by spectrophotometric method. PON1 activities were statistically significantly lower in obese individuals than normal-weight individuals (63.55 ± 2.27, 66.32 ± 1.78, $p < 0.001$ respectively). In our study, we found that PON1 activity was reduced in the obese group. Decreased PON1 activity indicates that the antioxidant properties of HDL are reduced. The basis of cardiovascular diseases caused by obesity may be a decrease in PON1 activity. The results of our study will shed light on studies on the prevention of cardiovascular diseases in obesity.

P-44-041
Royal jelly and rosmarinic acid reduce the damage caused by UVB radiation to skin cells and accelerate the wound healing process

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UVB radiation is known to cause DNA damage in skin cells, elevate levels of reactive oxygen species (ROS), induce inflammation, and lead to structural or functional abnormalities in proteins. Recently, there has been considerable interest in exploring the potential effects of natural products to mitigate these issues. In this study, we investigated the wound healing effects of single and combined treatments of rosmarinic acid (RA), a plant secondary metabolite, and royal jelly (RJ), which is bee product rich in bioactive peptides, on human fibroblast cell lines (HFF) and human keratinocyte cell lines (HaCaT). Treatment increased FGF2, VEGFA, and Ki67 (proliferation indicator marker) expression levels and ERK phosphorylation. ROS production, inflammation, apoptosis, ER stress, oxidative protein damage, and lipid peroxidation caused by UVB exposure significantly decreased after treatment with natural substances. Remarkably, the data obtained showed that the decrease in inflammation may be related to the down-regulated NUDT6/AKT/NF-κB pathway. RA and RJ treatment increased the E-cadherin/N-cadherin ratio, Twist1, Snail1, and TGF-β protein levels, accelerating the epithelial-mesenchymal transition (EMT). It also increased stem cell indicators (CD44, CD133, Nanog). Overall, RA and/or RJ treatments accelerate wound healing by down-regulating the NUDT6/AKT/NF-κB pathway and up-regulating the FGF2/ERK/EMT pathway. In particular, the combination of RA + RJ may be included in the composition of wound-healing topical drugs after future preclinical and clinical studies. This research may also help develop treatments for other health issues involving inflammation and oxidative stress. *The authors marked with an asterisk equally contributed to the work.

P-44-042
Dose-dependent effects of boron exposure on locomotor activity and oxidant-antioxidant status in zebrafish

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Boron is a trace element that, although not yet classified as an essential nutrient for humans, is suggested by several studies to play a critical role in maintaining homeostasis. However, at toxic doses, it may have detrimental effects. This study aims to investigate the potential dose-dependent effects of boron exposure in adult zebrafish focusing on locomotor activity and oxidant-antioxidant status in the brain. Zebrafish were divided into three groups: control group, 50 ppm boron-exposed group, and 100 ppm boron-exposed group. Exposure times were limited to a maximum of 2 weeks. At the end of the experimental period, distance traveled, exploration rate, and average speed were determined to evaluate locomotor activities. Then, fish were sacrificed under anesthesia and brain tissues were removed. In homogenates prepared from brain tissues, lipid peroxidation, nitric oxide levels, superoxide dismutase, and glutathione S-transferase activities were determined using spectrophotometric methods to assess the oxidant-antioxidant status. The results demonstrated that boron treatment had dose-dependent effects on the oxidant-antioxidant status, and locomotor activities. Our research continues to elucidate the mechanism underlying the relationship between changes in the brain's oxidant and antioxidant system and locomotor activity as a result of boron exposure.

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P-44-043
Chestnut shell-derived inhibitors of aldose reductase: from extraction to functional evaluation

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Aldose reductase (AR) is an enzyme belonging to the aldo-keto reductases (AKRs) superfamily, actively involved in the metabolism of toxic aldehydes implicated in pro-atherogenic processes, such as oxidative stress and inflammation. Considering the multifactorial nature of atherosclerosis, drugs which act on multiple pathways offer a promising therapeutic strategy. In this context, natural compounds are gaining attention, with agro-forestry residues emerging as valuable sources. *Castanea sativa* shells (CSS), a by-product of the chestnut industry, contain bioactive molecules with antioxidant and anti-inflammatory properties. Notably, CSS are a source of phenolics, such as catechins, with a demonstrated ability to inhibit AR [previously published in: Balestri F et al. (2022) Biology 11(9):1324], making them potential candidates for atherosclerosis multitargeting treatments. Our study was focused on the optimization of the extraction and fractionation techniques of bioactive compounds from CSS, followed by the assessment of their capacity as AR inhibitors and antioxidants. Given the overlapping substrate and inhibitory specificity of AKRs, we investigated whether these inhibitors could non-specifically target other reductases, using AKR1B10, an AKR involved in detoxification mechanisms, as a model. Inhibitory and antioxidant potentials of fractions were then correlated with the LC-MS characterization of their composition to select promising candidates with potential therapeutic applications. The impact of compounds with optimal inhibitory and antioxidant potentials on proliferation and ROS metabolism-related parameters of vascular smooth muscles cells is discussed. In conclusion, this research represents a promising opportunity for the valorisation of CSS agro-industrial by-products as a valuable resource for the extraction of bioactive compounds with therapeutic potential.

P-44-044
The role of naringenin and D-mannose in modulating oxidative stress and wound healing under hyperglycemic conditions

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Hyperglycemia represents a major pathological factor in diabetic wound healing, closely associated with systemic oxidative stress, which contributes to impaired tissue regeneration and diabetic complications. This study aimed to evaluate the potential of naringenin (NG) and D-mannose (D-M) in promoting wound healing under oxidative stress conditions induced by high glucose exposure. Human keratinocyte cells were cultured under normoglycemic (5 mM) and hyperglycemic conditions (25 mM and 50 mM D-glucose) and treated with NG and D-M (100–500 μM) for 72 hours. Cell proliferation and viability were assessed using the MTT assay, while intracellular reactive oxygen species (iROS) levels were quantified fluorometrically using H2DCFDA dye. The pharmacological interaction between NG and D-M whether synergistic, additive, or antagonistic was determined through the Chou-Talalay method. Wound healing was evaluated via scratch assay, and vasculogenic activity was assessed using a Matrigel-based tube formation assay. Furthermore, Nrf2 protein expression levels were analyzed via western blotting. The combined treatment of NG and D-M significantly reduced cell viability, migration, and vasculogenic activity in keratinocytes, while upregulating Nrf2 expression. These findings indicate that NG and D-M enhance wound healing by modulating oxidative stress responses under hyperglycemic conditions. The study provides insights into the therapeutic potential of NG and D-M in mitigating high glucose-induced oxidative damage, suggesting their possible application in diabetic wound management.

P-44-045
Biochemical and functional features of the body during experimental neuropathic pain under the influence of puncture photobiomodulation

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Oxidative stress, which develops with severe injuries and diseases accompanied by pain, is considered a common pathology of our time. Therefore, it seems relevant to monitor body parameters during pain stress under the influence of electromagnetic waves, which, with a minimum of contraindications, participate in intercellular interactions. The aim of the study was to evaluate the placebo-controlled effect of low-intensity electromagnetic waves in the infrared (IR) range on oxidative metabolism and vegetative-adaptive reactions in the acute phase of experimental pain stress. Pain stress was caused by double ligation of the sciatic nerve in 40 Wistar rats during a 10-day course of laser acupuncture (LA) using IR electromagnetic waves with a spectrum of 810±30 nm at an average radiation power of 0.35±0.07 mW. To evaluate the effectiveness of the procedure, a control group was formed, whose animals were exposed to radiation at a low radiation power of 0.01 mW, which excluded therapeutic effects and was evaluated as a placebo. A comparative assessment of the indicators of oxidative metabolism [lipid peroxidation products (LPO) and the activity of superoxide dismutase (SOD) and catalase] was performed by spectrophotometry, and vegetative reactions were performed by variational pulse measurement. It has been shown that pain stress leads to the development of oxidative stress in animals (a statistically significant increase in the content of malondialdehyde and a decrease in the activity of SOD and catalase). The study confirmed the adaptive, antitoxic and antioxidant potential of LA, which manifested itself in a decrease in the concentration of highly toxic malondialdehyde (antitoxic effect), normalization of

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indicators characterizing heart rate variability (adaptive effect), increased activity of SOD and catalase (antioxidant effect). Thus, it has been proven that the lotion is effective in correcting oxidative imbalances and increasing the body's resistance to stress.

LB-R-44-01
Antioxidant activities of *Sideritis galatica* Bornm

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Sideritis galatica, known as Ironwort herbal tea, belongs to the Lamiaceae family and has been used commonly as traditional medicine to treat gastrointestinal problems such as stomach pain and flatulence. *S. galatica* has several biological properties such as antimicrobial, anti-inflammatory, antioxidant and anti-mutagenic properties and might be used in the treatment of Alzheimer's disease, cardiovascular disease, arthritis and diabetes mellitus. It is considered that the antioxidants are significant nutraceuticals, showing a protecting effect in degenerative and chronic diseases. Antioxidative properties reduce oxidative stress by destroying biological molecules including DNA, lipids, and proteins produced by free radical overproduction, which causes those diseases. Therefore, the discovery of new antioxidants from natural sources is very important, especially to avoid illness. In this work, 2,2-diphenyl-1-picrylhydrazyl (DPPH) activity and total phenolic content of the methanol, ethyl acetate, and n-hexane extracts of *Sideritis galatica* were investigated. Even at low concentrations, the methanol extract and the ethyl acetate extract had significant phenolic and flavonoid content with powerful antioxidant activity. The 0.4 mg/mL methanol extract had 81% DPPH radical scavenging activity and 0.34 mg gallic acid equivalent. On the other hand, the n-hexan extract exhibited 10% radical scavenging activity and showed the lowest phenolic content values. All of these findings suggest the potential of *S. galatica* as a great source for the development of new antioxidant agents or products obtained from plants and completely natural, non-hazardous, and non-toxic. *The authors marked with an asterisk equally contributed to the work.

LB-R-44-02
Expanding the chemogenetic toolbox: a potential system for intracellular hydrogen sulfide production

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Chemogenetic tools utilize the expression of genetically engineered enzymes, receptors, and ion channels that are activated by exogenous substrates. These tools provide a powerful strategy for modulating cellular pathways with high specificity. Hydrogen sulfide (H_2S) has emerged as a key gaseous signaling molecule implicated in diverse physiological processes, including redox regulation, mitochondrial bioenergetics, and vascular homeostasis. Despite its biological significance, the ability to manipulate intracellular H_2S levels with spatial and temporal precision remains limited. Here, we present a novel chemogenetic tool for the controlled generation of H_2S in living cells, based on the bacterial enzyme *Salmonella typhimurium* D-cysteine desulfhydrase (stDCyD). By expressing stDCyD in mammalian cells and applying its substrate D-cysteine, we achieve tunable intracellular H_2S production. This approach enables real-time dissection of H_2S-dependent signaling events in a reversible and cell-type-specific manner. In addition, we have previously shown the application of stDCyD as a chemogenetic approach to manipulate intracellular pH levels [previously published in: Ghaffari Zaki et al. J. Am. Chem. Soc. 2023, 145 (22), 11899–11902]. We are currently investigating the potential of this approach for manipulating energy metabolism by targeting stDCyD to cristae of mitochondria and providing β-Chloro-D-alanine as a substrate. Altogether, this system offers a versatile platform for investigating the complex roles of H_2S and pH in biology and represents a significant addition to the chemogenetic toolbox.

LB-R-44-03
Oxidative stress responses in zebrafish (*Danio rerio*) exposed to perfluorooctane sulfonate (PFOS): might total oxidant status (TOS) and total antioxidant status (TAS) be a rapid biomarker?

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Permanent organic pollutants (POPs), including perfluorinated compounds, are known for their long environmental half-life, bioaccumulation, and toxic effects. Among these, perfluorooctane sulfonate (PFOS) is widely produced and poses significant risks to aquatic ecosystems. This study investigates the effects of PFOS exposure on zebrafish (*Danio rerio*) by assessing oxidative stress markers, specifically total oxidant status (TOS) and total antioxidant status (TAS), over different exposure periods. Zebrafish were exposed to PFOS at two concentrations (2 mg/L and 7 mg/L) for five different durations (2, 4, 7, 14, and 21 days). TOS and TAS levels were measured to evaluate oxidative stress responses. The fish were maintained under controlled laboratory conditions, and samples were collected at each exposure time for biochemical analysis using spectrophotometric methods. Exposure to PFOS led to a significant increase in total oxidative stress levels within the first 48 hours, particularly in the group exposed to 7 mg/L ($p < 0.01$). This oxidative imbalance persisted throughout the exposure period, with TOS levels remaining significantly elevated at most time points ($p < 0.05$). Total antioxidant status displayed a dynamic pattern, initially decreasing before significantly increasing on day 14 ($p < 0.05$), indicating a delayed antioxidant response. The rise in TAS was notably more pronounced at the higher PFOS concentration, suggesting that the antioxidant activation was dose-dependent. These findings highlight the potential ecological risks of PFOS contamination in aquatic environments. Further studies are needed to elucidate the long-term effects and underlying mechanisms of oxidative stress adaptation in zebrafish.

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LB-R-44-04
Diabetic microvesicles exacerbate Sestrin2 deficiency-induced endothelial dysfunction

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Chronic hyperglycemia and elevated reactive oxygen species (ROS) generation are hallmarks of diabetic vascular complications. Microvesicles (MVs), a subset of extracellular vesicles, along with lower Sestrin2 (SESN2) levels, have emerged as important mediators in its pathophysiology. This study examines the role of MVs in endothelial cell communication under diabetic conditions, focusing on their impact on oxidative stress signalling. To mimic diabetic injury, EA.hy926 endothelial cells were treated with methylglyoxal (MGO), and the resulting MVs were isolated and used to treat naïve cells. Western blot analysis assessed the expression and regulation of key antioxidant proteins and endothelial nitric oxide (NO) synthase (eNOS). The 2,3-diaminonaphthalene (DAN) fluorometric-based assay was used to assess NO levels, while flow cytometry-based methods [MitoSOX and dihydroethidium] were used to determine ROS production. To investigate the role of SESN2, cells were transfected with SESN2-specific siRNA. We found that MVs generated under diabetic conditions induced a complex antioxidant response in healthy endothelial cells. This response involves the upregulation of antioxidant proteins such as SESN2, superoxide dismutase 1 (SOD1), and heme oxygenase-1 (HO-1), suggesting a compensatory mechanism to mitigate oxidative stress and endothelial dysfunction caused by diabetic injury. MGO-induced MVs elevated ROS levels, leading to sustained oxidative stress that ultimately overwhelmed the initial upregulation of cellular antioxidant defenses. Furthermore, SESN2 knockdown alone significantly increased ROS production and reduced NO levels. These effects were further exacerbated upon exposure to MGO-induced MVs, underscoring the pivotal role of SESN2 in maintaining redox homeostasis. This study demonstrates that MVs influence diabetes complications by altering redox signalling through SESN2-dependent pathways, hence providing therapeutic targets for diabetic vascular disease. *The authors marked with an asterisk equally contributed to the work.

LB-R-44-05
Evaluation of advanced glycosylated end-products (AGE) levels to predict intrauterin insemination success

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Advanced glycosylated end-products (AGE) are end products of the glycosylation pathway that are known to increase in blood levels in cases of diabetes, insulin resistance and obesity, are involved in the pathophysiology of many diseases and can cause oxidative stress and activation of inflammatory pathways. In our study, we investigated whether there is a certain AGE level measured in serum and male semen that predicts pregnancy success in obese/non-obese patient groups in couples who underwent intrauterin insemination (IUI) in the infertility clinic of our hospital. Our study included 125 couples who were underwent IUI at Ankara City Hospital Infertility Clinic. HDL, triglyceride and fasting blood sugar values of the patients were examined. Height, weight and blood pressure measurements were made, BMIs were calculated. Patients' infertility causes, systemic disease diagnoses, smoking, alcohol and antioxidant/vitamin use were questioned. AGE levels were studied only in the blood samples of females and in the blood and semen samples of males. A positive betaHCG result after the IUI procedure was accepted as pregnancy success. When the patients who underwent IUI were examined as obese/non-obese in female and male genders, no significant relationship was found between post-procedure pregnancy success and blood and semen AGE levels. However, a significant relationship was found between semen AGE levels and metabolic syndrome, impaired fasting glucose and low HDL. A negative correlation was also found between sperm concentrations and blood AGE levels. Additionally, semen AGE values were found lower in men using antioxidants and/or vitamins. As a result of our study, it is thought that considering blood/semen AGE levels and their effects, especially in cases of male infertility, can increase pregnancy success with additional precautions and newly developed treatments. *The authors marked with an asterisk equally contributed to the work.

LB-R-44-06
Sestrin2 deficiency disrupts mRNA networks in diabetic endothelial dysfunction: implications for oxidative stress and metabolic pathways

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Diabetes significantly increases cardiovascular disease (CVD) risk through endothelial dysfunction driven by oxidative stress. Sestrin2 (SESN2), a stress-inducible antioxidant protein, is reduced in diabetes, worsening vascular impairment. While the cytoprotective roles SESN2 of are emerging, its function in endothelial cells under diabetic stress remains unclear. This study examined mRNA regulatory networks in *SESN2*-deficient endothelial cells exposed to diabetic injury. EA.hy926 endothelial cells were transfected with *SESN2* or control siRNA, then treated with methylglyoxal (MGO; 600 μM, 18 h), a glycolytic by-product implicated in diabetic vascular damage. Profiling of mRNA (Affymetrix Clariom D arrays) revealed 209 upregulated and 235 downregulated genes (FDR< 0.05, log FC≥1) in *SESN2*-silenced cells exposed to MGO, with *SESN2* silencing alone inducing 2.5-fold more differentially expressed genes (DEGs) than MGO treatment alone. Pathway enrichment analysis identified dysregulation in Nrf2-mediated antioxidant response (*SLC7A11* downregulation), p53 transcriptional regulation, ferroptosis, and PI3K-AKT-mTOR signaling. *SESN2* deficiency exacerbated MGO-induced pro-inflammatory cytokine upregulation (*IL-6*, *IL-8*, and *TNF-α*) and adhesion molecule expression (*ICAM-1*, *E-selectin*). Upregulated genes enriched fatty acid biosynthesis (*FASN*, *ACACA*) and cell cycle pathways, while downregulated genes impacted antioxidant defenses (*HMOX1*, *NQO1*) and apoptosis regulation. Protein-protein

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interaction networks highlighted 10 hub genes (e.g., *CDK1*, *TOP2A*, and *TGF-β*) central to these networks. These findings demonstrate that *SESN2* deficiency is a key transcriptional reprogramming regulator, altering mRNA networks that regulate redox homeostasis and metabolic processes, hence offering mechanistic insights into diabetic endothelial dysfunction. The study identifies potential therapeutic targets for CVD complications, particularly in pathways linking oxidative stress and metabolic dysregulation.

LB-R-44-07
Antioxidant activity of four local red-pigmented rice cultivars from the three southernmost provinces of Thailand: a combined *in vitro* and *in silico* study

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Rice is the most important staple food in Thailand. Each region of Thailand has its own local colored rice varieties. In the three southernmost provinces of Thailand (Pattani, Yala, and Narathiwat), several unique local red rice varieties are found, including Hawm Gra Dang Ngah 59 (HGDN 59), Hawm Mue Lau (HML), Lued Pla Lai (LPL), and Se Bu Kan Tang (SBKT). However, there is currently no published fundamental research that supports the further application of these local red rice cultivars. This study aimed to quantify antioxidant activity using the 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging method, investigate non-targeted metabolites and perform *in silico* predictions of antioxidant effects from ethanolic crude extracts of whole grain rice of HGDN 59, HML, LPL and SBKT. Among the tested cultivars, HGDN 59 exhibited the highest antioxidant capacity, reaching 897.31 ± 4.00 μM Trolox equivalents (TE)/g of dry extract, which corresponded to the highest total phenolic content at 190.76 ± 10.78 mg gallic acid equivalents (GAE)/g of dry extract. Based on natural product classification, the major compound groups identified included fatty acids, phenolics, vitamin E derivatives, lactones terpenoids and others. The relationship between $DPPH^{\bullet}$ scavenging activity and efficiency ($DPPH^{\bullet}$/total peak area) showed a strong positive correlation. Principal component analysis (PCA) revealed that HGDN 59 was distinctly associated with the highest antioxidant activity. Additionally, the ethanolic crude extracts of four cultivars exhibited varying predicted synergistic, antagonistic, and additive antioxidant effects, depending on their individual chemical compositions. The findings of this study enhance our understanding of the antioxidant activity and distinctive metabolomic profiles of four local red-pigmented rice cultivars. This knowledge provides a foundation for future research into the health-promoting potential of local colored rice varieties from the three southernmost provinces of Thailand. *The authors marked with an asterisk equally contributed to the work.

LB-R-44-08
Effects of novel mTOR inhibitors on oxidative stress and vascular senescence in progeria, a human aging disease

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This study investigates the senotherapeutic potential of two selective dual mTORC1/2 inhibitors using progeria iPSC-derived smooth muscle cells (iPSC-SMCs). Hutchinson-Gilford Progeria Syndrome (HGPS) is a rare human genetic disorder characterized by premature aging, caused by a point mutation in the *LMNA* gene leading to progerin accumulation. Progerin disrupts nuclear integrity, increases oxidative stress, and dysregulates mTOR signaling, accelerating senescence. We showed that these compounds shift transcriptomic longevity signatures and extend mouse lifespan [1]. These compounds' effects on oxidative stress, transcriptomic profile, aging-related molecular biomarkers including β-galactosidase activity were evaluated for their efficacy in premature senescence of progeria phenotype. Progeria iPS-SMCs treated with two novel mTOR inhibitors. To mimic vascular stress, cells underwent serial passaging or were cultured on prefabricated microfluidic chips and exposed to cyclic strain [2]. Intracellular reactive oxygen species (ROS) levels were measured using H2DCFDA fluorescence assay. Cellular senescence was quantified by β-galactosidase staining (X-Gal and C12FDG). Western blotting was employed to examine the expression of key proteins linked to cellular senescence and aging. RNA-sequencing examined transcriptomic changes including heatmap, volcano plot, and transcriptomic clock analyses [3]. Treatment with mTOR inhibitors significantly reduced intracellular ROS levels compared to DMSO-treated controls. Protein expression and transcriptomic analyses revealed downregulation of mTOR signaling pathway and senescence-associated markers, indicating a shift towards a less senescent phenotype. Dual inhibition of mTORC1/2 in progeria iPS-SMCs effectively reduced oxidative stress and modified the transcriptome profile with minimal cytotoxicity. These findings support the senotherapeutic potential of novel mTOR inhibitors for HGPS. *The authors marked with an asterisk equally contributed to the work. References [1] Tyshkovskiy, A., et al. (2023). *Cell*, 186(13), 2929–2949.e20. https://doi.org/10.1016/j.cell.2023.05.002. [2] Pitrez, P. R et al. (2020) *Nature Communications*, 11(1). https://doi.org/10.1038/s41467-020-17901-2. [3]

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Tyshkovskiy, A., et al. (2019). Cell *Metabolism*, 30(3), 573–593.e8. https://doi.org/10.1016/j.cmet.2019.06.018.

Toxicology

P-45-001
Long-term *in vitro* exposure to low-level perfluorooctanoic acid impairs human vascular endothelial cell function

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Perfluorooctanoic acid (PFOA), a persistent environmental contaminant often termed a "forever chemical", is recognized for its toxic effects on the nervous, endocrine, and reproductive systems, while emerging research suggests it may also impair vascular function, potentially contributing to cardiovascular diseases. However, the impact of PFOA on endothelial cell damage and endothelial dysfunction remains unclear. In particular, there is a lack of *in vitro* studies that mimic realistic exposure scenarios. In this study, we investigated the effects of long-term exposure to environmentally relevant concentrations of PFOA (1, 10, and 100 nM) on endothelial cell function using human vascular endothelial cell line EA.hy926 as a model. After 6 and 12 weeks of exposure, we assessed metabolic activity using the alamarBlue™ assay, cell proliferation through cell counting, apoptosis/necrosis via flow cytometry for annexin V and propidium iodide, endothelial permeability using two-compartment permeability assay with fluorescently labeled dextran, and monocyte adhesion using calcein AM-labeled U937 human monocytic cells. Our results showed no significant effect on metabolic activity at any concentration. However, after 12 weeks, exposure to 100 nM PFOA reduced cell proliferation. After 6 weeks of exposure to 1 nM PFOA, a slight decrease in live cells and an increase in dying cells were observed, which became more pronounced at all concentrations after 12 weeks. Additionally, a dose-dependent increase in endothelial permeability was seen after 12 weeks, and monocyte adhesion to endothelial cells was significantly elevated after 6 and 12 weeks of exposure to 10 nM and 100 nM PFOA, respectively. In conclusion, prolonged exposure to low-level PFOA adversely affects EA.hy926 cell proliferation, survival, endothelial permeability, and monocyte adhesion, supporting the hypothesis that exposure to PFOA may contribute to endothelial dysfunction.

P-45-002
Early-life exposure to di(2-ethylhexyl) phthalate (DEHP) disturbs reproduction and DNA methylation status of the ovary in adult female zebrafish

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Di(2-ethylhexyl) phthalate (DEHP) is ubiquitously present synthetic chemical added to PVC plastics, cosmetics, food and drink packaging, toys and babies' equipment. DEHP is well known endocrine disruptor and reproductive toxicant. Development of the reproductive system is highly sensitive to chemicals classified as endocrine disruptors. Our study aims to investigate the effects of exposure to DEHP during embryonic and early larval stage on reproduction in adult females, including the impact of DEHP on DNA methylation status of the ovaries. Zebrafish embryos were treated with DEHP (0, 10^{-8} M, 10^{-7} M, 10^{-6} M) from 5 h post fertilization until 5 days post fertilization and then allowed to mature in clean water. Fecundity and fertility were analyzed by counting all spawn eggs and by determining percentage of fertilized eggs, respectively. qPCR was used for the analysis of relative gene expression. Global DNA methylation status of the ovaries was analyzed by immunohistochemistry, using primary 5-mC antibody and secondary Alexa Flour 488 antibody. MSREqPCR was used to analyze gene specific DNA methylation. Level of 17β-estradiol was determined using ELISA. Results revealed a significant reduction in fecundity, fertility and blood estradiol level in DEHP treated groups. Gene expression analysis showed that DEHP inhibited ovarian expression of gonadotropin and estrogen receptors (fshr, lhr, esr1, esr2a, esr2b) as well as expression of ovarian DNA methytransferases dnmt1 and dnmt3aa. Analysis of lhr, fshr and esr1 promotor methylation status revealed trend of hypermethylation in 10^{-7} M group, while trend of global DNA hypomethylation was detected in ovaries in DEHP exposed groups. This study showed that early-life exposure to DEHP can affect reproduction in adult female zebrafish by decreasing the level of circulating estradiol, reducing the expression of ovarian genes involved in regulation of ovarian function and by disrupting ovarian DNA methylation status.

P-45-003
Evaluation of vegf-a, sp-d and muc-1 expression in baclofen poisoning and its combination with ethyl alcohol

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Baclofen (also known as Lioresal®) is a selective GABA-B agonist. Stimulation of these receptors causes a decrease in the release of glutamate and aspartate, which play a key role in the development of spasm. This process leads to a decrease in the intensity of skeletal muscle contraction, a decrease in pain sensitivity and a sedative effect. Baclofen is available in the form of tablets for oral administration, as well as solution for intrathecal administration. It is indicated for spasm treatment and as a part of the therapy in spinal cord injuries. Baclofen has a pronounced psychotropic effect and is often a subject to abuse especially among adolescents and young adults. In case of baclofen poisoning, a comprehensive study of the processes occurring in the body is necessary to provide effective medical care. One of the target organs in case of baclofen poisoning is the lung. The aim of the study was the assessment of MUC-1, VEGF-A, and SP-D expression in rat lungs 3, 4,5 and 24 hours after baclofen (85 mg/kg) and baclofen–ethanol (7 ml/kg 40% vol.) co-administration. The study was performed on 35 male Wistar rats (7 groups of 5 animals each). In animals of the control group, which were not treated with either baclofen or ethanol, no expression (-) of MUC-1, VEGF-A and SP-D was revealed. 3 hours after baclofen and baclofen–ethanol co-administration, 4,5 hours after baclofen and baclofen–ethanol

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co-administration, 24 hours after baclofen administration, moderate expression of MUC-1, VEGF-A and SP-D (++) was found. 24 hours after baclofen and ethanol co-administration the expression of MUC-1 and VEGF-A was strong (+++), the expression of SP-D was moderate (++). Understanding the processes occurring in the lungs when baclofen and its combination with ethanol is administered is necessary for the development of effective therapeutic measures in case of non-fatal poisoning, and in case of fatal poisoning it will be useful in determining the immediate cause of death.

P-45-004
Molecular and histopathological findings in salinomycin-induced cardiotoxicity in turkey

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The toxicity of Salinomycin (Sal), a commonly used antiparasitic agent in veterinary medicine, is dependent on the species, and turkeys are considered the most susceptible species in poultry. However, the underlying Sal-induced molecular toxicity mechanisms that could explain why turkeys are the most susceptible are still unclear in the heart, the target organ for toxicity. This study aimed to characterize Sal cardiotoxicity in turkey using histopathology and transcriptomic approaches. Turkeys were exposed to salinomycin in feed for 2 weeks (n = 6; 0.9 mg/kg b.w/day), and hearts were collected from Sal-exposed and control (n = 6) groups. For histopathological analysis, hearts were fixed in formalin, stained using hematoxylin and eosin, and evaluated by light microscopy. For molecular analysis, total RNA was isolated and sequenced, and differentially expressed genes (DEGs) were determined with the DESeq2 package ($\log_2$FC ≥ 1; p-adj< 0.05). Reactome was used to evaluate altered molecular signaling pathways between the groups. In the Sal-exposed group, the histopathological findings revealed that cardiomyocytes were fragmented and degenerated. Furthermore, numerous cardiomyocytes were vacuolated. As progressive findings, connective tissue hyperplasia and proliferation of myofiber nuclei were determined. The transcriptomic analysis resulted in 485 significant (188 up; 297 downregulated) DEGs in the Sal-exposed group compared to the control. Pathway analysis demonstrated that Sal cardiotoxicity triggered cell cycle arrest via downregulating cell-cycle checkpoint and mitotic pathways. The molecular findings of Sal cardiotoxicity correlated with histopathological findings. This is the first *in vivo* study that investigates Sal cardiotoxicity in turkey. Targeting cell-cycle pathways could be the promising mechanism for the early detection of Sal-induced cardiotoxicity in turkey. Acknowledgements: The work was funded by NCN project 020/38/E/NZ7/00260. *The authors marked with an asterisk equally contributed to the work.

P-45-005
Screening of carcinogenic and antitumor properties for benzimidazole and azafluorene derivatives as a heterocyclic polyamine's structural analogs

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Substances inhibiting an oxidative deamination of polyamines (PAs) are likely to manifest carcinogenic properties. Chemical compounds activating the process of oxidative disintegration of PAs may have an antitumoral potential. The aim of the present work was to measure the influence of some benzimidazole and azafluorene derivatives on the rate of oxidative deamination of PAs in tissue with a high proliferative rate. The specific activity of the main enzymes of PAs' oxidative degradation – spermine oxidase (SMO), polyamine oxidase (PAO) and diamine oxidase (DAO) – was determined by the acellular test-system from regenerating rat liver. The tested compounds were: (1) 7-aminopyrido[1,2-α]benzimidazole, (2) 7-nitropyrido[1,2-α] benzimidazole, (3) 1-amino-4-azafluorene, (4) 1-amino-4-azafluorenole, (5) 9-dicianomethylene-4-azafluorene, (6) 9-[α-(β-hydroxiethyl)aminomethylene]-4-azafluorene, (7) 9-[α-pyridilaminomethylene]-4-azafluorene, (8) 1-amino-9-phenylamino-4-azafluorene, (9) 1-amino-4-azafluorenone-9, (10) 1,4-diazoacetonaphtyleno[1.2-f]-fluorantene, (11) 2-methoxycarbonyl-(β-benzoylethyl)aniline, (12) 5-(2-methoxycarbonyle)-phenyle-α-furfurole. DAO, PAO, and SMO activity were inhibited by substances 1, 2 and 11, suggesting that they have shown cancerogenic properties. Antitumor properties were revealed for substances 3, 4, 7, 9, 10 and 12, those that have activated DAO, PAO, and SMO. This is the probable reason for the proliferation suppression of cancer (CaOv) cells growth in culture by the same compounds. Docking with yeast PAO enzyme Fms1 using Molegro Virtual Docker software was performed with flexibility in torsion angles of ligands. A few critical atoms in the sphere radius 12Å of the PAO active center were identified: His67, Tyr450, His191, Trp174, Gly487. The results revealed the structural basis for the design and synthesis of novel activators of polyamine catabolism as potential antitumor agents. This study has been supported by the RUDN University Strategic Academic Leadership Program.

P-45-006
Cadmium disrupts blood-brain barrier integrity via mitochondrial dysfunction and iron homeostasis dysregulation

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The blood–brain barrier (BBB) is a critical protective barrier that regulates the selective transport of substances between the bloodstream and the brain. Composed of endothelial cells, astrocytes, pericytes, microglia, and neurons, the BBB plays a key role in maintaining brain homeostasis. Disruption of BBB integrity accelerates neuroinflammation and neurodegeneration.

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Cadmium (Cd), a toxic heavy metal found in food and the environment, is increasingly recognized as a risk factor for neurological and cerebrovascular diseases. This study investigated the cytotoxic effects of cadmium on BBB integrity using a murine brain endothelial cell line. After 24-hour cadmium exposure, cadmium reduced cell viability, decreased tight junction protein expression, and significantly lowered trans-endothelial electrical resistance (TEER), indicating BBB dysfunction. To uncover the underlying mechanisms, we evaluated mitochondrial function and iron-dependent cell death pathways. Cadmium exposure impaired mitochondrial respiration and ATP production, disrupted autophagy, increased intracellular iron accumulation, and elevated lipid peroxidation. These findings are characteristic of ferroptosis, a form of iron-induced programmed cell death. In conclusion, cadmium exposure compromises BBB integrity by inducing mitochondrial dysfunction and dysregulating iron homeostasis, ultimately triggering ferroptosis in brain endothelial cells. This study highlights cadmium as an environmental neurotoxicant that poses a significant threat to cerebrovascular health. This research was supported by a grant from the National Research Foundation (RS-2022-NR070655) of Korea.

P-45-007
The impact of glyphosate on galanin receptor expression in the uterine wall of sexually immature gilts

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Glyphosate is a widely used herbicide that has been shown to disrupt reproductive functions and cause neurotoxic effects. The galanin peptide family, particularly the neurotransmitter galanin (GAL), regulates uterine function both in normal and pathological conditions through interactions with G protein-coupled receptors (GALRs). This study aimed to assess the impact of glyphosate on the expression and localization of galanin receptors in the uterine wall of sexually immature gilts. Fifteen sexually immature gilts were divided into three groups: control – animals receiving empty gelatin capsules; E1 – animals receiving a low dose of glyphosate corresponding to the theoretical maximum daily intake (TMDI) of 0.05 mg/kg body weight/day; E2 – animals receiving a higher dose of glyphosate corresponding to the acceptable daily intake (ADI) of 0.5 mg/kg body weight/day, in gelatin capsules for 28 days along with morning feeding. GALR receptor localization was determined using single immunofluorescence staining, and receptor expression in the uterine wall was assessed by western blotting. Receptor proteins were labeled using specific antibodies. Galanin receptor expression (GALR1 and GALR2) was observed in the smooth muscle cells of the uterus as well as in the endothelial and muscular layers of blood vessels in the control group and the groups receiving glyphosate (E1 and E2). Glyphosate supplementation led to changes in GALR expression in the uterine wall of gilts. Statistically significant changes were observed only in the E2 group. The observed alterations in galanin receptor expression may result from the direct neurotoxic effect of glyphosate or from oxidative stress induced by glyphosate. Funded by the National Science Centre in Poland (grant no. 2022/45/N/NZ7/01853) and the Minister of Science under the Regional Initiative of Excellence Program.

P-45-008
Polystyrene microplastics-induced extracellular vesicles disrupt the kidney microenvironment

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The COVID-19 pandemic has intensified plastic waste accumulation, primarily due to the increased use of personal protective equipment, single-use food packaging, and disposable utensils, straining global waste management systems. Recent studies emphasize extracellular vesicles (EVs), including exosomes, mediating intercellular communication. This study explores the impact of polystyrene microplastics (PS-MPs) on EV composition and their regulatory effects on LGALS3BP and fibronectin (FN) expression in kidney cells, as well as the subsequent influence of PS-MP-modified EVs on recipient cells. Quantitative proteomic analysis revealed a significant elevation of LGALS3BP and FN levels in EVs from PS-MP-exposed kidney cells compared to controls. PS-MP exposure also led to increased intracellular expression of these proteins. Functional knockdown of LGALS3BP and FN attenuated the upregulation of PS-MP-induced IRE1α and phosphorylated GSK-3β. Notably, EVs derived from PS-MP-conditioned media of LGALS3BP-deficient kidney cells exhibited increased antioxidant pathways Nrf2 and Hsp27. In vivo, oral PS-MP exposure in mice resulted in significant upregulation of LGALS3BP and FN, coupled with suppressing Nrf2 and Hsp27 in renal tissues. These findings suggest that PS-MP-induced EVs disrupt the kidney microenvironment by upregulating fibrotic markers and suppressing key antioxidant defenses, potentially contributing to renal dysfunction and fibrosis.

P-45-009
Particulate matter promotes the development of atopic dermatitis by inhibiting TREX1 and activating the inflammasome pathway

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Atopic dermatitis (AD) is one of the most common skin diseases, with its incidence and prevalence increasing over the past few decades, particularly in developed countries. Recent epidemiological studies have indicated a positive correlation between environmental pollutants and various pre-existing diseases. Particulate matter (PM) has been shown to adversely affect the integrity of the skin barrier, leading to the generation of reactive oxygen species (ROS) and triggering inflammatory responses. However, the specific mechanisms by which PM exacerbates AD symptoms have yet to be clearly elucidated. Analysis of gene expression in AD patients using data from the

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GEO database revealed significant upregulation of TNF-α and interferons (including IFN-α and IFN-γ), along with suppressed expression of the TREX1 gene. This suppression was associated with the activation of the inflammasome/pyroptosis pathway. The present experimental results demonstrated that PM exposure increased transepidermal water loss (TEWL) in AD mouse models, elevated serum levels of IgE and IFN-γ, and activated mast cells. Additionally, under simulated AD conditions, PM exposure led to a marked upregulation of key proteins involved in the cGAS–cGAMP–STING and pyroptosis pathways compared to the AD alone group. Current research findings confirm that PM promotes AD progression by enhancing cellular inflammatory responses, potentially through TREX1 suppression, which induces activation of the cGAS–cGAMP–STING and inflammasome/pyroptosis pathways.

P-45-010
Metabolomic analysis of sulfur mustard derivatives

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Sulfur mustard (SM), a potent vesicant and chemical warfare agent, poses severe health risks through its toxic effects on the skin, eyes, and respiratory system. Despite extensive research on its acute effects, understanding of its long-term biochemical and metabolic impacts remains limited. This metabolomic study aimed to identify and characterize the metabolic alterations induced by sulfur mustard exposure *in vitro*, with a focus on the identification of specific biomarkers and metabolic pathways affected by the compound. Based on the cytotoxicity data, GC-MS-based non-targeted metabolomic analyses were performed in the A549 cell line in which different concentrations of HD (100 μM, 300 μM, 1000 μM) were applied for 24 hours. The results of metabolic profiling and subsequent pathway analysis are evaluated. When the different concentrations of HD were compared with the control group, it was observed that the metabolomic profiles were significantly distinct; the most significant metabolomic differences were evident at 300μM concentration compared to the control, at 100 μM it was very similar to the control, and at 1000 μM it was very different due to very significant cell toxicity. Furthermore, the pathways involved in HD toxicity were also supported by pathway analyses performed at the end of metabolomic analyses, and it was shown that glutathione metabolism, aspartate, glutamate metabolism and mitochondrial energy pathways were affected, and thus oxidative stress and apoptosis could be induced, DNA damage could develop, and inflammatory response could occur. We also evaluated potential biomarkers for exposure to sulfur mustard, including specific metabolites linked to cellular damage and inflammation. This research not only enhances our understanding of the toxicological mechanisms of sulfur mustard but also contributes to the development of diagnostic tools for detecting exposure and assessing the severity of poisoning.

P-45-011
Identifying cholinesterase inhibitors among commercially available pesticides by bioinformatic analysis and *in vitro* inhibition

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The residual presence of pesticides (herbicides, insecticides, etc.) in various crops, vegetables, and fruits often exceeds the allowed limits. Exposure to pesticides through direct contact, ingestion, inhalation, or residues in food, can have harmful effects on human health. Pesticides that inhibit cholinesterases (ChE) pose significant health risks due to their neurotoxicity. Acetylcholinesterase (AChE) is a crucial enzyme in terminating cholinergic transmission, and a related enzyme, butyrylcholinesterase (BChE), is a backup to AChE and is involved in the metabolism of various xenobiotics. Inhibition of ChE by pesticides induces cholinergic crisis and can lead to long-term cognitive impairments and neurodegenerative disorders. We screened a library of 346 commercially available herbicides listed in the HRAC open-access online source to identify potential ChE inhibitors. For the herbicides identified, we conducted *in silico* analyses of their structural similarity with ChE inhibitors as well as physicochemical and ADME/Tox parameters to assess their ability to inhibit ChE and cross the blood-brain barrier (BBB) and potentially affect the central nervous system (CNS). The physicochemical and ADME analysis revealed that most herbicides structurally similar to ChE inhibitors also had profiles favorable for BBB permeability with physicochemical parameters by values recommended for CNS-active drugs. Molecular docking studies were performed to evaluate the antiChE activity of the compounds. Morfamquat, Profoxydim, and Lactofen exhibited the highest docking scores, indicating potential antiChE activity against both enzymes. Furthermore, we assessed the inhibition constants for selected herbicides to clarify their mechanism of ChE inhibition. Assessments made within this study will help understand the health risks of commercially available pesticides and may contribute to their replacement with safer alternatives. This work was supported by the Croatian Science Foundation (IP-2023-10-6685).

P-45-012
Skin sensitization study of bee venoms collected from honeybee ecotypes from Türkiye and Cyprus

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Bee venom can be produced by all species of honeybees. It is a complex product that contains proteins, peptides, biogenic amines, amino acids, sugars, volatiles, and minerals. The medicinal effects of bee venoms have been known and used since ancient times. This study aimed to sensitization potential of eight bee venoms collected from honeybee ecotypes (ArtvinMacahel,

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Düzce, Gökceada, HatayAntakya, KıbrısMallıdag, Kırklareli, MuglaIkizce and SırnakFarasin) in Turkey and Cyprus. To evaluate the sensitization potential of bee venoms collected from eight honeybee ecotypes in Turkey and Cyprus, the Local Lymph Node Assay (LLNA) was performed following the OECD 442B guideline (BrdUELISA). The LLNA is a validated method to assess skin sensitization by measuring lymph node cell proliferation, a key indicator of allergic reactions. Female CBA/J mice (6–8 weeks old) which were acclimatized under controlled conditions (22 ± 3°C, 50 ± 20% relative humidity, and a 12-hour light/dark cycle). were used in this study. For each bee venom, three treatment groups were established: a venom treatment group (low, medium, and high concentrations), a positive control group (hexyl cinnamal), and a vehicle control group [acetone:olive oil (4:1)]. The test substances were topically applied to the ears of mice for three consecutive days. On day 5, bromodeoxyuridine (BrdU) was administered via intraperitoneal injection to quantify lymphocyte proliferation in the auricular lymph nodes using an ELISA-based assay. The Stimulation Index (SI) was calculated for each concentration, with an SI ≥ 1.6 considered indicative of sensitization. The results showed that bee venoms collected from Düzce, Kırklareli, and Artvin exhibited sensitizing properties, whereas those from Muğla, Hatay, Şırnak, Çanakkale, and Cyprus did not demonstrate sensitization potential. This study provides critical insights into the sensitization profiles of bee venoms from diverse geographic regions.

P-45-013
Metallomic and oxidative stress alterations in testicular cancer: tracking changes up to one-year post-chemotherapy

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Bleomycin, etoposide and platinum (BEP) chemotherapy is the standard therapeutic regimen in men with testicular germ cell tumours (TGCT). Although most patients achieve excellent outcomes, the therapy is less effective for those with advanced disease. The administration of Pt-based drugs significantly increases the concentration of Pt in the body where it may circulate for years post-treatment and affect key physiological activities. The aim of the study was to determine the differences in the metallomic profiles in whole blood, serum and urine of men with TGCT undergoing BEP chemotherapy. The study population consisted of 86 men with newly diagnosed TGCT. Blood and urine samples were obtained before any type of cancer treatment, after two cycles of chemotherapy and one year after chemotherapy. In all three time points, concentrations of 18 elements in whole blood, serum and urine, and blood activities of glutathione peroxidase (GPx) and superoxide dismutase (CuZnSOD) was measured. In serum, significant discrimination was observed (Wilks' $\lambda = 0.210$, $p < 0.001$), with key contributing elements including Mg, Fe, Co, Zn, Se, Ca, Tl, Hg, and Pb. In whole blood, K, Mg, Ca, Cu, Co, Mn, Na, Al, and Cr were the primary contributors to differentiation (Wilks' $\lambda = 0.191$, $p < 0.001$), while urine profile demonstrated significant differences (Wilks' $\lambda = 0.312$, $p < 0.001$), with Cu, Cr, Pb, Na, K, and Al playing a major role. The results of Friedman ANOVA also showed the significant change in the activity of oxidative enzymes SOD ($p < 0.001$) and GPx ($p = 0.002$). The results suggest that BEP chemotherapy induces substantial shifts in metal homeostasis, up to one-year post-treatment, potentially reflecting drug-related toxicity, oxidative stress, and metabolic changes. The observed alterations in essential elements may have implications for antioxidant defence and immune function, while increased levels of toxic metals could indicate altered renal clearance or mobilization from tissue stores.

P-45-014
Toxicological effects of tetracycline on *Danio rerio* larvae: developmental, physiological, and molecular responses

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Tetracycline, a widely used antibiotic, is frequently detected in freshwater ecosystems, raising concerns about its potential impact on aquatic organisms. Due to its persistence in aquatic environments, tetracycline can interfere with early developmental processes in fish. This study investigates the developmental effects of tetracycline exposure in *Danio rerio* larvae by assessing morphological, behavioral, and molecular responses. *Danio rerio* embryos at 4 hpf were exposed to environmentally relevant concentrations of tetracycline until 96 hpf. Locomotor activity was analyzed using Noldus EthoVision XT. Developmental toxicity was assessed through hatching delay, reduced body length, and enlarged yolk sac area. To explore molecular effects, quantitative RT-PCR (qRT-PCR) was used to quantify stress-related genes involved in oxidative stress (SOD), resistance mechanisms (ABCC1), and drug biotransformation (CYP1A). Tetracycline exposure above 10 μg/L significantly delayed hatching, reduced body length, and increased yolk sac area compared to controls ($p < 0.05$). Behavioral analysis revealed decreased locomotor activity, prolonged inactivity, and abnormal swimming patterns. Gene expression analysis showed upregulation of SOD, ABCC1, and CYP1A ($p < 0.05$), indicating oxidative stress and activation of detoxification pathways. These findings demonstrate that even relatively low tetracycline concentrations can induce physiological and molecular disturbances in *Danio rerio* larvae, highlighting their potential as a bioindicator for aquatic pollution. The observed alterations provide valuable biomarkers for assessing pharmaceutical contamination in freshwater ecosystems. Further research is needed to explore the long-term consequences of antibiotic pollution on fish development. Funding: This research was supported by the RUDN University Scientific Projects Grant System, project 202789-2-000.

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P-45-015
Particulate matter 2.5 impairs retinal organoid growth and development via AMPK activation

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Particulate matter ($PM_{2.5}$) is a major environmental pollutant that induces oxidative stress and inflammation, yet its effects on ocular tissues remain unclear. Here, we utilized human-induced pluripotent stem cell (hiPSC)-derived three-dimensional (3D) retinal organoids to investigate $PM_{2.5}$-induced retinal toxicity. $PM_{2.5}$ exposure resulted in significant cytotoxicity, with particle adhesion and accumulation within organoids. Key retinal marker proteins (CRX, Recoverin, NR2E3, Rhodopsin) were downregulated, while pluripotency markers (SSEA, KLF4, OCT4, SOX2) remained unchanged, indicating impaired retinal differentiation. Gene set enrichment analysis (GSEA) revealed activation of inflammation, apoptosis, and oxidative stress pathways. Pro-inflammatory cytokines (IL-1β, TGF-β, VEGF) were upregulated, alongside increased NF-κB phosphorylation. $PM_{2.5}$ also induced cellular senescence, as evidenced by upregulated p53, p21, and p16 expression and enhanced SA-β-gal staining. Autophagy activation was confirmed by increased AMPK phosphorylation, decreased mTOR expression, and elevated LC3 and p62 levels. Mitochondrial dysfunction and ATP depletion further suggested autophagy-mediated cell death. Notably, AMPK inhibition using Compound C suppressed autophagy, reduced inflammation and senescence, and partially restored retinal differentiation. These findings suggest that $PM_{2.5}$ induces retinal toxicity via AMPK-mediated autophagy, inflammation, and senescence, ultimately impairing retinal development. This study highlights the ocular risks of air pollution and provides a foundation for protective strategies against $PM_{2.5}$-induced retinal diseases.

P-45-016
Mechanisms of aflatoxin B1-induced hepatic injury: insights from an *in vitro* human hepatocellular carcinoma (HepG2) cell model

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Aflatoxin B1 (AFB1) is a mycotoxin known to contaminate agricultural products, animal feed, and animal-derived food products, exerting deleterious effects on human and animal health. AFB1 is a significant contributor to liver damage and the development of hepatocellular carcinoma. Given that toxicity and biotransformation of AFB1 occur in the liver, the HepG2 cell line was used in this study as an appropriate and well-characterised model of the human liver because its physiological properties are similar to those of the primary hepatocytes. Moreover, the liver is the primary target organ for AFB1 metabolism and toxicity, necessitating further elucidation of the mechanisms underlying liver injury. Consequently, this study investigated an *in vitro* toxicity model of AFB1-induced inflammation and mitochondrial dysfunction in HepG2 cells. HepG2 cells were cultured in MEM supplemented with 10% foetal bovine serum, 1% L-glutamine, and 1% penicillin/streptomycin. Subsequently, the cells were exposed to varying concentrations of AFB1 (5, 3.5, 2.45, 1.72 μM) and the positive control, lipopolysaccharide (LPS), at 1 μg/ml. Cell viability and cytotoxicity were assessed after 72 h using 3-[4, 5-dimethylthiazol-2-yl]-2, 5-diphenyltetrazolium bromide (MTT) and lactate dehydrogenase (LDH) assays. Mito Tracker was employed for flow cytometry to evaluate mitochondrial content and function. Quantitative real-time polymerase chain reaction (qRT-PCR) was used to quantify the expression of pro-inflammatory cytokine genes (NF-κβ and TNF-α) and mitochondrial biogenesis genes (TFAM, NRF1, and SIRT1). A reduction in cell viability was observed at all tested concentrations of AFB1 and LPS (positive control). Concurrently, there was an upregulation of pro-inflammatory cytokine genes, specifically NF-κβ and TNF-α. AFB1 further diminished the expression of the genes associated with mitochondrial biogenesis, TFAM, NRF1, and SIRT1. In conclusion, AFB1 triggered inflammation and mitochondrial dysfunction by increasing cytotoxicity and decreasing cell viability compared with LPS, a known inducer of both inflammation and mitochondrial dysfunction. Thus, this study suggests that inflammation and mitochondrial dysfunction may be potential therapeutic targets against AFB1-induced liver injury. *The authors marked with an asterisk equally contributed to the work.

P-45-017
Ecological and human health impacts of isothiazolinone biocides: investigating emerging pollutants and their risks

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Isothiazolinones (IS), which are used in pesticides, disinfectants, detergents, and personal care products, present effective biocides though not at acceptable human health and environmental risks. Despite their antimicrobial activity, IS are classified as very strong sensitizers, producers of skin irritation and allergies, as well as ecotoxicological including the inductive formation of reactive oxygen species and increased cytokine release as well as a pro-inflammatory response leading to toxic effects to both humans and fish. The increasing use of IS especially in COVID-19 medical supplies should be followed by a study of the mechanisms of their actions and effects in due time. The relevant substances are the 5-chloro-2-methyl-2H-isothiazolin-3-one plus 2-methyl-2H-isothiazolin-3-one (CMIT/MIT) which is very common in consumer products such as shampoos, toothpaste, and disinfectants, in addition to other products. Therefore, the objective of this study is to evaluate the toxicity of CMIT/MIT toward human neuroblastoma cells, SH-SY5Y and *in vivo* model. The exposure of the cells to increasing concentrations of CMIT/MIT for 24 hours inhibited cell proliferation while augmenting LDH release and lipid peroxidation and reducing antioxidant defenses. Furthermore, it induced Nrf-2/HO-1 cellular pathway and pro-inflammatory cytokines (IL-1β, IL-6, TNF-α). Also, ERK1/2, p38, and JNK1/2 were activated in response to pro-inflammatory stress. These pro-inflammatory pathways connected with cell cycle-regulating genes (p53, p21) and apoptosis. CMIT/MIT appears to induce cellular damage and accelerate neuronal aging through the Nrf-2/HO-1 and MAPK signaling pathways. Additionally, we performed *in vivo* studies to evaluate the

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toxicity of these compounds. Animals were exposed via aerosol to different doses of the tested compounds for 28 days. At the end of the experiment lungs and brains were harvested to assess the possible long-term impacts on human and ecological health.

P-45-018
Evaluation of the protective effects of silibinin against mycotoxin-induced toxicity using patient-derived intestinal organoids

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Mycotoxins, toxic secondary metabolites produced by fungi, are common contaminants in food and feed, posing significant cytotoxic and genotoxic risks. In recent years increasing attention has been directed toward the protective role of silibinin in mycotoxin-induced cellular damage. In our study [previously published in: Tran, Van Nguyen, *et al.* (2020) Toxins, 12(3), 148], we evaluated the cytotoxic and genotoxic effects of various mycotoxins and their mutual combinations. Additionally, we assessed the potential of silibinin to suppress acute toxicity of these mycotoxins. We also determined the permeability of mycotoxins and silibinin across the intestinal barrier in a two-compartment Transwell system comprising Caco-2 and RAW 264.7 cells. Following our previous study, we aim to evaluate this results in an advanced *in vitro* model of the intestinal barrier. For this purpose, intestinal organoids derived from patient tissues have been prepared. So far, suitable conditions for the preparation of organoids have been optimized, including digestion enzymes, growth factors and culture conditions. The prepared organoids will be used to test the additive cytotoxic and genotoxic effects of mycotoxins and the protective effect of silibinin. Furthermore, the bioavailability of the test compounds will be verified using these intestinal organoids. Overall, this research will enhance our understanding of silibinin and its potential therapeutic applications in mitigating mycotoxin-induced toxicity. This work was funded by the Grant Agency of the Czech Republic (GACR), No. 2410000S.

P-45-019
Effects of perfluorooctanesulfonic acid on steroidogenesis in human granulosa cell line HGrC1

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Infertility has become a global health challenge, affecting millions of people worldwide, and exposure to endocrine disruptors has been recognized as a significant contributor. One such endocrine disruptor is perfluorooctanesulfonic acid (PFOS), a "forever chemical" widely distributed in the environment. Current data highlight a link between PFOS exposure and female reproductive system disorders; however, little is known about the mechanism through which PFOS interferes with ovarian function. The primary aim of this study was to explore the effects of PFOS on steroidogenesis in human granulosa cell line HGrC1. HGrC1 cells were exposed to 0.1 μM, 1 μM, 10 μM and 25 μM PFOS for 6 h and 48 h. Alamar Blue assay revealed that none of the tested concentrations affected viability of HGrC1 cells after 48 h of exposure. Western blot analysis showed that exposure to 1 μM PFOS for 6 h led to a significant increase in the levels of steroidogenic acute regulatory protein (StAR), that controls the rate-limiting step in progesterone biosynthesis. Levels of two other enzymes involved in progesterone synthesis, cholesterol side-chain cleavage cytochrome P450 and 3-beta-hydroxysteroid dehydrogenase, remained unchanged after 48 h of treatment. Subsequently, the signaling pathways involved in the regulation of StAR expression in HGrC1 cells were analyzed. One μM PFOS significantly increased phosphorylation of protein kinase B (AKT) and cAMP response element-binding protein after 15 minutes of exposure. Pretreatment with H-89, protein kinase A (PKA) inhibitor, prevented PFOS-induced increase in StAR protein levels, indicating involvement of the cAMP/PKA signaling pathway in PFOS action. Interestingly, results of the enzyme-linked immunosorbent assay (ELISA) showed that 1 μM PFOS did not induce changes in the levels of progesterone in HGrC1 cells after 48 h of exposure. In order to reveal a more detailed mechanism by which PFOS affects ovarian function, additional research is required.

P-45-020
Long-term nanoplastic exposure triggers cellular stress and inflammatory pathways in lymphatic endothelial cells

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The emergence of micro- (MPLs) and nano-plastics (NPLs) has become a significant concern regarding their impact on human health. This study aims to investigate the potential toxic effects of polystyrene NPLs (PS-NPLs) on axillary lymph node endothelial cells (SVEC4-10) following long-term repeated exposure of 15 days. Therefore, SVEC4-10 cells were exposed to 1.6, 16, and 160 μg/mL PS-NPLs for 1, 3, 11, and 15 days. Cell viability (by flow cytometry-based Live/Dead assay) and morphology (by optical microscopy) were analyzed in both treated and untreated cells (control). The cellular redox status was assessed by determining the level of reactive oxygen species (ROS) (by flow cytometry) and by measuring the concentration of malondialdehyde (MDA). The level of proinflammatory cytokines (IL-6/-1β, TNFα) was evaluated by indirect ELISA. The protein expression of oxidative stress (Hsp27/60/70) and proliferation (MCM2, PI3K) markers was revealed by western blot. SVEC4-10 cells exposed to PS-NPLs showed an early inflammatory burst (first 3 days) with peak TNFα (+325% relative to control) and IL-1β (+125% vs. control), PI3K and MCM2 suppression. By 11 days, Hsp27/70 and PI3K were downregulated, marking the transition toward cellular exhaustion. By 15 days, a persistent inflammatory state was observed with continued IL-6 elevation, significant lipid peroxidation indicated by the MDA level, persistent ROS production, and further reductions in Hsp27/60, and PI3K. Optical microscopy revealed cell volume increase from day 1 (160 μg/mL), and persistent vacuolization (16-160 μg/mL) from day 3. Live/Dead assay confirmed a dose-dependent decrease in

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viability, correlating with prolonged exposure. These findings suggest that sustained exposure to PS-NPLs induces a biphasic response in SVEC4-10 cells, characterized by acute inflammation and stress adaptation, followed by suppressed proliferation, and chronic inflammatory signaling.

P-45-021
Long-term nanoplastic exposure induces oxidative changes in RTgill-W1 cells

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Understanding the extent of nanoplastic (NPL) damage in freshwater ecosystems is essential yet challenging, due to their diverse distribution patterns and complex internalization mechanisms. This study aimed to evaluate the cytotoxic effects induced by repeated treatment with polystyrene-NPLs (PS-NPLs) on the rainbow trout (*Onchorynchus mykiss*) RTgill-W1 cell line. The repeated treatment involved 100 nm-PS-NPLs (0.5, 25, and 100 μg/mL). Untreated cells served as control. Tests were performed after 24 hours, 3, 6, and 9 days of exposure. Cell viability and morphology were assessed using fluorescent microscopy (Live/Dead, F-actin staining), while membrane integrity was evaluated by estimating the activity of lactate dehydrogenase (LDH). Assessment of oxidative potential was performed by measuring the level of reduced glutathione (GSH), malondialdehyde (MDA), and the enzymatic activity of glutathione peroxidase (GPX). The generation of reactive oxygen species (ROS) was estimated using flow cytometry. Fluorescence microscopy revealed morphological alterations and cytoskeletal disorganization (day 3 at 25 μg/mL and day 1 at 100 μg/mL). LDH levels increased significantly at 100 μg/mL, indicating membrane damage, while at 0.5 and 25 μg/mL, no significant changes were observed. GSH concentration increased in day 3 at 25 μg/mL, suggesting an antioxidant response, but decreased with prolonged exposure. MDA levels rose significantly at 100 μg/mL, reflecting lipid peroxidation. GPX activity progressively increased at 25 and 100 μg/mL, reflecting oxidative stress adaptation. ROS production showed that certain concentrations did not significantly increase ROS levels vs. control. These findings suggest that 0.5 μg/mL PS-NPLs induce minimal effects, 25 μg/mL activates antioxidant defenses, while 100 μg/mL triggers severe cytotoxicity. The results highlight concentration-dependent toxicity and underscore the need for further research into PS-NPL impact on freshwater species.

P-45-022
MiRNA 34a-5p in acute model of anthracycline cardiotoxicity in rabbit

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Anthracycline (ANT) cardiotoxicity is a well-known complication of cancer treatment, and miRNAs (mainly miRNA-34a-5p) play very important role in the development of ANT induced cardiomyopathy resulting in heart failure. Our previous study described that the first subclinical signs of cardiotoxicity (characterized by significant increase in plasma cardiac troponin T, cTnT) were observed after 5 weeks of daunorubicin treatment. At this time point, miRNA-34a-5p was significantly upregulated (fold change 6.2, $p < 0.001$). After 10 weeks of daunorubicin treatment, when a further rise in cTnT was accompanied by significant left ventricle systolic dysfunction, the miR-34a-5p was also even higher (76-fold change, $p < 0.001$). The pathogenesis of anthracycline cardiotoxicity is linked to their effect on topoisomerase IIβ. The aim of this study was to investigate if the miRNA-34a-5p can be also involved in acute stage of ANT cardiotoxicity, which is characterized by the process of DNA damage, and the subsequent DNA damage response. Acute ANT cardiotoxicity was induced in male rabbits using a single dose of daunorubicin (DAU, n = 6; 3 mg/kg i.v.) and compared with controls (n = 6, saline 1mg/kg i.v. in the same schedule). In myocardial samples of the left ventricle 1.5 hours after DAU administration, double-strand break marker γH2AX was significantly elevated in DAU groups compared to controls ($p < 0.001$). After 6 hours after DAU administration, p53 – the main regulator of DDR, was significantly elevated in animals treated with daunorubicin compared to controls ($p < 0.001$). However, it was not accompanied with significant change of miR-34a-5p. In conclusion, miR-34a-5p is not involved in the early stage of DAU induced cardiomyopathy, which is related to DNA damage response.

LB-R-45-01
The study of ritonavir on embryotoxicity: preliminary study

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Ritonavir (RTV), a protease inhibitor against HIV, has also been used with nirmatrelvir for COVID-19. Previous studies examining the effects of Ritonavir on the male reproductive system have shown that it increases oxidative stress and suppresses sperm functions during capacitation. Developing *in vitro* alternatives is essential to reduce and replace animal use in toxicity assessments. We used the mouse embryo assay (MEA) for assess the potential for embryotoxicity of Ritonavir. The present study aimed to investigate the effects of different concentrations of RTV (Sigma PHR1734) added to the culture media, the quality and in vitro development rate. Single-cell embryos were flushed from superovulated CBF1 female mice. The single-cell embryos were randomly divided into groups and cultured in life global medium + 0, DMSO, 0,1, 1, 10, and 100 μM RTV in 5% CO2, 37°C until 96-120 h at the blastocyst stage. The two-cell development rates of 0, DMSO, 0,1, 1, 10, and 100 μM RTV were 90% (31/34), 95.56% (43/45), 78.39% (65/83), 85.71% (54/63), 92.06% (58/63) and 84.12% (53/63), respectively. The blastocyst development rates of 0, DMSO, 0.1, 1, 10, and 100 μM RTV were 63.51% (22/34), 80% (36/45), 68.87% (57/83), 74.60% (47/63), 87.30% (55/63) and 39.68% (25/63), respectively. The hatched blastocyst development rates of

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0, DMSO, 0,1, 1, 10, and 100 μM RTV were 35.71 (5/14), 47.38 (17/36), 79.58 (28/35), 58.27 (19/33), 53.05 (30/55) and 11.01 (4/25), respectively. Reproductive toxicity testing is essential for the rational and safe use of pharmaceuticals. By the 3Rs principles, reducing animal use while increasing research efficiency through *in vitro* methods. This study has shown that hatched blastocysts decreased as RTV concentration increased and RTV 100 μM seems to embryotoxic effect on the *in vitro* mouse embryo development.

LB-R-45-02
High methamphetamine concentration interferes with amphetamine on LC-MS/MS: internal standard and product ion signal suppression

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Chromatographic techniques are confirmatory for illicit drug tests; however they can also be prone to interferences. Methamphetamine accounts for 37% of all addiction-related admissions in Türkiye, making it the most prevalent drug in emergency cases; those mostly have very high blood or urine concentration. In this report we detail interference from high methamphetamine concentration in an LC-MS/MS assay. Ten urine samples were selected with methamphetamine concentrations ranging from 22 887 to 56 263 ng/mL, exhibiting amphetamine-d5 suppression, poor peak shape and an amphetamine peak characterized by the presence of the quantifier ion (136.1 > 119.0), but absence of the qualifier ion (136.1 > 91.0). Sample preparation was performed using the "dilute and shoot" technique on an Agilent 6475 LC/MS-MS system, in positive MRM and electrospray ionization mode. Methamphetamine-d5 and amphetamine-d5 were employed as internal standards. Each sample was 10 to 100 for diluted using blank urine and subsequently reanalyzed. Quantitative analysis was performed using a seven-point calibration curve ranging from 0 to 1000 ng/mL. Qualifier ion and internal standard peak areas, and analyte concentrations were compared before and after dilution. While the amphetamine qualifier ions were not detected prior to dilution, both quantifier and qualifier ions became detectable after dilution. Additionally, the peak shapes and response of amphetamine-d5 aligned with those of the calibrators. Before dilution, the samples would have been interpreted as positive for methamphetamine but falsely negative for amphetamine. However, following dilution, the results were evaluated as positive for both methamphetamine and amphetamine, and quantification was made possible due to the correction of the amphetamine-d5 internal standard response. Awareness and management of such interferences are critical for accurate toxicological interpretation.

Virology

P-46-001
Niemann-Pick C1 protein modulates hepatitis B virus entry

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There are more than 296 million people world-wide chronically infected with hepatitis B virus (HBV) resulting in 800.000 annual deaths. Currently, there is no curative treatment. Accumulating evidence suggest a relationship between the HBV life cycle and host cell lipid metabolism. Niemann-Pick C1 (NPC1) is an endosomal/lysosomal membrane protein that when dysfunctional leads to Niemann-Pick disease type C (NPC), a rare, inherited lysosomal storage disease. The cellular phenotypes of NPC include cholesterol and sphingolipid accumulation, low luminal lysosomal Ca^{2+} levels and impaired phagosome-lysosome fusion. Owing it to the crucial role played in lipid metabolism, NPC1 is involved in the life cycle of several unrelated viruses. Our hypothesis is that NPC1 could modulate host cell pathways that are essential to HBV. To investigate this possibility, *NPC1* expression was down-regulated in hepatoma cell lines permissive for HBV replication (HepG2.2.2.15 and Huh7) and infection (HepG2-NTCP) and further confirmed by western blot and immunofluorescence. Analysis of the HBV life cycle in NPC1-depleted cells showed a significant inhibition of HBV internalization. Further investigation will focus on the mechanism underlying the modulation of HBV entry by NPC1, possibly involving changes in lipid distribution, attachment to the plasma membrane, regulation of HBV receptors or envelope endosomal uncoating.

P-46-002
Exploring viral spike–glycosaminoglycan interactions on a glycocalyx mimetic bilayer with dynamic mass photometry

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Glycosaminoglycans (GAGs) are multifunctional components of the glycocalyx that play an important role in a range of functions ranging from cell-cell signalling to cellular protection. GAGs also act as the first point of contact between a virus and host cell, which is an important aspect of viral attachment and fusion, yet we little know about this vital interaction interface. Importantly, there are examples of GAGs binding to viral spikes such as HIV-1, SARS-CoV-2 and Ebola spikes but the precise nature of this interaction, namely the effect of GAG structure, charge and density is unclear. Equally, the binding stoichiometry and effect specific GAGs play in infectivity are challenging to study. To study such interactions, we have developed dynamic mass photometry which is a single molecule measurement technique that efficiently quantifies the mass, diffusion and

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interactions of non-labelled biomolecules on a lipid bilayer. The modification of phospholipids in the system allows us to create a synthetic glycocalyx by immobilizing GAGs to the bilayer, in which we can measure the interactions with viral spikes over time. Here, we recently showed that HIV-1 envelope (Env) spike trimer confers a high GAG selectivity that is dependent on sulfation pattern and length. These data provide a better mechanistic understanding of molecular specificity of GAG binding to HIV-1 Env. In this way, we can unreveal how GAGs interact with viral spikes in a physiologically relevant environment, thus identifying viral attachment progress.

P-46-003
Host factors enhancing influenza A virus replication in engineered cell lines

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The effective replication of the influenza A virus (IAV) depends on multiple host cell factors. The maturation of viral particles requires the presence of serine proteases such as trypsin, which is added to cells *in vitro*. Experiments on cellular models require stable expression of proteases to optimize the reinfection process. IAV is sensitive to interferon-induced antiviral states, so deletion of IFNAR1 avoids a reaction to interferon during further infection cycles. In addition, the knockdown of caspase 2 (CASP2) delays apoptosis, which makes it possible to obtain virulent offspring before cell death. We compared the impact of transmembrane serine protease 2 (TMPRSS2) expression in HEK293T, knock-out of IFNAR1, and knockdown of CASP2 into the replication of 7 strains of different subtypes (H1N1, H3N2, H5N3, H7N2). Also, Madin-Darby canine kidney (MDCK) cells expressing TMPRSS2 were tested. Stable sublines expressing TMPRSS2 and CASP2 shRNA were obtained by lentiviral transduction, as well as control sublines expressing GFP. IFNAR1-knocked-out cells were obtained by CRISPR/Cas9 technology. TMPRSS2, CASP2 expression, and viral RNA levels were determined using quantitative RT-PCR and western blotting. Expression of TMPRSS2 enhances viral replication without the addition of trypsin during infection for strains H1N1 and H7N2. For other strains, the effect was either lower or absent. IFNAR1 knock-out enhances sensitivity for all of the studied strains, while CASP2 knockdown leads to a 20-fold increase of sensitivity for H1N1 and H7N2 strains. By combining these effects, it is possible to obtain a permissive IAV cell line, allowing the development of highly attenuated vaccine replication-competent IAV strains. This work was supported by the Russian Science Foundation (grant no. 23-74-10102).

P-46-004
Dependence of Puumala hantavirus infection on cellular cholesterol levels

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One of the difficulties in developing treatments for viral infections is the lack of understanding of the cellular mechanisms and factors that influence virus internalization. Hantaviruses are dangerous human pathogens that cause acute, often fatal, viral diseases for which there is no effective treatment. Studying cholesterol metabolism may be important in developing specific antiviral drugs against hantaviruses. We demonstrated that Puumala hantavirus infectivity depends on cellular cholesterol levels. Treatment of Vero E6 cells prior to infection with drugs that reduce intracellular cholesterol levels by blocking NADPH-dependent HMG-CoA reductase, statins (simvastatin, mevastatin) at a concentration of 0.6-10 μM, and PF-429242 as a sterol regulatory element binding protein (SREBP) inhibitor at a range of 15-80 μM reduced Puumala virus infectivity. The drugs demonstrated unequal and dose-dependent effects. A 50% decrease in Puumala virus internationalization was observed when cells were treated with statins at a concentration of 3-5 μM and 50-60 μM PF-429242, which corresponded to a decrease in the level of cellular cholesterol by up to 40% compared to untreated cells. Mevalonate at a concentration of 5 mM restored up to 90% of hantavirus infectivity in Vero E6 cells treated with statins and PF-429242. Treatment of Vero E6 cells with methyl-β-cyclodextrin, which extracts cholesterol from the membrane, at a concentration of 10 mM before infection led to a 30% decrease in total cellular cholesterol and a 95% loss of Puumala virus infectivity. The results of the study indicate an important role of cholesterol in the life cycle of Puumala viruses, its necessity for penetration into the host cell and, possibly, for virus replication. The possibility of using cholesterol-lowering drugs shows promise for the treatment of hantavirus infection.

P-46-005
Photosensitizing fluorescent dyes based on BODIPY scaffold as broad-spectrum antiviral drugs

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Aromatic fluorescent dyes have recently been widely applied in photodynamic cancer therapy due to their ability to produce singlet oxygen and other reactive oxygen species. Antiviral photosensitizers are less studied, but equally promising direction of photodynamic therapy. Earlier we demonstrated that derivatives of pentacyclic aromatic hydrocarbon perylene exhibit high activity against enveloped viruses. The mechanism of their antiviral activity is based on the generation of singlet oxygen, which oxidizes the lipid envelope of the virion and prevents fusion with the host cell. Despite extensive studies, further application of perylene derivatives has been limited by challenges related to structural modification and hydrophilization [previously published in: Mikhnovets et al. (2023) Int. J. Mol. Sci., 24, 16483]. To overcome this problem, we focused on

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boron-dipyrromethene (BODIPY) dye derivatives. Although BODIPY compounds are readily modified and widely employed in cancer photodynamic therapy, their potential as broad-spectrum antiviral agents remains largely unexplored. We synthesized a BODIPY-based series of compounds, that demonstrate strong antiviral activity and high singlet oxygen generation yield. We also conducted molecular dynamics simulation of membrane positioning and investigated the fluorescence anisotropy of the synthesized dyes. Correlating these data with photophysical parameters allowed us to further explore the photosensitization mechanism underlying their antiviral activity [previously published in: Maryewski et al. (2025) ACS Appl. Mater. Interfaces., 17, 3, 4502–4528]. Additionally, we developed near-infrared-absorbing BODIPY dyes that retain antiviral efficacy and enable deeper tissue penetration for photodynamic activation. This work was supported by the Russian Science Foundation (Project No. 23-15-00158). *The authors marked with an asterisk equally contributed to the work.

P-46-006
Downregulation of Toll-like receptor transcription factors towards human norovirus host cell entry

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Human norovirus (HuNoV) is currently the leading cause of non-bacterial acute gastroenteritis. Despite its substantial socioeconomic and health burden, HuNoV pathobiology remains poorly understood, attributable to the virus non-replicative nature *ex vivo*. Toll-like receptors (TLRs) are involved in host cell invasion of enteric viruses, leading to immune response activation by specific transcription factors. However, neither the factor nor the mechanism is known with regards to HuNoV. Moreover, commensal bacteria have been implicated in enhancing enteric virus invasion. Thus, this study aims to investigate the role of TLRs and their corresponding transcription factors in HuNoV infection. Firstly, virus replication in the microfold (M) cells 3D-Alcart© (a cultivation system established by our group previously) was confirmed via transmission electron microscopy (TEM) performed at 48-hour post-inoculation with HuNoV-VLP. The result showed the presence of HuNoV indicated by small and round, non-enveloped particles, widely distributed in the cytoplasm. The induction of pattern recognition receptor (PRR) signaling in response to virus stimulation was measured by Real Time PCR of the transcription genes; *NF-κB* and *IRF3/7* involved in the signaling pathway of TLR7. The result showed significant downregulation in the expression of *IRF3*, *IRF7* and *NF-κB1* genes of the group HuNoV infection with presence of commensal bacteria, *E. coli*. In conclusion, the results substantially suggest the involvement of TLR7 for HuNoV host cell entry via downregulated expression of the transcription factors towards mucosal immune response. Further work is ongoing to determine the expression level of TLRs as potential targets for a more comprehensive understanding of HuNoV mechanism of infection. *The authors marked with an asterisk equally contributed to the work.

P-46-007
A novel vaccine production platform: microalgae-expressed HCV E2 induces protective immunity in mice

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Hepatitis C Virus (HCV) remains a global health challenge, with no approved vaccine despite decades of research. The viral envelope glycoprotein E2 is a prime target for vaccine development due to its critical role in host cell entry and its immunogenic potential. However, producing recombinant E2 with native-like folding and glycosylation remains a major bottleneck in vaccine research. This study presents a novel approach for the production and purification of functional HCV E2 antigens, leveraging an alternative expression system to overcome existing limitations. We developed an optimized workflow for the expression, purification, and characterization of recombinant viral glycoproteins in microalgae. The antigen was extracted and purified or used in immunization studies by feeding algae biomass. Antigenicity was assessed via ELISA using E2-specific conformational antibodies. Structural integrity and glycosylation patterns were analyzed to evaluate the fidelity of the produced antigen in microalgae. The purified E2 antigen retained its native conformation and key neutralizing epitopes, as confirmed by binding to conformation-sensitive monoclonal antibodies. Intramuscular and oral immunization studies demonstrated strong immunogenicity. Furthermore, neutralization assays showed that sera from immunized animals effectively blocked HCV pseudoparticle infection, highlighting the functional relevance of the antigen. This study provides a novel and scalable strategy for producing functional viral antigens with native-like properties. The approach overcomes existing expression and purification challenges, paving the way for improved subunit vaccine candidates. Our findings contribute to the field of viral glycoprotein-based vaccines, offering new perspectives on antigen design and production for difficult-to-target pathogens. Previously published in: Hammel A. & Cucos L. et al. (2024) Proc. Natl. Acad. Sci. U.S.A. 121 (24) e2400145121. *The authors marked with an asterisk equally contributed to the work.

P-46-008
Deciphering PICIs activation in Gram-negative bacteria: AlpA as a transcriptional activator

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Phage-inducible chromosomal islands (PICIs) are small, mobile genetic elements widely distributed in bacteria. They are clinically important because they carry and spread genes involved in virulence, host adaptation, antibiotic resistance, and immunity

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(Fillol-Salom A et al. (2020) Microbiol Infect 22, 362–367; Fillol-Salom A et al. (2018) ISME J 12, 2114–2128). PICIs are satellite phages that parasitize the life cycle of bacteriophages, also known as helper phages, hijacking their machinery to induce their own life cycle and dissemination. To induce the phage life cycle, they must transcribe their genes, a process well studied in Gram-positive bacteria but not yet understood in Gram-negative bacteria. This study aims to decipher the mechanism by which this occurs, focusing on an activator protein named AlpA, whose transcriptional role remains unclear. The *Klebsiella pneumoniae* strain Kp DSM30104 (KpCIDSM30104) was induced with mitomycin, which activated a PICI island in its genome, confirmed by a Southern blot. Mitomycin triggers the bacterial SOS response, activating the present phages and inducing the lytic cycle. Mutations were made in the phages to identify which one activated the island, revealing that phage KP 31290 was responsible. Sequencing its genome revealed AlpA, an activating protein. Overexpressing AlpA from both the island and phage induced island activation, with phage-derived AlpA being more effective. This suggests that phage AlpA plays a crucial role in activating the PICI island, which has led to a focus on AlpA from the phage *in vitro*. Footprinting experiments confirmed that the two AlpA proteins bind to three distinct sites near the PICI integrase gene, with PICI AlpA binding most strongly to site 1, while phage AlpA had a higher affinity for sites 2 and 3. Additionally, crystallography experiments revealed the structure of the PICI protein, which contains a helical domain that binds to DNA and has one DNA-binding domain (DBD). *The authors marked with an asterisk equally contributed to the work.

P-46-009
Future updates on NeoRdRp for comprehensive RNA virus detection from metatranscriptome data

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RNA viruses are distributed across various environments, and most RNA viruses have recently been identified through metatranscriptome sequencing data analyses. However, due to the high nucleotide diversity of RNA viruses, identifying novel RNA viruses from metatranscriptome data remains challenging. To address this issue, we developed a dataset named NeoRdRp, comprising hidden Markov models (HMMs) of RNA-dependent RNA polymerase (RdRp) domains, which are essential for nearly all RNA viruses. The current version (v2.1) contains 19 394 RdRp HMM profiles constructed from 557 197 RdRp-containing amino acid sequences [previously published in: Sakaguchi et al. (2024) Front Virol 4, 1378695]. Since the release of this version, a huge number of novel RNA viruses with diverse RdRp sequences have been reported. Notably, RdRp sequences identified from RNA viruses discovered in hot springs differed significantly from previously known sequences and were undetectable using the current NeoRdRp datasets. Additionally, the current version has too many HMM profiles, leading to excessively long search times. To overcome these limitations, we collected additional datasets and simultaneously refined our bioinformatics pipeline to facilitate the identification of a broader diversity of RdRp sequences. The amino acid sequences, HMM profiles, and annotations of NeoRdRp are available at https://github.com/shoichisakaguchi/NeoRdRp.

P-46-010
Expression of soluble ectodomain of envelope protein (sE) in *Escherichia coli* cells

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Tick-borne encephalitis (TBE), a neurological disease caused by the tick-borne encephalitis virus (*Orthoflavivirus encephalitidis*), is one of the most prevalent arboviral diseases in Europe. Currently, there are no approved medications for TBE treatment. A promising antiviral target is the envelope (E) protein, which plays a key role in viral entry. However, producing soluble E protein remains challenging due to its propensity to degrade and aggregate during expression. In this study, we successfully expressed and purified the soluble ectodomain (sE) of the E protein, avoiding refolding step, and evaluated its ability to bind TBE antibodies. The gene encoding sE protein was cloned into the pET-28a(+) vector. When expressed in *E. coli*, the protein was insoluble due to misfolding. To address this issue, we co-expressed sE protein with chaperones using Chaperone Plasmid Set (Takara Bio), which contains five plasmids encoding different chaperone sets. Western blot analysis showed that only one chaperone set helped the sE protein to fold correctly and remain soluble. Next, we optimized expression and purification conditions to maximize soluble protein yield. To confirm the functionality of the purified sE protein, we performed ELISA assays using mouse and human serum samples containing TBE antibodies. In all cases, higher sE protein concentrations led to a significant decrease in optical density, indicating protein binding to antibodies. We further validated these results using surface plasmon resonance (SPR), which confirmed sE protein's affinity for a chimeric monoclonal TBE antibody. Therefore, the key findings of this study were the successful expression and purification of soluble sE protein and the demonstration of its antigenic activity via ELISA and SPR. These results pave the way for future studies where sE protein will be used as a target for screening potential antiviral compounds, helping identify promising candidates for TBE treatment. *The authors marked with an asterisk equally contributed to the work.

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P-46-011
Impact of SARS-CoV-2 spike protein on hemostasis: insights from platelet aggregation, coagulation, and fibrin polymerization assays

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The SARS-CoV-2 spike (S) protein plays a crucial role in viral entry by interacting with host receptors, but emerging evidence suggests its involvement in coagulation and hemostasis. This study aimed to investigate the effects of spike protein and its subunits (S1 and S2) on primary and secondary hemostasis, fibrin polymerization, and viscoelastic properties of blood clots using *in vitro* assays. Human platelet-rich plasma (PRP), platelet-poor plasma (PPP), and whole blood samples were incubated with recombinant spike proteins at 1, 5, and 50 ng/mL for varying durations (15 min, 30 min, 1 h, and 2 h). Platelet aggregation assays, coagulation tests (PT, aPTT, D-dimer, thrombin-antithrombin complex), thromboelastography (TEG), and fibrin polymerization imaging via confocal microscopy were conducted. Results demonstrated that spike proteins did not significantly alter platelet aggregation responses to ADP, collagen, or epinephrine. However, prolonged incubation with S1 at 50 ng/mL resulted in a decrease in fibrinopeptide A levels, suggesting an inhibitory effect on fibrin formation. Confocal microscopy confirmed that fibrin networks were disrupted in the presence of spike proteins, with increased fibrin aggregation. Thromboelastographic measurements indicated an acceleration of clot formation, potentially due to direct interactions between spike proteins and coagulation factors. Factor XII activation assays revealed that spike proteins could trigger intrinsic pathway activation. These findings highlight the prothrombotic potential of spike proteins and provide insights into their role in COVID-19-associated coagulopathies. Further studies are required to elucidate the clinical implications of these observations.

P-46-012
Production of Triatoma virus caspids from individual structural proteins

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Triatoma virus (TrV) is a member of the insect virus family Dicistroviridae and consists of a small, non-enveloped capsid that encloses its positive-sense ssRNA genome. TrV virus like particles (VLPs) have been previously obtained after genome release from native virions [1]. The capsid polyprotein has also been obtained in a baculovirus-based expression model [2]. Due to many advantages that *E. coli* protein expression could represent versus the mentioned methods and to the variety of nanotechnological applications of VLPs, we are optimizing the expression in bacteria, purification and *in vitro* assembly of individual viral subunits. Each of the structural proteins of the capsid, VP1, VP2 and VP3 can be expressed in bacteria to high levels. The 3C protease and the RNA-dependent RNA polymerase from non-structural ORF1 have also been produced and purified. The stability and solubility of the individual proteins under different buffer conditions has been investigated. *In vitro* virus-like-particles (VLP) self-assembly from protein subunits have been assayed under different conditions (protein concentration, temperature, pH, ionic strength, presence of macromolecular crowding agents or biopolymers). Ultimately, we aim to identify the best viral capsid functionalization loci and determine the size of insertions that do not compromise VLPs assembly and stability. The development and redesign of virus-based platforms for biomedical and biotechnological applications will benefit from easily obtained and modified recombinant viral proteins.

LB-R-46-01
Bioinformatics analysis of eventual novel ORFs within genomes of ssRNA phages

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Phages belonging to family Leviviricetes typically have a short 3.5-4 kb ssRNA genome, enclosed in a T = 3 capsid, built from 89 coat protein (CP) dimers and one or two copies of maturation or "A" protein (AP), responsible for attachment to cellular receptors – various kinds of bacterial pili. Genomes of all cultivated Leviviricetes phages have three core ORFs, encoding AP, CP and replicase subunit (RP), always in that order. Additionally, most ssRNA phage genomes have a separate ORF for lysis protein (LP). In some metagenomic studies putative additional ORFs have been noticed, although the existence of actual proteins has not been confirmed. We have performed extensive bioinformatic analysis of more than 94 000 available ssRNA phage genome sequences to evaluate likelihood of hypothetical ORFs to encode biologically relevant proteins. In combination with protein expression and structural analysis we confirmed that many hypothetical ORFs form well-structured proteins of unknown function. We have also searched for ssRNA phages with similar genomes in locally available environmental samples and confirmed presence of similar ORFs. Currently, we are isolating the ssRNA phages in order to establish the biological function of discovered ORFs.

LB-R-46-02
Development of reference materials for the validation of influenza A/B and RSV nucleic acid detection kits using digital PCR

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Validation of diagnostic methods requires quantitative standards. Whole-virus materials allow full assay evaluation under real

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conditions. However, commercial quantitative standards for respiratory virus nucleic acid tests remain limited. In this study, we aimed to develop quantitative nucleic acid standards for influenza (inf) A/B and respiratory syncytial virus (RSV) using digital PCR (dPCR). In the study, the amount of viral nucleic acids in samples prepared by pooling nasopharyngeal swab samples known to be RSV, infA, infB RNA positive were determined by dPCR using commercial primer/probe sets. Nucleic acid extraction was performed using a commercial kit. The analytical sensitivity (LoD) and lower limit of quantitation (LoQ), intra- and inter-study reproducibility and linearity of the dPCR method for inf A/B and RSV were determined. Samples were analysed by both dPCR and real-time RT-PCR (qRT-PCR), and the relationship between Ct values and dPCR quantification results was evaluated by linear regression. Statistics were performed using GraphPad Prism 10.4.0. The LoD values of the dPCR method for InfA, infB and RSV were 93.75, 15.59 and 26.23 copies/mL, respectively. The intra-study reproducibility (CV%) of the dPCR method ranged from 0.06 to 7.97, being higher in samples with low viral load. Inter-study reproducibility was 0.73 to 5.41. Linearity analysis performed with dilutions in the range of 3-4 $\log_{10}$ for inf A and inf B and 7 $\log_{10}$ for RSV showed $R^2 \geq 0.99$ for all three viruses. The concentrations measured by dPCR were correlated with qRT-PCR Ct results. When the reproducibility results of dPCR and qRT-PCR tests were compared, the CV% value of dPCR was significantly lower ($p = 0.0312$). In conclusion, standard materials obtained from sample pools using the dPCR method can be reliably used in the validation and verification of new tests due to their low limits of detection, high repeatability, and near-perfect linearity performance. *The authors marked with an asterisk equally contributed to the work.

LB-R-46-03
Elucidation of host-dependent antiviral targets of diphyllin analogues via integrative proteomic approaches and biophysical validation

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In our search for bioactive compounds from plants in the Lingnan region of China, we discovered a series of diphyllin analogues from Justicia plants as potent antiviral lead molecules with broad-spectrum antiviral properties [1, 2]. However, their precise molecular targets remain incompletely characterized, hindering structure-activity optimization. This study utilized proteomics-based approaches to systematically identify and validate molecular targets of diphyllin analogues, focusing on elucidating their role in antiviral mechanisms. Several potential targets were identified through affinity-based pull-down assays with biotin-conjugated diphyllin. These proteins are involved in viral trafficking, endosomal acidification, and host-pathogen interactions. Validation via label-free binding assays including, drug affinity responsive target stability (DARTS) and cellular thermal shift assays (CETSA) confirmed compound-induced stabilization of myosin-9, indicating direct engagement. Further characterization using microscale thermophoresis (MST) demonstrated a high-affinity interaction between diphyllin analogues and His-tagged Myosin-9, with binding constants (Kd) ranging from 0.59 to 3.29 μM, suggesting a strong and specific binding. These results indicate myosin-9 as a key functional target, with diphyllin analogues acting as potent modulators of its activity. Overall, our findings advance the mechanistic understanding of diphyllin-based antivirals and show how integrative proteomic and biophysical approaches can accelerate target-driven antiviral drug discovery for clinical translation.
Acknowledgements: This work is supported by the Research Grants Council of the Hong Kong Special Administrative Region, China (Project No. HKBU12103021), and the Innovation and Technology Commission of Hong Kong Special Administrative Region, China (GHP/206/22GD). References : 1. Zhang HJ et al. (2017) J Nat Prod 80, 1798-1807. 2. Martinez-Lopez A et al. (2019) EBioMed 47, 269-283. *The authors marked with an asterisk equally contributed to the work.

LB-R-46-04
Antibody–nanodisc conjugate (ANC) as a virucidal platform to overcome limitation of broadly neutralizing antibodies (bNAb)

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The recent resurgence of highly pathogenic avian influenza H5N1, characterized by outbreaks in wild birds, spillover into domestic animals, and increasing infections in mammals, has renewed concern over its pandemic potential. Although human cases remain rare and often mild, H5N1 has historically shown a fatality rate of nearly 48%, underscoring the urgent need for broadly protective antiviral strategies. Broadly neutralizing antibodies (bNAbs) have emerged as promising agents due to their ability to target conserved regions across viral strains. However, their efficacy often depends on Fc-mediated immune activation, which can be suboptimal during infections such as Influenza A and SARS-CoV-2. To address this limitation, we developed a strategy conjugating bNAbs to membrane scaffold protein (MSP)-based nanodiscs (NDs). These NDs possess a phospholipid bilayer that enables virucidal activity by binding to viral membrane proteins. Upon attachment, membrane fusion between the ND and viral envelope induces structural disruption and causes a puncture in the viral membrane, leading to lysis. An Fc III-binding domain was engineered into MSP to stably attach the nanodisc to the Fc region of bNAbs, forming an antibody–nanodisc conjugate (ANC). We constructed ANC using the MEDI8852 sequence in scFv-Fc format and demonstrated enhanced *in vitro* neutralization against Influenza A subtypes H1N1, H3N2, and H5N2. bNAbs targeting conserved regions of the SARS-CoV-2 spike also showed increased efficacy against pseudovirus when delivered via ANC. Importantly, ANC acts through an Fc-independent virucidal mechanism, disrupting viral membranes by physical rupture, and may be advantageous in immunocompromised settings. Future work will evaluate ANC's *in vivo* efficacy and optimize its components for therapeutic development. These findings support ANC as a promising next-generation antiviral platform combining epitope-specific targeting with direct membrane-disruptive activity.

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LB-R-46-05
Chimeric W-PreS-O vaccine provides protection against SARS-CoV-2 Omicron BA.1 in Syrian hamsters

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Despite the official end of the pandemic, COVID-19, caused by SARS-CoV-2, remains a significant health concern due to ongoing viral circulation and the emergence of new variants with increased transmissibility and immune escape. Therefore, designing a vaccine platform with easy antigen switching, high safety, strong neutralizing effects and broader protection has become a major priority. In this study, we evaluated the protective efficacy of the chimeric fusion protein-based vaccine W-PreS-O in a Syrian hamster model. W-PreS-O comprises receptor-binding domains (RBDs) from both the Wuhan and Omicron variants of SARS-CoV-2, fused to the PreS surface antigen of Hepatitis B virus, a modular carrier enhancing immunogenicity, and adsorbed to aluminum hydroxide. Hamsters were divided into vaccine, placebo, and intact (untreated) groups, immunized three times at three-week intervals, and infected with the Omicron BA.1 variant. Body weight and clinical condition were monitored, and neutralizing antibody titers, viral loads in the upper and lower respiratory tracts, and lung pathology were assessed by macroscopic and microscopic examination. Vaccinated animals showed milder clinical symptoms and recovered faster, regaining body weight and general condition sooner. These improvements were accompanied by robust neutralizing antibody responses to Omicron BA.1, significantly reduced infectious virus titers in the lungs, and a marked reduction of viral RNA in the nasal cavities compared to the placebo group. Histological analysis demonstrated that W-PreS-O immunization almost completely prevented pneumonia and lung edema, while placebo-treated animals displayed pronounced lung pathology. These findings demonstrate prove of concept that the W-PreS-O vaccine provides protection against SARS-CoV-2 Omicron BA.1 infection in Syrian hamsters and supports the further development of chimeric fusion protein-based vaccines as a promising strategy for broad-spectrum COVID-19 prevention.
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Miscellaneous

P-47-001
Investigation of the relationship between placental grade and the immature platelet fraction

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Immature platelets with high RNA content, newly released from the bone marrow, are the youngest platelets that are indicators of platelet function. Increased blood coagulation at the tissue level of the placenta, which is important for fetal growth and a healthy pregnancy, is related to advances in placental grade. This study was performed to investigate the correlation between immature platelet fraction (IPF) and placental maturation. A total of fifty women who attended fetal assessment during their pregnancy at the Gynecology and Obstetrics Clinic of Konya City Hospital were included in the study. Twenty of the patients in the study were in the second trimester and thirty were in the third trimester. Placental changes were evaluated ultrasonographically. Placental sonographic images were graded according to the Grannum scoring system, and synchronous haemogram samples were taken from patients. Platelet indices were measured using an automated hematology analyzer. Maternal IPF, mean platelet volume (MPV), platelet distribution width (PDW) and platelet count were compared with placental grades. The relation between week of pregnancy and placental Grannum score was significant ($p < 0.001$); i.e. placental Grannum score increased with gestational age. The association between Grannum score and IPF was analysed by Kruskal-Wallis test and a significant positive relationship was found ($r = 0.385$; $p < 0.001$). While a statistically significant correlation was found between PDW and Grannum score ($r = 0.311$; $p < 0.05$), MPV and platelet count were found to have no significant relationship with Grannum score ($p > 0.05$). The results of this study, which demonstrated the relationship between hypercoagulation and placental grade through IPF, suggest that IPF can be used as a new potential biomarker in the early detection of placental aging.

P-47-002
Comparison of different statistical algorithms for continuous reference intervals of alkaline phosphatase in the pediatric population

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Continuous reference intervals (RIs) can provide a more precise evaluation of dynamic physiological changes, potentially improving laboratory result presentation. This study aimed to visually examine and compare different models used to establish continuous RIs for alkaline phosphatase (ALP) in the pediatric population. ALP data of children aged 0–18 years from a tertiary hospital, covering the period between March 1, 2024, and

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February 1, 2025. LMS-based statistical modeling was employed to determine continuous RIs. For the parametric additive terms, we used GAMLSS with third- and fourth-degree polynomials. For non-parametric additive terms, we used GAMLSS with splines and machine learning. Model comparison was performed using the Generalized Akaike Information Criterion (GAIC), where the optimal model is identified by the lowest GAIC value, while a higher Pseudo R^2 indicates a better model fit. The analyses were conducted using the application available at github.com/SandraKla/AdRI_GAMLSS with R v. 4.4.2. For males, the LMS model was superior to the other models, with the lowest GAIC values of 61 592 and R^2 of 0.457. For females, the P-splines model was superior to the other models, with the lowest GAIC values of 60 256 and R^2 of 0.737. In these models, the reference range is initially broad after birth, followed by a period of relative stability throughout childhood. At the onset of puberty, a noticeable widening occurs, primarily due to an increase in the upper reference limit. As individuals transition from puberty to adulthood, both the upper and lower limits decrease, resulting in a progressive narrowing of the reference range. The utilization of continuous RIs may present notable benefits over traditional age-based partitioning methods, especially in detecting sudden age-related shifts within pediatric populations. However, their practical implementation and usability may be limited by challenges, including integration into the laboratory information system.

P-47-003
Population genetic studies of *Apis mellifera* L. Kazakhstan using STR markers

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The honeybee (*Apis mellifera* L.) is an important pollinator of many agricultural crops and wild plants, making it an integral part of the ecosystem and agriculture. Globally, the honeybee is a key subject of scientific research aimed at preserving its populations and studying the genetic diversity necessary for environmental adaptation. In Kazakhstan, with its diverse climatic and geographical conditions, honeybee populations represent unique genetic groups shaped by local environmental factors. Honeybees collected from the Almaty, Zhambyl, West Kazakhstan, Akmola, Zhetysu and Turkestan regions of Kazakhstan were used for this study. Genomic DNA was isolated using the DNeasy Blood & Tissue Kit (Qiagen, Germany). Microsatellite analysis was performed on nine loci: A24, A28, A43, A88, A124, A007, A113, Ap55 and Ap43. Fragment analysis was performed on the SeqStudio analyzer and analyzed using GeneMapper v.6.0 software. The population structure was examined using STRUCTURE 2.3.4 software using the standard algorithm. As a result, the AP43 locus was identified as the most informative, showing high polymorphism. Analysis of allelic diversity revealed that the highest number of effective alleles was recorded in the Turkestan population (Ne = 5.99 ± 0.78), and the lowest – in the populations of West Kazakhstan and Zhambyl region. The Shannon index also confirms the high genetic diversity of Turkestan bees. Heterozygosity assessment showed that Almaty_2 population had the highest observed heterozygosity, while Almaty_3 had the lowest. The genetic structure of the populations, determined using the Delta K method, revealed five genetically distinct groups with clear differences between regions. Thus, this study highlights significant differences in the genetic structure of honeybees in Kazakhstan, which is important for conservation and management of populations in different regions.

P-47-004
Is it sufficient to use a heterophilic blocking tube in case of suspicion of heterophile antibody interference in terms of precocious puberty?

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Immunoassay methods are still widely used for the analysis of hormone tests. Due to the properties of the reagents used in immunoassay analyses and components other than the measured analyte, deviations may occur in clinical results. There are many factors that cause this condition called interference, and heterophile antibody (HA) is among the most common ones. If luteinizing hormone (LH) values are above 0.3 mIU/mL in pediatric patients under 8 years of age, girls and under 9 years of age, it suggests a preliminary diagnosis of precocious puberty. The reliability of the laboratory analysis result is important. Therefore, we included 48 serum samples belonging to the age group with LH values of 0.3 mIU/mL above, which can be diagnosed with precocious puberty. In order to evaluate the suspicion of HA interference, we measured the samples again by binding the antibodies and removing them from the matrix as recommended by the Clinical and Laboratory Standards Institute (CLSI) I/LA30 guideline. For this, we used a heterophile blocking tube (HBT). We analyzed the samples first with Beckman Coulter UniCel DxI 800 and then with Roche Cobas e 601 immunoassay systems. We separated the supernatants of the samples we processed according to the HBT application protocol into aliquots and measured them on both autoanalyzers. Although there was a significant difference in the results obtained from two different autoanalyzers without HBT pretreatment ($p < 0.001$), we found that the measurements after HBT pretreatment did not create a statistically significant difference between the two devices ($p = 0.751$). We concluded that the HBT method is not suitable for the LH test in detecting HA interference. In the future, with the widespread use of biosensor-labeled methods and their application in routine patient sample analysis, it may be possible to report more cost-effective, accurate analysis results that do not allow interference in a shorter time.

P-47-005
New chlorpyrifos-degrading bacteria isolated from Turkish soil

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Pesticides are widely used chemicals in agriculture to control pests, but their uncontrolled and intensive use increases their persistence in ecosystems and causes harm to non-target

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organisms. Chlorpyrifos (CP), an organophosphate pesticide, is widely used as an insecticide; however, it accumulates in soil and water, presents a significant threat to both ecosystems and human health, primarily due to its neurotoxic effects. Biodegradation of pesticides is a critical process to reduce ecotoxicity, promote sustainability in agriculture, and protect public health, thus warranting support. For this purpose, the study was conducted to identify bacteria with pesticide-degrading capabilities. To identify bacteria with this ability, soil samples were collected from a citrus orchard in the Mediterranean region of Türkiye, where various pesticides, including CP, were used. After enrichment for CP-degrading bacteria, morphologically distinguished isolates were identified using 16S rDNA characterization. Isolates HB10 and HB24 showed 99.29% and 99.43% molecular similarity to *Olivibacter jilunii* and *Pseudomonas oryzihabitans*, respectively. Although there are limited biodegradation studies on some *Olivibacter* species, no research has been conducted on pesticide degradation by *O. jilunii*. While *P. oryzihabitans* has known degradation capabilities for some pollutants, no studies have explored its potential for degrading CP or other organophosphate pesticides. This study presents the first report on pesticide degradation by both *O. jilunii* and *P. oryzihabitans*, contributing new insights into their potential roles in bioremediation efforts. The findings suggest that both bacterial strains may play an important role in the biodegradation of CP and may have potential applications in bioremediation to remove pesticide contaminants from ecosystems. Additionally, this study contributes to the limited number of biodegradation studies conducted in Turkish soils.

P-47-006
Investigation of the biological properties of *Rubus sanctus* aerial parts extracts

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Medicinal plants have a long history of traditional use and constitute a large pool for identifying bioactive compounds or mixtures with a therapeutic effect. Many modern pharmaceuticals have been derived from plant-based compounds, demonstrating the immense potential of natural sources in drug discovery. *Rubus sanctus* (RS) has been traditionally used in herbal medicine, particularly in Mediterranean and Middle Eastern regions for its antioxidant, antimicrobial, and anti-inflammatory effects. Here, we aim to further elaborate on the properties of RS by using plant material from Cyprus. We generated six distinct RS fractions using dichloromethane, methanol, and water. Specifically, three extracts were prepared from the leaf material and three from the remaining aerial parts. Then, we assessed their antioxidant, anti-cholinesterase, antimicrobial, and anti-neurotoxic potentials. Chemical characterization of the extracts is underway by ultra-performance liquid chromatography tandem mass spectrometer with electron spray ionization in either positive or negative mode. Specific RS extracts (methanolic) showed a strong antioxidant activity that correlated positively with their corresponding flavonoid and phenolic contents. Some extracts demonstrated a considerable anti-cholinesterase potential against acetylcholinesterase and butyrylcholinesterase, while an important antimicrobial potential was also documented. Dichloromethane extracts showed that they could uplift cell viability in neuroblastoma cells. Finally, the extracts originating from leaves showed a slightly better performance than those from the remaining aerial parts. Our results suggest that particular RS mixtures possess properties that can be useful to the pharmaceutical industry for developing herbal drugs and functional food products. These abilities can be attributed to the presence of specific bioactive compounds.

P-47-007
Investigation of the possible role of Tool-like receptor 4 (rs4986790) polmyhorpism in patients diagnosed with immune thrombocytopenic purpura (ITP)

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Immune (idiopathic) thrombocytopenic purpura (ITP) is an acquired hemorrhagic syndrome characterized by decreased platelet count, increased megakaryocytes in the bone marrow, and shortened platelet lifespan due to antiplatelet factors in the spleen and circulating IgG structure. The etiology of ITP is not yet clear, but genetic and environmental factors are thought to play an important role in the development of the disease. Toll-like receptors (TLRs), which play an important role in the innate response to infections, are also involved in acute and chronic inflammatory processes induced by endogenous and exogenous ligands. The TLR4 rs4986790 variant may play a role in pITP by contributing to increased inflammation and/or inhibiting the anti-inflammatory effects of therapeutic agents (e.g. steroids). Therefore, this study aimed to investigate the possible role of TLR4 (rs4986790) polymorphism in patients diagnosed with ITP. The study included 24 patients diagnosed with ITP and 24 patients without any systemic disease. DNA was isolated from blood samples taken into EDTA tubes from the individuals participating in the study. Analysis of TLR4 (rs4986790) polymorphism was performed using a Real Time PCR (LC480, Roche) device. In TLR4 rs4986790 polymorphism in ITP disease; AA, AG and GG were found to be 76%, 16% and 8% in the patient group, and 84.6%, 7.7% and 7.7% in the control group, respectively. No statistically significant relationship was found between the disease and genotype ($p = 0.648$). In addition, when examined in terms of alleles, no significant difference was found between the groups ($p = 0.513$). This study is the first to evaluate the TLR4 (rs4986790) polymorphism in adults diagnosed with ITP, and it is thought that the data obtained may be clinically important when supported by further studies including disease severity and treatment response. Key words: Toll like receptor, PCR, Immune (idiopathic) thrombocytopenic purpura.

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P-47-008
Nonsense mutations in mitochondrial DNA: correlative pairs associated with atherosclerosis

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Mutations in mitochondrial DNA (mtDNA) can lead to mitochondrial dysfunctions. Mitochondrial dysfunction can be found in many pathologies, including atherosclerosis. In this study, we sequenced mitochondrial DNA isolated from macrophages of patients with atherosclerosis (n = 12) and controls (n = 7) to identify mutations correlating with atherosclerosis. For mtDNA extraction, we used the mtDNA Extractor CTKit (Fujifilm). We employed nanopore sequencing technology (Oxford Nanopore Technologies) for mtDNA sequencing. The NCBI Reference Sequence: NC_012920.1 was used as the reference human mitochondrial genome. Sequencing depth and genome coverage analysis were performed using our original custom computer programs written in Python. It was established that sequencing was performed with 100% coverage and a read depth of more than 20 000×. Genomic data processing was conducted using the "Bio" module, bootstrap analysis and statistical analysis using the "scipy" and "statsmodels" modules, and data visualization using the "matplotlib" and "seaborn" modules. The analysis did not reveal a significant correlation of any single mutation with atherosclerosis. However, we identified 22 pairs of mutations correlating with atherosclerosis. Moreover, mutation m.3333C>T was present in 15 pairs, while m.6548C>T was present in the remaining 7 pairs. Both mutations are nonsense mutations; m.3333C>T causes the formation of a truncated, non-functional form of NADH dehydrogenase complex I subunit 1, while m.6548C>T affects cytochrome c oxidase subunit 1. Both of these enzymes play a critical role in OXPHOS, therefore nonsense mutations in their coding genes can lead to mitochondrial dysfunction. Thus, we found "nonsense-modifier mutations" that correlate with the presence of atherosclerosis.
This work was supported by Russian Science Foundation Grant # 22-15-00064-P.

P-47-009
Developing a method for targeted ACPA profiling that allows discrimination of rheumatoid arthritis versus juvenile idiopathic arthritis

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The second-generation anti-cyclic citrullinated peptide (anti-CCP2) test is a cornerstone of rheumatoid arthritis (RA) diagnostics, valued for its specificity and sensitivity. However, it uses artificial peptide motifs unrelated to any natural citrullinated protein. Our study presents an alternative ELISA method targeting citrullinated chemokines directly linked to arthritis for improved diagnostics. Biological samples from RA (n = 45), juvenile idiopathic arthritis (JIA) (n = 36) patients, and healthy donors were analyzed. Anti-citrullinated peptide antibodies (ACPA) were detected using ELISA and western blot, while total chemokine levels were assessed with standard ELISA. In RA patients, we identified ACPA against citrullinated ENA-78/CXCL5, MIP-1α/CCL3, and IL-8/CXCL8. ENA-78/CXCL5 levels were higher in early and severe RA compared to progressed RA and elevated in JIA serum versus synovial fluid, suggesting its diagnostic potential. Elevated IgA and IgG ACPA levels in advanced RA indicate their role in disease progression. Thus, we successfully detected the ACPA targeting chemokines strongly related to RA development and progression. The method we developed could improve RA and JIA or other autoimmune disease diagnostics and enable personalized treatments.

P-47-010
Azithromycin induces oxidative stress and detoxification enzyme GST activity in the fruit fly *Drosophila melanogaster* (Meigen) (Diptera: Drosophilidae) larvae reared on an artificial diet

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Antibiotics are widely used in insect rearing as phagostimulants or deterrents to influence the consumption of artificial diets. The newer macrolide antibiotic azithromycin (AZR) as an alternative chemical for managing the model pest fruit fly *Drosophila melanogaster* (Meigen) (Diptera: Drosophilidae) was evaluated in this study. The effects of AZR on oxidative stress parameters, malondialdehyde (MDA) and protein carbonyls (PCO) levels, and detoxification enzyme, glutathione-S-transferase (GST) activity of model pest fruit fly *D. melanogaster* last instar larvae were investigated at dietary concentrations of 100, 200, 400 and 800 mg/L. At the highest concentration, AZR significantly increased MDA level and while high concentrations increased PCO level. Detoxification enzyme GST activity was increased in the larvae by approximately about 3-fold at the highest concentration. These findings refer that antibiotic-based dietary formulation induces oxidative stress and detoxification capacity of the fruit fly larvae, as promising alternative chemical management strategies.

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P-47-011
Association of *MTHFR* genotypes with serum uric acid levels

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Methylene tetrahydrofolate reductase (MTHFR) gene mutations and their association with uric acid metabolism remain unclear. This retrospective study aimed to examine whether the C677T and A1298C *MTHFR* mutations are risk factors for hyperuricemia by analyzing serum uric acid levels and *MTHFR* genotypes in 109 females from the Turkish population. Patients were compared to an age-matched healthy control group. Statistical significance was tested with the t-test, and clinical significance was assessed using bias percentages based on biological variation data provided by the European Federation of Clinical Chemistry and Laboratory Medicine (EFLM). The mean uric acid levels for the homozygous and heterozygous genotypes were 3.89 ± 0.86 and 4.35 ± 1.21 mg/dL for C677T, and 4.16 ± 1.21 and 3.75 ± 1.16 mg/dL for A1298C, respectively. In compound heterozygous patients, the mean uric acid level was 4.15 ± 1.28 mg/dL. When compared to healthy control group, a clinically and statistically significant increase in uric acid levels was observed in females with the heterozygous C677T genotype, while only a clinically significant difference was observed in A1298C homozygous and compound heterozygous genotypes. In conclusion, the C677T and A1298C *MTHFR* mutations may cause higher uric acid levels in females and may be a risk factor for hyperuricemia. *The authors marked with an asterisk equally contributed to the work.

LB-R-47-01
Development of a personalized aerobic exercise program to improve the quality of life for cancer survivors

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The aim is to develop a personalized aerobic exercise program to improve the quality of life for cancer survivors. The exercise characteristics of cancer survivors were categorized into four types, and an aerobic exercise program was designed to match each physiological characteristic. After expert consensus involving medical professionals and exercise physiologists, the following programs were finalized: Type 1 has a smaller skeletal structure and lacks muscle mass compared to other types, resulting in overall weakness in muscle strength and power. The aerobic exercise focuses on enhancing cardiorespiratory endurance and basic fitness. Exercise duration is 30 min, with a target heart rate (HR) of 60–80% of maximum heart rate (MaxHR), and a Borg's Rating of Perceived Exertion (RPE) of 3–6. Type 2 has excellent instantaneous cardiorespiratory power but weak cardiorespiratory endurance. Upper body strength is generally good, but lower body muscular endurance is weak. The aerobic exercise focuses on enhancing cardiorespiratory endurance and lower body strength. Exercise duration is 15 min, with a target HR of 80–90% of MaxHR, and an RPE of 7–8. Type 3 has generally good muscle strength, but excessive weight can lead to weak cardiorespiratory and muscular endurance. Reducing weight can improve both cardiorespiratory and muscular endurance. The aerobic exercise is designed as interval training to burn body fat and increase energy expenditure. Exercise duration is 30 min, with a target HR of 70–90% of MaxHR, and an RPE of 5–8. Type 4 excels in explosive power, such as cardiorespiratory and muscular power, but is very weak in cardiorespiratory and muscular endurance. The head, neck, and thoracic regions are developed, but the lower back, buttocks, and lower limbs are weak. The aerobic exercise focuses on enhancing cardiorespiratory endurance and lower body strength. Exercise duration is 15 min, with a target HR of 80–90% of MaxHR, and an RPE of 7–8.

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POSTERS – EDUCATION

Undergraduate teaching/ learning

P-E-01-01
Using digital escape rooms to enhance data analysis skills and student experience

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Active and gamified teaching methods have received significant interest within higher education. One active approach that has recently gained popularity is that of escape rooms and, in particular, digital escape rooms. In biochemical sciences generally, the ability to analyse and interpret data is an important learning outcome, however, undergraduate students often possess limited experience in utilising key analytical skills. The use of escape rooms may offer a playful route to supporting students with this key threshold concept. Therefore, this project aims to develop a digital escape room to enhance students' analytical skills and assess its impact on student experience. The digital escape room was created using the University of Salford's digital escape room platform "UoScape"[1]. Students perceived data analysis skills and experience were evaluated using pedagogical surveys comprising either open-answer or 5-point Likert scale questions[2]. This study was approved by the University of Salford's ethical review board (14273). Most students (n = 104) reported an enhancement of their knowledge of data analysis (74.0%). Students (67.3%) reported a positive experience completing the digital escape room. Students described positive experiences with gamified learning (74.0%), and that it enhanced the value of their degree (68.3%). Students (76.0%) stated that the digital escape room would support their upcoming assessments. Students reported puzzles containing mathematical elements (51.8%) were more challenging than image analysis puzzles (73.3%). When responses were stratified based on socioeconomic factors and higher education entry qualifications, we observed no significant difference between group responses. These data show the pedagogical benefits of digital escape rooms in enhancing students' knowledge of data handling and interpretation, student experience, and overall degree value. References: 1 Morgan N & Jones M (2025) Pedagogy: LTEC Learning and Teaching Showcase, 1(1) https://doi.org/10.57898/pedagogy.279. 2 Hussain et al. (2023) British Journal of Biomedical Science, 80, 11731.

P-E-01-02
A quasi-experimental study on the impact of active learning strategies in molecular life sciences education

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Traditional lecture-based (TLB) teaching in molecular life sciences often results in passive learning and limited retention, but it remains widely used. Active learning strategies (ALS), such as problem-based learning (PBL) and flipped classrooms, are believed to improve comprehension and engagement. However, more evidence is needed to confirm their impact on student performance, particularly in diverse settings, hence the need for this study. A quasi-experimental study was conducted with 34 fourth-year pharmacology students at Kaduna State University, Nigeria. The participants were divided into two groups: Group A (n = 17) received TLB teaching, while Group B (n = 17) was taught using active learning techniques, including PBL, case studies, and flipped classroom activities. Both groups studied core pharmacology topics such as Signal Transduction Pathways, Pharmacogenomics, and Biological Therapeutics over one semester. Pre- and post-tests assessed knowledge gains, and end-of-semester exams measured overall academic performance. Student engagement was gauged through anonymous surveys. Statistical analyses were performed using paired t-tests and chi-square tests, with significance set at $p < 0.05$. Group B, which experienced active learning, showed significantly higher post-test scores compared to Group A (mean: 78.5% ± 8.6 vs. 67.2% ± 9.4, $p < 0.001$). Group B also had a greater pre- to post-test score improvement (+22.3% vs. +10.1%, $p < 0.001$). End-of-semester exam results further favored Group B (mean: 81.4% ± 7.5 vs. 72.6% ± 8.3, $p < 0.001$). Also, 86% of Group B students reported feeling more engaged and confident in applying molecular pharmacology concepts, compared to 54% in Group A ($p < 0.01$). ALS significantly enhance student performance, engagement, and retention of molecular life sciences concepts. Integrating these methods into curricula may enhance deeper learning & better prepare students for clinical and research environments.

P-E-01-03
Challenge-Based Learning: the role of university in promoting meaningful learning in high school education

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Challenge-Based Learning (CBL) is an educational approach that engages students in solving real-world problems through collaboration, critical thinking, and innovation. CBL promotes deeper learning and student empowerment by making education relevant and impactful. CBL follows a structure where teachers (here called teamcher) or stakeholders propose a Big Idea and students must identify a challenge (engage phase), research solutions (investigation phase), and implement actionable outcomes (action phase). We piloted a CBL program with Reproductive Health as the Big Idea, in 2 high schools, engaging 51 students from 11th and 12th grade classes. Students explored how everyday factors influence human fertility. The groups were guided by 4 teamchers (2 teachers and 2 university students). The program was settled using Padlet, a collaborative digital platform enabling real-time sharing on virtual boards facilitating teamwork and project management and real time feedback. Students created their own Padlet and shared it within the CBL program's Padlet, making it accessible to the entire class and teamchers. The final oral presentation to the whole class was assessed according to immediate feedback using a well-defined criteria grid that was presented to the students in advance. Feedback questionnaires evaluated the method's effectiveness, gathering student insights on the learning experience and possible improvements. For example, students suggest that the programme should be carried out over a longer period, allowing them to implement the projects they have developed. The students enjoyed

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taking responsibility in developing the challenge they had chosen themselves. Overall, the experience was highly positive for both students and teachers, as students demonstrated engagement, enthusiasm, and high-quality contributions. This CBL program has the potential to be scaled across various educational levels and subjects, enriching the learning experience and increasing its impact.

P-E-01-04
Navigating e-learning challenges: data-driven insights from a mandatory Moodle course in medical chemistry

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The increasing popularity of e-learning has fostered innovative teaching approaches while simultaneously revealing certain drawbacks that may affect student engagement and learning outcomes. This study investigates student behaviour in a compulsory e-course on the Moodle platform within the subject 'Medical Chemistry and Biochemistry' at the Faculty of Medicine in Pilsen, Czech Republic. The Moodle course consists of ten sequential weekly topics that expand upon high school chemistry knowledge, including chemical nomenclature and basic chemical calculations. Each topic incorporates both required and optional learning activities. Completion of a control test is required to unlock the next topic. Analysis of user engagement data indicates that while the obligatory components are consistently completed by the students, the supplementary instructional materials and non-compulsory tasks are largely neglected, with only a small percentage of students accessing these resources. In the academic year 2023/24, a total of 431 General Medicine students fulfilled the course. Only 37 of them (8.6%) opened at least one non-compulsory part. Most students just filled in the answers to the questions mandatory for successful completion of the course. Furthermore, a dichotomous pattern emerges wherein participants either complete all optional components or none at all. There were 10 students (2.3%) who completed all non-compulsory tasks. Their average score in the control tests was significantly higher (94.5%) than the average score of all participants (83.1%). A score of 70% is required to pass the control tests. These findings suggest that while e-learning platforms are effective in facilitating the delivery of core content, they may inadvertently encourage a minimalistic approach to learning, thereby necessitating further efforts into strategies that could enhance the intrinsic motivation to engage with such comprehensive educational materials.

P-E-01-05
Validating biomolecular visualization assessments: a mixed methods study incorporating classical test theory and semi-structured interviews

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For over a decade, the BioMolViz group has been working to improve biomolecular visualization instruction and assessment. Through workshops that engaged educators in visual assessment writing and revision, this community has produced hundreds of assessment items, a subset of which are freely available to educators through an online repository, the BioMolViz Library. Assessment items are at various stages of a validation process developed by BioMolViz. To establish evidence of validity, these items were iteratively revised, reviewed by an expert panel, and tested in classrooms. Here, we describe the results of the final phase our process, which involved classroom testing across 10 United States-based colleges and universities with over 700 students. Classical test theory was applied to evaluate 26 multiple-choice or multiple-select items divided across two assessment sets. The results indicate that the validation process was successful in producing assessments that performed within our defined ideal range for difficulty and discrimination indices, with only four items outside of this scale. However, some assessments showed performance differences among student demographic groups. Thus, we added an interview phase to our process, which involved 20 student participants across three institutions. In these semi-structured interviews, small groups of students described their problem-solving strategies, adding their unique insights as the discussion progressed. As these interview transcripts were qualitatively coded, areas to further improve assessment items were identified. We will illustrate the progression of several items through the entire validation process and discuss how student problem solving strategies can be leveraged to guide effective assessment design. *The authors marked with an asterisk equally contributed to the work.

P-E-01-06
3DP-Jmol – an automatic tool for generating 3D printable molecular models from structural data

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Physical models of macromolecules have been shown to improve learning gains under various settings and to be well received by students and educators alike. Various systems to produce physical models are available, but coupling the available structural databases with 3D printing is by far the most flexible. Hypothetically, in this approach, any life science educator would be able to select any PDB entry, generate a custom physical model adapted to his teaching goals, and fabricate it using consumer-grade 3D printers. The major bottleneck in this approach is the conversion of the structural data into reliable printable files. This step requires knowledge in structural biology and 3D printing technology and is key not only for the printability of the model but also for its success as an educational or demonstration material. Here we present 3DP-Jmol, a Jmol/JSmol script that automatically loads structural data from known databases, evaluates the size of the model, atom types and bond lengths and generates printable files with minimum user input. The script allows users to select specific models of 3D printers and provides instructions related to adjusting the maximum and minimum size of the final physical model, as well as indicating suitable rendering styles that the user can choose from. Generated models have been tested for printability on consumer-grade FDM (fused deposition modeling) printers from 3 known brands (Prusa Research, Creality and BambuLab). Experimental support for multilateral

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printing is also available, which would allow multicolor printing with simple hardware setups. The script can be easily integrated into HTML web pages, and its code is available at https://github.com/mariusmihasan/3DP-Jmol/releases under an MIT License.AMM, AS and MM are supported by EDUMOL3D, Project PN-IV-P7-7.1-PED-2024-0343 financed by the Ministry of Research, Innovation and Digitization, UEFISCDI, Romania.

P-E-01-07
Student perceptions of pre-assessment: a barrier or a learning tool?

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Pre-assessment (PA) is designed to evaluate students' foundational knowledge and skills relevant to a course, helping educators tailor teaching methods and content accordingly. This study analyzed PA results from three consecutive years of pharmacy and medical biochemistry students enrolled in the Immunology course at the University of Zagreb Faculty of Pharmacy and Biochemistry. Kahoot quizzes were used to assess knowledge and gather student perspectives before and after the PA. Students perceived their prior knowledge in basic subjects, such as physiology and pathophysiology, to be lower than their course grades suggested. However, despite recognizing this gap, most did not take steps to reinforce their knowledge before the Immunology course. While students generally support active participation in shaping their education, they displayed a predominantly negative attitude toward PA. Even high-achieving students failed to recognize its benefits, often viewing it as an unnecessary burden rather than a valuable learning opportunity. Only a small proportion of students considered poor PA performance as a motivator for improvement, while the majority found the process stressful and discouraging. Interestingly, although PA aimed to enhance self-reflection and encourage independent learning, students rarely used it as a tool to address knowledge gaps. This discrepancy between students' self-reported learning needs and their reluctance to engage with PA suggests a need for better communication regarding its purpose and benefits. These findings emphasize the importance of fostering a more positive perception of PA, as it provides students with a structured opportunity for self-assessment while offering educators critical insights to optimize teaching strategies and improve learning outcomes.

P-E-01-08
Summary of a three-year program of changes gradually introduced in the short Biochemistry course for Emergency Medical Services students

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A stimulating learning environment enhances student satisfaction. Modern teaching should prioritize engagement techniques and student-centered approaches. Short courses with a limited number of students can present challenges, as participants often begin with varying levels of prior knowledge. Active teaching ensures participation from all learners. Changes were implemented gradually over 3 years within the biochemistry course for paramedic students. Each year, the same questionnaire was distributed to students. The survey was anonymous, voluntary, and conducted post-course but pre-exam. A total of 100 first-year students took part: 34 before the changes, 28 in the first year of changes, and 38 in the final year. Active learning methods enhanced engagement and facilitated practical knowledge application. The paramedic student perspective was adopted. Course topics were aligned with future professional tasks, including drug dose calculations in ambulances, eye injury safety protocols, and buffer systems in alkalosis/acidosis. Case studies from the paramedic journal, translated as "At Rescue", were integrated into the curriculum, and students completed real-life medical incident report forms. A blended learning approach was developed, incorporating the university e-platform. This included gamification elements, exam-preparatory and student-generated question banks, enabling them to earn points contributing to their final exam. The course modifications improved student satisfaction. Top ratings increased from 3% to 58%. More students found materials well-delivered (37%→66%), while those struggling with content decreased (27%→19%). There was a growing demand for clinical case-based practicals (79%→88%), particularly paramedic-specific ones. Despite increased lab sessions, 69% of students expressed a desire for further practical opportunities. However, seminar interest declined following the introduction of e-learning, indicating a preference for self-directed learning. *The authors marked with an asterisk equally contributed to the work.

P-E-01-09
Evaluation from a student's perspective: an individual opinion report on 1st-year biophysics courses in a Turkish medical program

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This report presents an individual evaluation of the 22-hour biophysics course taught by a single professor in a first-year Turkish medical program. The course content aligns well with the high school curriculum, making it accessible for students who already have a foundational interest in the subject. Additionally, visual materials and engaging course titles, such as "Cell as a Machine," enhance student interest and comprehension. One of the course's strengths is interactive participation, which encourages discussion and deeper understanding. However, some students perceive this as a form of assessment rather than engagement, leading to reluctance in participation. To address this, inviting senior students to the first session to share the benefits of active participation could encourage a more comfortable learning environment. A key challenge students face is not knowing how to study effectively for biophysics. To resolve this, clear study strategies should be incorporated into the course structure to help students prepare for both learning and exams. Overall, this report highlights the strengths and areas for improvement in the biophysics course, with recommendations to increase student engagement and provide better study guidance for first-year medical students. *The authors marked with an asterisk equally contributed to the work.

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P-E-01-10
Incorporating project-based learning through scientist interviews and student reflection in an undergraduate neuroscience course

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Project-based learning (PBL) is a widely adopted approach in undergraduate programs, taking various forms such as laboratory research, literature reviews, and academic service learning. Real-world exposure in PBL and neuroscience education is crucial for bridging the theory with practice, deepening students' understanding of the complexities of the field. This study explores the design and implementation of PBL through scientist interviews and student reflections within an undergraduate neuroscience course at Uskudar University, Istanbul, Turkiye. The PBL, designed as a group task, spanned 14 weeks and involved four deliverables, focusing on interviewing neuroscientists to learn about their scientific practices, views on interdisciplinarity, and career trajectories, and synthesizing this information into a reflection essay. Student work was assessed through rubric-assisted evaluation and qualitative feedback. Results indicate that students showed improvement in scientific communication, demonstrated enhanced team work and interdisciplinary thinking skills, gained a more comprehensive understanding of career paths. These findings align with the course's learning objectives by promoting critical thinking, teamwork, communication, and information literacy, while deepening students' understanding of neuroscience, interdisciplinarity, and career perspectives. The real-world exposure gained through conducting interviews allowed students to bridge theory with practical applications, fostering deeper engagement and critical thinking. This approach demonstrates strong potential for enhancing neuroscience education and could be adapted to other STEM programs by involving professionals from diverse fields, facilitating cross-disciplinary learning, and providing students with insights into various career paths. Expanding this approach could also be used in international settings to further enrich the learning experience and encourage global perspectives in scientific education.

P-E-01-11
Bridging the gap in interdisciplinary education: the ONCOLIGHT approach to cancer management training

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Despite the increasing implementation of next-generation sequencing (NGS) and molecular data in cancer management, European university education seems to remain fragmented, lacking interdisciplinary integration between medicine, biochemistry, biology, biotechnology, bioinformatics, and economics. To test this hypothesis, a comprehensive needs analysis was conducted across multiple European universities in Croatia, Spain, Netherlands, Germany and Türkiye, incorporating survey research from 46 faculty members and 88 students. The analysis assessed related curricular gaps, faculty readiness, existing interdisciplinary collaboration, and digital learning infrastructure. Data were collected anonymously through structured surveys via Google Form between 1st and 15th February 2025, and analyzed using GraphPad Prism. Findings indicate that only 8.7% of lecturers engage in interdisciplinary teaching, and over 50% of students lack familiarity with genomic technologies and healthcare policies. More than 45% of students were unfamiliar with early cancer diagnostics policies, while 43.2% had no exposure to NGS applications. Additionally, 40.9% felt unprepared to advocate for policy changes, and 48.8% lacked experience in interdisciplinary teamwork. Faculty members identified curricular rigidity, lack of faculty training, and insufficient digital infrastructure as major barriers. The ONCOLIGHT project Needs Analysis showed a strong necessity of creating interdisciplinary, online courses integrating molecular biology, biochemistry, bioinformatics, health economics, and clinical applications. The curriculum should emphasize case-based learning, hands-on genomic data analysis, and policy simulations to enhance student readiness for cross-disciplinary collaboration. These conclusions align with EU strategies such as the Digital Education Action Plan, and Horizon Europe, intending to ensure a new generation of professionals trained in evidence-based, cost-effective cancer management.

LB-E-01-1
Virtual labs and self-assessment integration in a customized web platform for biochemistry education in the Universidad de León

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To address the increasing demand for dynamic and accessible educational resources in university-level science education, we have designed and implemented a personalized online platform aimed at supporting Biotechnology students enrolled in the subject of "Proteomics and Protein Engineering" at the Universidad de León. This digital tool was developed to strengthen both conceptual understanding and technical proficiency through a dual approach. The first module of the platform offers interactive virtual lab experiences that replicate key experimental procedures and molecular techniques covered in the coursework. These simulations provide students with an opportunity to engage in protocol execution, data interpretation, and conceptual reinforcement within a risk-free, flexible digital environment, particularly valuable in contexts where access to physical laboratories may be limited. Complementing this, the second module serves as a private academic support hub. It fosters student interaction through peer-to-peer question forums and offers a self-assessment area featuring a comprehensive bank of practice questions. This resource is designed to aid students in identifying knowledge gaps, consolidating theoretical content, and preparing more effectively for evaluations. The platform also integrates analytical tools for instructors, enabling them to track student engagement and detect areas in need of pedagogical reinforcement. By aligning technological innovation with educational needs, this customized digital platform contributes to a more interactive and autonomous learning experience. It represents a practical step toward modernizing teaching strategies in the life sciences and promoting a more student-centered approach to learning in higher education.

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LB-E-01-2
Microbial identification by MALDI-Biotyper as a university practical training on mass spectrometry

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Nowadays, the huge variety of mass spectrometers configurations and applications, depending on the final target molecules, offered by the different providers makes it complex to be updated on this equipment, even for specialists. Thus, the undergraduate teaching of mass spectrometry to the Biotechnology Degree students [course: 'Proteomics and Protein Engineering' (Universidad de León)] could be a tough task. However, a MALDI instrument, which is a relatively simple mass spectrometer, due to a lack of a chromatography system (upstream of the apparatus) or gases as additional requirements to achieve a proper activity, appears as an optimal candidate for teaching. Microbial identification is a activity in high demand in human and animal health, as well as in environmental samples. Currently, the MALDI-Biotyper is one of the most popular procedures, which also includes the detection of antibiotic resistances. Thus, this microbial taxonomy assisted by a mass spectrometer seems to be an optimal way to ease its training for university students. A simple multi-response survey was conducted before and after the practical and theoretical teaching on different aspects of MALDI-Biotyper mass spectrometry to monitor the evolution of the students. The obtained results demonstrate the increase in knowledge gained by the students after these sessions, which increases students' skills in working with a mass spectrometer.

LB-E-01-3
Diverse outcomes of peer learning

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Peer learning is increasingly recognized as a powerful educational approach that enhances student comprehension and academic performance more effectively than traditional instructional methods. Functioning similarly to the "just-in-time teaching" method, peer learning facilitates the timely identification of conceptual gaps, enables immediate feedback, and promotes error correction. This approach maximizes student outcomes in terms of both efficiency and academic achievement. This study aimed to evaluate the effectiveness of peer learning in improving understanding and academic success in a biochemistry laboratory setting. The research was conducted with third-year medical students enrolled in a biochemistry laboratory course. A quiz comprising key conceptual questions was administered, followed by structured peer learning sessions. The question with the highest frequency of incorrect responses was re-administered during the term committee examination to assess long-term learning outcomes. Statistical analysis was performed using paired sample t-tests, with significance set at $p < 0.05$. In the initial quiz, the correct response rate for the selected question was 48.52%, while the incorrect response rate was 51.47%. Upon reassessment during the committee exam, the correct response rate increased to 70.41%, indicating a statistically significant improvement ($p < 0.05$). The findings demonstrate that peer learning significantly enhances academic performance and conceptual understanding in biochemistry education. Furthermore, it promotes long-term knowledge retention, improves communication and critical thinking skills, and fosters active student engagement through reciprocal teaching and shared responsibility.

LB-E-01-4
Blended learning in biomedical education: virtual tools for practical skill development

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In recent years, biomedical teaching has faced the challenge of adapting to digital environments, especially in the context of the COVID-19 pandemic. Practical courses, which are essential for developing experimental and analytical competencies, were particularly affected due to restrictions on face-to-face activities. This project presents the design and implementation of a hybrid teaching model in the Clinical Biochemistry course at the University of Alcalá, aimed at maintaining academic quality and enhancing student engagement through the integration of virtual and hands-on laboratory experiences. The strategy combined three stages: (1) a preliminary online module using active methodologies such as problem-based learning and flipped classroom, where students used biomedical databases and bioinformatic tools to design experimental protocols; (2) face-to-face laboratory work using cooperative learning techniques, where students executed their protocols and analyzed experimental results; and (3) a final stage of scientific writing and oral presentation, promoting collaborative work and critical thinking. Our results demonstrate that students not only acquired key practical skills but also improved their ability to apply theoretical knowledge to real biomedical challenges. Surveys revealed high satisfaction rates, particularly highlighting the value of using real-world tools and designing experiments as key motivators. This methodology also allowed a reduction in laboratory occupancy, supporting blended learning models. In conclusion, virtual tools can enhance the quality and flexibility of biomedical education, preparing students for current research demands while adapting to future academic challenges.

LB-E-01-5
Revolutionizing teaching of microbiology laboratories in undergraduate education through inquiry-based and digital learning

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Undergraduate microbiology education often struggles to connect foundational concepts, such as microbial physiology, to practical applications. To address this, two complementary approaches – an inquiry-based laboratory design and virtual laboratories (VLs) – offer innovative solutions to enhance student engagement and learning outcomes. A redesigned microbiology laboratory series utilizes problem-based learning centered on a food poisoning case study conducted over seven experiments. Students assume the role of microbiology technicians, employing skills such as aseptic techniques, bacterial cultivation, Gram staining, and antibiotic testing. This hands-on, inquiry-based methodology fosters active learning and critical thinking, using affordable materials and nonspecialized equipment. Successful implementation has shown improved student motivation and deeper conceptual understanding. As a digital complement, virtual laboratories provide additional opportunities for microbiology teaching. The ERASMUS+ VHEalthLab project offers an open-access platform with scenario-based interactive experiments relevant to microbiology, alongside other biological topics. These VLs promote inclusivity by supporting diverse learners through self-paced, multimodal materials in multiple languages and culturally adaptable formats. Topics such as microbial safety, metabolism, and clinical scenarios align with laboratory teaching goals, fostering key skills like problem-solving and digital literacy. By combining inquiry-based laboratory practices with the flexibility of digital learning tools, microbiology education can be transformed into a more accessible, engaging, and effective learning experience, preparing students for future scientific and clinical endeavors.

Postgraduate teaching/learning

P-E-02-01
DEGRADATOR: A gaming expedition into targeted protein degradation therapies

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In an era of rapid scientific advancements, engaging educational tools are essential for bridging the gap between complex concepts and general public understanding. DEGRADATOR, a 2D computer game, immerses players in the ubiquitin-proteasome system, a key pathway in cellular protein degradation, and introduces innovative therapeutic strategies like PROTAC drugs. Designed for players aged 12 and above, the game features 10 levels combining interactive gameplay with educational quizzes. Alongside the gameplay details, I will present insights from evaluating DEGRADATOR with 97 high school students (aged 15–19) during biology classes. Over 75% of participants completed the game in under 30 minutes, and 63% rated it highly for improving their understanding of protein degradation. Quiz results showed 59% scoring 10 or more out of 12 points on the final test, indicating significant knowledge retention. Teachers praised the game for deepening student comprehension and highlighted the value of accompanying materials for classroom integration. Freely accessible at https://degradator-game.com and incorporated into the LabXchange ecosystem, DEGRADATOR is complemented by teacher resources and multimedia content, ensuring its broad applicability in diverse educational settings. By blending education with entertainment, the game demystifies molecular processes and addresses potential future misconceptions about emerging targeted protein degradation-based technologies like PROTACs, fostering curiosity and understanding beyond traditional academic environments.

P-E-02-02
Design of a project-based learning strategy for biochemistry master teaching labs: a case study at University of Aveiro

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A project-based learning (PBL) strategy has been implemented in the Biochemistry Master's laboratory classes at the University of Aveiro, fostering active and student-centered learning. Each academic year, students engage with a new research question, promoting problem-solving, critical thinking, and hands-on learning. In the first semester of 2024/2025, students focused on investigating the side effects of an anticancer drug. A total of 28 students, divided into two classes of 13 and 15, collaborated in small groups of 2–3 to analyze the drug's effects on a specific organ (heart, liver or kidney), comparing results from control and treated animals. To assess these changes, students examined various biochemical parameters in biological samples, such as serum, markers for organ injury assessment, and organ extracts. A range of experimental techniques, including electrophoresis, western blotting, enzymatic activity assays, and mass spectrometry-based proteomics, was employed for tissue extracts analysis, ensuring a multidisciplinary and inquiry-driven approach. At the end, students synthesized their findings into a scientific report and engaged in peer discussions to interpret the physiological significance of their results and evaluate the applicability of the experimental approaches used. This pedagogical strategy significantly enhanced students' practical laboratory skills, critical data analysis abilities, and scientific reasoning. Student feedback was very positive, with a rating of 8.57 out of 9 for the curricular unit's overall functioning. Students particularly highlighted greater engagement, increased autonomy, and enhanced confidence in conducting experimental work. This case study underscores the effectiveness of PBL in fostering a research-oriented learning environment, promoting collaborative and self-directed learning, and bridging the gap between theoretical knowledge and experimental practice, ultimately preparing students for future challenges.

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LB-E-02-1
Advancing technical expertise in molecular diagnostics for educators and researchers "MolecuLearn"

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Our study project, MolecuLearn, was aimed at university teachers, researchers, and students with an interest in molecular diagnostics. The workshop carried out as part of the project was designed to provide training in modern genetic analysis techniques, including mutation research and diagnostics, the study of various disease types, and DNA sequencing analysis. Through practical, workshop-based sessions, participants used advanced laboratory equipment and gained both theoretical knowledge and hands-on experience in molecular and biomedical diagnostics. One of the initial outcomes was a significant improvement in the technical skills of teachers and researchers in performing analyses using the ABI 3500 sequencer, as well as enhanced competencies among students in the field of molecular diagnostics. As part of the laboratory training, students conducted research and analyzed novel biomarkers associated with the painful form of diabetic polyneuropathy in patients with diabetes mellitus, using molecular biology techniques. These skills will prepare our students to effectively utilize emerging technologies and respond to the growing demand for rapid and accurate diagnostics in clinical practice. The innovative educational approaches implemented at our university, along with the hands-on training offered through this project, are expected to benefit the broader community. *The authors marked with an asterisk equally contributed to the work.

LB-E-02-2
Critical thinking in action: debate as a teaching strategy in postgraduate biomedicine

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This study explores the effectiveness of debate-based learning in a postgraduate biomedical program at the University of Alcalá, Spain. A common challenge for students is writing the discussion section of theses or scientific papers. To address this, an educational intervention involved 18 master's students participating in structured debates based on unpublished scientific preprints. Students, acting as reviewers, were divided into groups with opposing views on whether a paper should be published. The debate sessions required thorough preparation, evidence-based argumentation, rebuttals, and audience participation. The activity was designed to foster critical thinking, communication, and teamwork. Each student was evaluated individually on presentation, argumentation, language, enthusiasm, and respect for opponents, using a detailed rubric. A post-activity survey indicated high student satisfaction; 91.7% found it valuable, and 100% supported integrating such methods into biomedical curricula. Students reported improvements in literature research, experimental design, scientific reasoning, and oral presentation. Academic performance also improved compared to prior cohorts (mean score increased from 8.0 to 9.3). Faculty feedback echoed the students' enthusiasm, emphasizing the debate's utility for developing essential scientific skills. The study concludes that debate is a powerful, underused pedagogical tool in postgraduate biomedical education that promotes active, collaborative, and analytical learning, and recommends its broader adoption in science curricula. *The authors marked with an asterisk equally contributed to the work.

Faculty and career development

P-E-03-01
Navigating career transitions in life sciences: the role of psychosocial counseling

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Career transitions in the life sciences can be challenging, often requiring scientists to navigate uncertainty about their next steps —whether in academia, industry, or alternative fields. While technical expertise is essential, career decision-making is deeply intertwined with personal values, motivations, and perceived self-efficacy. Psychosocial counseling offers a structured, reflective approach that helps scientists clarify their career goals, reduce anxiety about the unknown, and develop a concrete plan for the next steps. Here we explore how workshops incorporating psychosocial counseling techniques can facilitate career decision-making. By integrating structured discussions, small-group work, and self-reflection exercises, these workshops create a supportive environment where participants can explore their professional identities, assess career options, and develop actionable roadmaps for their future paths. Working in both small and larger groups, demonstrates how peer exchange and guided counseling support a deeper understanding of individual aspirations. A key outcome of this approach is that all participants leave the workshop with a personalized action plan, outlining clear next steps for their career development—whether continuing in academia, transitioning to industry, or exploring alternative career paths. This model offers a scalable, structured, and supportive approach to career development in the life sciences, providing scientists with the tools to make confident and informed decisions about their professional future.

P-E-03-02
Resilience in research: transforming the PhD journey amidst the pandemic

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The COVID-19 pandemic presented unprecedented challenges to the academic community, particularly for doctoral students. Disruptions to research activities, the closure of laboratories, and the uncertainty surrounding future academic progress led to

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heightened stress and anxiety. This study aims to explore how resilience strategies can help doctoral students navigate such crises and turn adversity into opportunity. Specifically, we examine how one of our PhD students, faced with the loss of access to the laboratory, overcame feelings of panic and distress, ultimately contributing to a series of significant academic publications. We applied a resilience-based approach to support the student in managing the psychological and professional challenges brought on by the pandemic. Initially, we encouraged the student to adopt a positive outlook, emphasizing that difficult circumstances could still yield productive outcomes. Instead of focusing on the limitations caused by the lab closure, we redirected our efforts toward theoretical work, literature reviews, and online collaborative research. This shift in focus led to the development of new research questions, culminating in the publication of a key opinion piece titled "Is Glucose-6-Phosphate Dehydrogenase Deficiency a Factor in Coronavirus-19 (COVID-19) Infections and Deaths?" which gained recognition as one of the top publications in its field at the time. The student's shift in perspective, from a sense of despair to a proactive, solution-oriented mindset, resulted in the production of several academic works despite the ongoing challenges. Our first publication during the pandemic quickly gained international attention, being ranked among the top 65 or 67 publications globally. Following this success, additional opinion articles and research papers were published, further solidifying the academic impact of the student's work during this challenging period. This experience highlights the importance of resilience in academic success, particularly for doctoral students. Adapting to negative circumstances and transforming them into opportunities for growth is a crucial skill in both academic and professional contexts. By embracing challenges and maintaining a positive, solution-oriented mindset, students can achieve remarkable academic outcomes even in the face of adversity. We conclude that resilience is essential for overcoming immediate setbacks and preparing students for future academic and professional challenges. *The authors marked with an asterisk equally contributed to the work.

LB-E-03-1
Artificial intelligence awareness and usage among medical residents

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Artificial intelligence (AI) is rapidly transforming clinical laboratory medicine by enhancing accuracy, efficiency, and decision-making. This study investigates the current state of AI awareness, usage patterns, and training needs among medical residents. A cross-sectional online survey was conducted among 44 residents from 14 different institutions. The questionnaire covered AI-related knowledge, laboratory use, academic applications, and educational expectations. Findings indicate that residents are generally receptive to AI integration, especially in post-analytical processes such as data interpretation and academic writing. However, knowledge levels were variable: 34% reported moderate knowledge, another 34% indicated limited knowledge, and only a small minority described their understanding as good or very good. The most commonly used AI tools were academic writing assistants, modeling platforms, and image analysis software. Post-analytical stages were reported as the most frequent entry point for AI use in laboratory workflows. Notably, 98% of participants agreed that structured training is necessary for effective AI use, and 88.8% supported its inclusion in residency education. These findings highlight the need for targeted curriculum development and institutional support to promote effective AI integration. The study contributes practical insights that can guide educational strategies and policy planning related to AI in clinical laboratory settings.

LB-E-03-2
Biomed News – a solution to keep up with the latest biomedical research literature using machine learning and expert curation

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Approximately 30 000 abstracts are published each week on PubMed, a database covering life sciences and biomedical topics. This enormous resource of new information needs to be retrieved by individuals who desire access to it – researchers, industry, funders, policy makers, clinicians, patients, and the public. This information is conventionally retrieved using searches or tables of contents. However, these methods can be overwhelming, broad and irrelevant. We aim for Biomed News to be a free user-friendly platform covering all areas of biomedical research that allows new abstracts to be discovered rapidly, shared with interested parties, recorded permanently and available openly. Biomed News uses selected abstracts to determine features that are relevant to each report topic through training. This allows for a broader set of features instead of those used in conventional searches. This enables a relevance score to be generated for each abstract which are then presented to editors in a ranked order. The ease of use of Biomed News has widespread impact as there are users based all over the world at different career stages and organisations, who are maintaining over 100 active reports with more than 1700 subscribers.

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POSTERS – ADDENDUM

This addendum comprises abstracts whose inclusion in the *FEBS Open Bio* supplement was delayed during the Congress administration.

Cell fate determination

LB-R-04-03
Positive correlation between homocysteine and ischemia-modified albumin in subjects with multiple sclerosis

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Multiple sclerosis is a progressive autoimmune disease that continues with the degeneration of the myelin sheath. Oxidative stress plays a central role in many diseases. Homocysteine and ischemia-modified albumin (IMA) are biomarkers of oxidative load. This study aimed to find out the connection between IMA and homocysteine. 44 relapsing remitting, 31 secondary progressive MS patients, and 39 healthy control individuals who applied to the Neurology outpatient clinic of Selcuk University Faculty of Medicine were included. Serum homocysteine concentrations were measured using liquid chromatography-tandem mass spectrometry (LC-MS/MS), following protein precipitation and derivatization protocols standardized for high-throughput biomarker quantification. Serum IMA levels were determined using a colorimetric assay based on the cobalt binding method, with absorbance measured spectrophotometrically at 470 nm. Statistical analysis was performed with SPSS statistical software package version 21.0. A one-sample Kolmogorov-Smirnov test was performed to find out the distribution. Student's t-test was used to compare the mean values between the two groups. One-way ANOVA analysis and Spearman correlation were also performed. $p < 0.05$ was accepted as statistically significant Serum homocysteine (10 ± 7 vs 7 ± 2 μmol/L, $p = 0.025$) and IMA levels (0.55 ± 0.13 vs 0.49 ± 0.08 ABSU, $p = 0.010$) were significantly higher than those of controls. Serum homocysteine levels were positively correlated with IMA levels ($r = 0.313$, $p = 0.005$) and EDSS scores ($r = 0.230$, $p = 0.041$) in the patient group. The observed association between elevated serum homocysteine and IMA levels supports a potential link between oxidative stress and multiple sclerosis. These findings align with existing literature implicating redox imbalance in multiple sclerosis and provide additional biochemical evidence for oxidative mechanisms contributing to disease processes.

Genome organization and stability

LB-R-08-01
RUVB-1 Is essential for genomic stability and cell cycle regulation in the *Caenorhabitis elegans* germline

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Maintaining genomic integrity is critical for proper cellular functions and ensuring accurate transmission of genetic information. Cells rely on complex signaling pathways that coordinate chromatin remodeling, DNA repair, and cell cycle regulation to protect against DNA damage. RUVB-1 is a highly conserved ATP-binding protein that functions as a scaffold and molecular motor, assembling and regulating diverse complexes related to chromatin remodeling, transcription, DNA repair, and cell cycle regulation. Despite extensive research, several aspects of RUVB-1 function remain unclear, including its contribution to specific complex activities and its context-dependent roles in different tissues. In this study, we aim to investigate the role of *ruvb-1* in maintaining genomic integrity in *C. elegans*. RNAi-mediated knockdown of *ruvb-1* triggered robust activation of the DNA damage response pathway as evidenced by elevated levels of ATM/ATR activity in the *C. elegans* germline. In addition, cell cycle progression was disrupted in *ruvb-1*-depleted germlines. Immunostaining for the mitotic marker pH3Ser10 revealed aberrant mitotic cells extending into the pachytene region, while EdU incorporation showed inappropriate DNA replication beyond the mitotic region of the germline. These defects were accompanied by the accumulation of RPA-1 at the chromatin and increased germline apoptosis. Notably, the apoptotic phenotype partially depended on CEP-1/P53 activity, as apoptotic cells were identified in cep-1 null mutants. Altogether, our results demonstrate that RUVB-1 is essential for preserving genomic stability and plays a critical role in cell cycle regulation. Our ongoing work aims to elucidate the molecular mechanisms underlying these findings and to define how RUVB-1 interacts with other proteins in genome maintenance pathways.

Protein chemistry

LB-R-21-03
Two magnesium ion binding sites of a pyruvate aldolase from *Achromobacter xylosoxidans*

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C1 compounds – such as methane, formic acid, methanol, CO_2, and CO – serve as substrates for various metabolic pathways, with formaldehyde representing a key intermediate due to its high reactivity. Pyruvate aldolases catalyze the reversible aldol reaction between pyruvate and aldehydes, producing 4-hydroxy-2-ketoacids with high stereoselectivity. These enzymes are of high interest because some of them can incorporate formaldehyde into pyruvate. We characterized a pyruvate aldolase from

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Achromobacter xylosoxidans (AxADL) that catalyzes the condensation of formaldehyde and pyruvate to produce 2-keto-4-hydroxybutyrate (2-KHB). AxADL exhibited optimal activity at pH 9.0 and 50°C in a Mg^{2+}-dependent manner. Crystal structure analysis revealed a typical triosephosphate isomerase (TIM)-barrel fold and hexamer formation through exchange of C-terminal α-helices between two trimers. The TIM barrel of each protomer features a deep and wide, solvent-accessible pocket containing several conserved residues essential for catalysis and substrate recognition. Structural comparison of the Mg^{2+}-bound and Mg^{2+} and pyruvate-bound forms exhibits two metal positions away by approximately 2.5 Å. Magnesium ion reorganizes the active site, positioning the metal to directly coordinate with the substrate and creating an environment suitable for forming enolate intermediate. Since AxADL shows modest activity, this structural feature will provide a basis for improvement through protein engineering to enhance the product yield.

Cancer – basic research

LB-R-30-25
Membrane HER2 localization associated with drug resistance in breast cancer cell lines

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HER2+ breast cancer is notable for its high invasiveness and poor clinical prognosis. Around 70–88% of HER2+ metastatic breast cancer patients do not respond to initial Trastuzumab (Herceptin) treatment. Moreover, most cases who initially respond to Trastuzumab develop secondary resistance within a year. Therefore, it is important to develop new strategies to overcome drug resistance. The HER2 tyrosine receptor kinase is located on the plasma membrane, and it is extremely complex to engineer anti-HER2 therapies because the intracellular pathways and biological effects, resulting from receptor activation, are particularly complex. In this study, we assessed the membrane HER2 localization and lipid structure, with particular attention to the architecture of lipid rafts, isolated from cell models of HER2+ breast adenocarcinoma characterized by different sensitivity to Trastuzumab. The data suggested significant differences relative to the total and membrane lipid profile among sensitive and resistant HER2+ breast adenocarcinoma cells, especially in fatty acid composition. Moreover, the resistant HER2+ breast cells showed a significant enrichment of the receptor also in the raft domains. This confirms the hypotheses that the ordered membrane microdomains could stabilize the receptor homodimers-heterodimers, favouring the persistence and expression of HER2 in the membrane, consequently increasing the transduction of neoplastic signalling and promoting the development of drug resistance. The specific and peculiar lipid and protein composition of lipid rafts contributes to the formation of lipid–protein and protein–protein interactions, responsible to HER2 membrane localization. In the future, the results emerging could contribute to identify biomolecular mechanisms implicated in resistance associated with membrane lipid domains and to develop new pharmacological therapies to alter the raft structural integrity and counteract the onset or progression of drug resistance.

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