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POSTERS

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Abstracts submitted to the 48th FEBS Congress from 29th June to 3rd July 2024 and accepted by the Congress Scientific Committee are published in this Supplement of *FEBS Open Bio*. Late-breaking abstracts are not included in this supplement. The abstracts are available as three PDF files: Talks (Plenary Lectures, Symposia and Speed Talks), Posters and Posters Annex.

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* Each poster has been given a unique number beginning with the letter P; the next part relates to the session in which the poster will be presented.

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POSTERS – RESEARCH

Advanced Methods of Structural Biology

P-01-001
Modulating the *in vitro* oligomerization of SMAD4

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SMAD proteins are the family of transcription factors that mediate transforming growth factor beta (TGFβ) signaling. The transcriptionally active unit of this signaling pathway is thought to be a heterotrimer made up of two receptor-activated SMADs (R-SMADs), such as SMAD3, and one chain of SMAD4. R-SMADs and SMAD4 are composed of approximately 500 amino acids and consist of two globular domains, the DNA-binding Mad Homology 1 (MH1) at the N-terminus and the C-terminal MH2 domain, which mediates protein-protein interactions. The MH1 and MH2 domains are connected by intrinsically disordered linkers comprising around 100 residues. To date, the interactions of isolated globular regions and linkers of SMADs with cofactors and/or DNA have been extensively studied. However, there are no data on the interactions of full-length SMADs, either in the context of transcriptionally active heterotrimers or in other complexes. To address these gaps, we plan to use constructs derived from llama heavy chain-only antibodies that we have discovered in collaboration with the VIB-VUB Center for Structural Biology in Brussels thanks to an Instruct-ERIC project. So far, we have identified a nanobody that binds the MH2 domain of SMAD4 in the nanomolar range and we have grafted it into larger rigid scaffolds to generate megabodies. We hope these megabodies will be useful to increase the mass of full-length heterotrimers or other complexes, and help in the identification of SMAD4 in structural studies by cryogenic electron microscopy (cryoEM).

P-01-002
Deep learning-based prediction of protein-carbohydrate interfaces

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Carbohydrates play a key role in essential energy cycles, support cell architecture, participate in cellular recognition and modulate host-pathogen infection. Their unique characteristics also make them potential sustainable material alternatives to fossil fuels. Despite significant technological advances, experimental resolution of protein carbohydrate interfaces still faces numerous challenges due to the flexibility and chemical diversity of carbohydrates, as well as the generally low affinity of such interactions. As a result, protein-carbohydrate complexes are significantly underrepresented in the Protein Data Bank, and there is a critical lack of atomic-level details on the interfaces of interaction between proteins and sugars, essential for a precise understanding of fundamental biochemical mechanisms. Our study consists of two main parts: thorough annotation of the available information of existing protein-carbohydrate structures and prediction of carbohydrate-binding residues in proteins using deep learning models. We have developed a new database including more than 2 million carbohydrate-binding sites and used this data to train two deep learning models. The first one is based on protein sequence data encoded by residue-wise embeddings derived from the state-of-the-art Ankh protein language model, while the second one integrates structural details in the form of a protein graph. We demonstrate that our models outperform every current tool in predicting carbohydrate-binding, as well as allows identifying missed carbohydrate-binding residues in biologically significant proteins. This methodology paves the way for understanding carbohydrate-related biochemical processes and can significantly impact the development of new sustainable materials.

P-01-003
The potential of coumarin-chalcone compounds in inhibiting EGFR and ERα: structural design and molecular docking studies for novel drug candidates in breast cancer treatment

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This study investigated the potential of coumarin-chalcone derivatives to inhibit estrogen receptor α (ERα) and epidermal growth factor receptor (EGFR) in breast cancer. Current therapies targeting hormone receptors often have limitations, including side effects and resistance. Here, we synthesized four novel chalcone-coumarin derivatives with different substituent groups via Claisen-Schmidt condensation. Structural characterization was

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performed using IR, NMR, and elemental analysis. *In silico* molecular docking with Autodock Vina identified interactions between the synthesized compounds and both ERα (PDB ID: 2JF9) and EGFR (PDB ID: 1M17) from the Protein Data Bank. This suggests the compounds may influence these receptors in cancer cells. Virtual screening evaluated ADMET properties, blood-brain barrier permeability, gastrointestinal absorption, and Lipinski's rule of five compliance. Pharmacokinetic parameters, physicochemical properties, and lipophilicity were also estimated. Our findings demonstrate that certain synthesized coumarin-chalcone derivatives possess greater inhibitory potential against breast cancer compared to existing drugs. These results warrant further investigation and evaluation of these molecules as potential therapeutic agents for breast cancer.

P-01-004
Acid-base equilibria govern the activity of the SARS CoV-2 main protease

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The chymotrypsin-like main protease (3CLpro) of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) is responsible for cleavage of two viral polyproteins into 16 nonstructural proteins. Its catalytic activity is critical for the life cycle of the SARS-CoV-2, since the inhibition of the 3CLpro can effectively block the whole replication of the virus. The 3CLpro has a bell-shaped activity-pH profile with a peak performance set at a value of approximately 7.4. Molecular dynamics and X-ray crystallography suggested that the enzyme undergoes some conformational changes due to the pH change of the environment. Our goal was to characterize the effects of pH on structure and dynamics of the 3CLpro in solution using nuclear magnetic resonance (NMR) spectroscopy. We obtained high quality NMR spectra recorded at pH between 5.2 and 8.5 for perdeuterated protein samples, selectively 1H, 13C-labelled on the methyl groups of Ile, Leu, Val and Met (ILVM) residues. Moreover, selective isotopic labelling of the histidine residues provided us with the residue specific pKa values for the individual imidazole moieties within this 66 kDa protein molecule. Since four out of seven histidines are located within the enzyme active site, these data allowed us to correlate the protonation states of the His side-chains with the proteolytic activity of the 3CLpro. Interestingly, we found that the protonation state of the histidines distant from the catalytic center also affects the 3CLpro proteolytic activity, leading to the discovery of the new allosteric pathway. Taken together, our results improve our understanding of the 3CLpro regulation and may facilitate the design of new potent inhibitors.

P-01-005
Double-helical crystal packing of potassium-independent L-asparaginase from *Phaseolus vulgaris*

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Plant-type, or Class 2, L-asparaginases are a group of Ntn-hydrolazes, subdivided into potassium-dependent and potassium-independent enzymes. Ntn-hydrolazes utilize an N-terminal nucleophilic residue (Thr, Ser or Cys) in their catalytic mechanism and share the same β-sandwich fold with flanking α-helices. The enzyme is comprised of two identical subunits, which are further split into α and β subunits upon autoproteolytic activation. The mature enzyme is thus an $(\alpha\beta)_2$ dimer. The maturation cleavage exposes the catalytic nucleophile at the N-terminus of subunit β and leaves the linker dangling at the C-terminus of subunit α. The potassium-dependent L-asparaginase from *Phaseolus. vulgaris*, PvAIIIK(+) was characterized previously [1]. However, the potassium-independent PvAIIIK(-) isoenzyme, with a lower affinity for L-Asn, is poorly studied. Here, we present the bewildering crystal structure of the PvAIIIK(-) protein, comprised of eight (αβ)2 dimers. The crystals have the rare P2 symmetry, unusual helical packing. The eight dimers are segregated into two helical arrangements. Within each helical assembly, the huge 18-stranded central molecular β-sheet of each dimer is extended in both directions by similar β-sheets of its neighbors. In this fashion, an infinite β-sheet helix is generated throughout the entire crystal. Each "protein helix" is then complemented by its antiparallel copy generated by the crystallographic twofold axis. A linker at the C-terminus of subunit α from one chain makes a molecular bridge to the complementary chain in the protein "double helix". In this work we present the unique oligomerisation pattern observed in the crystal structure of potassium-independent L-asparaginase PvAIIIK(-) from common bean, and its interactions and catalytic properties. Work supported by National Science Centre (NCN, Poland) grants 2020/38/E/NZ1/00035 and 2020/37/B/NZ1/03250. Reference: 1. Bejger, M. et al. (2014). Acta Cryst. D70, 1854–1872.

P-01-006
Toward a dependable structural database for L-asparaginase research

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The Protein Data Bank (PDB) is a carefully curated treasury of experimentally derived structural data on biological macromolecules. Such information is fundamental for projects that involve large-scale data mining and/or detailed evaluation of individual structures of importance to chemistry, biology, and medicine, as

the foundation for structure-based drug discovery. However, despite extensive validation mechanisms, it is almost inevitable that among the ~215 000 entries, there will be some suboptimal or incorrect structure models. It is thus vital to apply careful verification procedures for those segments of the PDB that are of direct medicinal interest. We carried out such an analysis for crystallographic models of L-asparaginases, enzymes that include approved and candidate drugs for the treatment of leukemia. Our main focus was on the adherence of the atomic coordinates to the rules of stereochemistry and their agreement with the experimental electron density maps. We identified 189 asparaginase entries in the PDB and found that the majority of the deposits are without any serious errors, oversights, or misinterpretations. However, ~30 models posed various kinds of problems, from trivial but annoying inconsistent placement in the asymmetric unit, to misrepresentations of the solvent area, to inconsistencies between the deposited and published data, or the perpetual "to be published" declaration. Ultimately, and these were the most serious cases, we found crystal structures where parts of the protein were modeled without any support from the electron density or even in stark defiance against such evidence, or where – on the contrary – stretches of evident protein electron density were left unmodeled. In-between were very frequent cases of incorrect modeling of side chain rotamers, of impossible interatomic contacts, or misidentification of metal cations. We hope that the revised structures will help in search for improved L-asparaginase drugs.

P-01-007
A multiscale approach to study angiotensin-converting enzyme 2 (ACE2) and its peptide inhibitor DX600

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Most of the computational techniques employed in drug design have been originally introduced and optimized for the case of a small molecule interacting with a protein. As pharmaceutical research is gradually shifting its interest towards the use of short peptides instead of small molecules, the need to modify old methods or devise new ones is becoming more urgent. In this contribution, we show how a multiscale approach combining atomistic and coarse grained simulations is able to clarify the (as yet unresolved) molecular details of the interaction between ACE2, the membrane protein acting as a receptor for SARS-CoV-2 spike, and the peptide inhibitor of its enzymatic action called DX600. Apart from the intrinsic applicative interest of this complex, which is strong due to the roles played by ACE2 both in COVID-19 infection and in the function of the renin-angiotensin-aldosterone system, the strategy we adopted lends itself to be easily accommodated to the study of similar protein-peptide interactions.

P-01-008
Structural studies of the HOMER1 protein: combining experiments and modeling

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The postsynaptic density (PSD) is a protein rich network beneath the postsynaptic membrane. This network has a role in memory formation, learning processes. Alteration of the PSD has been linked to numerous neural disorders. HOMER1 is a major postsynaptic scaffold protein containing a long-coiled coil region attached to a globular EVH1 (Ena/VASP homology domain1) domain via a disordered linker. The EVH1 domain binds proline-rich regions on partner proteins such as the Shank family. Here we report the functional characterization and stucture investigation of the EVH1 domain and two of its mutants, M65I and S97L that have been observed in patients with autism spectrum disorder (ASD). We have determined the structure and dynamics of the wild-type domain by solution NMR spectroscopy. We characterized the partner binding of the wild-type and mutant domains. We have also performed multidimensional NMR and SAXS measurements, as well as molecular dynamics simulations to determine the structural effect of the mutations. Our results suggests that the mutations primarily affect the stability and partner binding affinity of the EVH1 domain. We also modeled the full-length tetrameric Homer1 protein by building theoretical structures of the coiled coil and disordered regions and assembling them with the experimentally determined EVH1 and tetramerization domains. Detailed analysis of the model allowed us to characterize the local stability and its changes along the coiled coil segment and estimating the distances that the full-length protein can bridge within the PSD network.

P-01-009
Deciphering lipid binding: unveiling novel interaction motifs in the C-terminal domain of *Schistosoma mansoni* septin10

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The interaction between phosphatidylinositols (PI) and proteins plays a crucial role in recruiting proteins to specific sites and inducing membrane deformation events. This process depends on the intrinsic properties of interacting proteins and their interactions with the lipid bilayer. Septins, ubiquitous cytoskeletal proteins found in animals and fungi, are associated with important cellular events involving membrane reshaping. Septins engage in

specific binding motifs with membranes, facilitated by PI interactions. Previous research has identified polybasic regions (PB1 and PB2) linked to lipid interactions, while the C-terminal domain (CTD) may also be implicated. To elucidate the relevant residues or groups of residues for PI interaction, especially within the CTD, we conducted an analysis of the lipid-binding profile of *Schistosoma mansoni* Septin10 (SmSEPT10) through PIP membrane assays and Langmuir monolayer experiments. Our findings highlight the CTD as the primary lipid-interacting domain in SmSEPT10, exhibiting a preference for PI(3,5)P2, PI(4,5)P2, and PI(3,4,5)P3, as well as phosphatidic acid. We identified a novel polybasic region, PB3, within the SmSEPT10 CTD, conserved across animal and fungal septins. Furthermore, we demonstrate the significance of a specific lysine (K367) within its putative amphipathic helix (AH) for lipid binding. Deletion of PB3 or mutation of K367 significantly impairs lipid interaction. Interestingly, the AH, within a construct lacking the final 43 amino acid residues, proves insufficient for lipid binding. Our approach, encompassing cross-linking experiments, CD spectroscopy, SEC-MALS, and SEC-SAXS data analysis, reveals that the CTD primarily assembles as a parallel coiled-coil homodimer. This study delineates the lipid-binding region in SmSEPT10, offering insights into the molecular mechanisms of septin-membrane binding, and it holds particular relevance for less-explored parasite septins, such as SmSEPT10.

P-01-010
Structural characterization of the postsynaptic Drebrin protein

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The postsynaptic density (PSD) of excitatory synapses is a complex network of nervous system proteins involved in postsynaptic signaling. It modulates and regulates several functions of nervous system, thereby being responsible for a few molecular mechanisms fundamental to learning and memory [1]. Our group focuses on the function of proteins in PSD organization. The Drebrin protein is an essential component of the cytoskeleton, and its presence is required for actin polymerization of synapses and recruitment of CXCR4 chemokine receptors [2], and for the morphogenesis of the dendritic spike. Drebrin also plays an important role in synaptic plasticity associated with hippocampal memory and establishes several key interactions with other proteins present in PSD [3]. In this work we characterize the structure and function of different truncated Drebrin constructs that are responsible for connecting to the cytoskeleton through the F-actin and their interactions with another postsynaptic protein (Homer). We investigate the previously predicted single alpha-helical motif of Debrin [4]. We have optimized bacterial expression of the corresponding constructs and already performed structural analysis using a combination of CD (circular dichroism), NMR (nuclear magnetic resonance) spectroscopy and SAXS (small angle X-ray scattering). Our results demonstrate that the isolated motif does indeed form a single alpha-helix and can spatially sample a limited number of extended conformational states in solution. In addition, we have also successfully performed resonance assignment of the intrinsically disordered actin-binding core of Drebrin. Molecular interactions with other PSD proteins and F-Actin will also be investigated with BLI (biolayer interferometry) and ITC (isothermal titration calorimetry) measurements. References: 1. Ho, VM et al. (2011) Science 334, 623–8. 2. Pérez-Martínez, M. et al. (2010) J. Cell Sci. 123, 1160–1170. 3. Kovács, Á., et al. (2018). J Struct Biol. 204, 109–116.

P-01-011
Applying time-resolved serial crystallography at a 4th generation synchrotron source to dissect the mechanisms of enzyme inhibition and activity at room temperature

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Cryocrystallography provides unique insights into macromolecular recognition and enzyme activity but understanding these dynamic processes at physiologic temperatures has been lacking. Cryogenic temperatures limit conformational flexibility and protectant molecules can hinder ligand binding at enzyme active sites. Serial femtosecond crystallography at XFEL sources revolutionized macromolecular structure determination with unprecedented temporal resolution and the "diffraction before destruction" principle, allowing experiments at room temperature. Fourth generation synchrotron sources now offer bright beams and small cross sections, enabling serial crystallography experiments for a wider community. We will use a state-of-the-art synchrotron beamline to study protein-protein interactions and enzymatic processing dynamics. Using time-resolved serial crystallography at room temperature, we will investigate the interactions of enzymes with specific inhibitors and synthetic substrates (including photocaged compounds). Contrary to conventional cryocrystallography experiments, where large single crystals are preferred, serial crystallography experiments require a homogenous preparation of microcrystals with a maximum dimension of 5 μm. To produce these crystalline samples, we will systematically optimize crystal nucleation to produce a large number of microcrystals instead of a few large crystals. Once the crystallization protocol is optimized, it will be scaled up using batch methods before recording diffraction data in serial crystallography experiments and determine the corresponding crystal structures in ambient conditions. Our aim to establish a benchmark for the performance of the ESRF ID29 beamline and provide an experimental approach for future studies on protein-protein interactions and enzyme mechanisms using synchrotron crystallography. (Work funded by FCT, Portugal [https://doi.org/10.54499/2022.03363.PTDC]) * The authors marked with an asterisk equally contributed to the work.

P-01-012
Structural characterisation of pathological protein aggregates in a cat affected by systemic AA amyloidosis

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AA amyloidosis is caused by the misfolding and aggregation of serum amyloid A in the extracellular matrix, leading to organ failure. Specifically, the kidneys, spleen, and liver are the most affected organs in cats. Interestingly, previous studies reported a high prevalence of the disease in captive cats and cheetahs, plausibly caused by horizontal transmission [Previously published in: Ferri et al. (2023) PLoS One 10.1371/journal.pone.0281822]. This project focuses on cryo-electron microscopy structural analyses of *ex-vivo* samples extracted post-mortem from diseased cats, who therefore potentially constitute a model for the pathology. Intriguingly, post-mortem analysis revealed an unparalleled distribution of AA fibrils present in the majority of the internal organs. Therefore, herein we report two AA amyloid fibril structures extracted from the heart of a deceased cat. Interestingly, three different polymorphisms coexist, named polymorphisms I, II, and III, which represent 80%, 15%, and 5% of the sample, respectively. The amyloid folds of the three polymorphisms are totally different, with polymorphisms I and II composed of two protofilaments and characterised by C2 symmetry, and polymorphism III composed of three protofilaments and presenting C1 symmetry. Moreover, the polypeptide arrangement is unique compared to cat, mouse, and human AA amyloid structures previously deposited [Previously published in: Schulte et al. (2022) Nat Commun 10.1038/s41467-022-34743-2; Bansal et al (2021) Nat Commun 10.1038/s41467-021-21129-z]. The investigation and comparison of AA amyloid structures from different organs and species will deepen the knowledge of the molecular mechanisms of amyloidogenesis. Moreover, this project ultimately aims to design treatments to increase the life expectancy of individuals affected by AA amyloidosis.

P-01-013
Molecular binding processes using robot mechanics molecular simulation

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Considering the rapid advancement of AI driven molecular modeling [as published in Jumper, J. et al (2021), Nature 596, 583–589], the challenge in structural bioinformatics shifts from structure generation to recovering their conformational spaces. Given the rugged conformational space of biomolecules, classical molecular simulation cannot sample major conformational changes in feasible computer times. To overcome this hurdle, we developed Robosample [previously published in Spiridon L. et al (2020), Biochim Biophys Acta Gen Subj 1864(8)], a molecular simulation software which uses Gibbs sampling and robot mechanics to efficiently sample rare transitions between conformational pools. Briefly, Robosample models the system as a robot, defined as a set of rigid bodies connected by joints, and changes the topology of this set to create different Hamiltonian Monte Carlo trial moves. This allows sampling with large integration timesteps (computed in linear time by using robotics algorithms). Using Gibbs sampling, Robosample is able to achieve ergodicity and correctly reproduce the Boltzmann distribution. Current work shows Robosample's ability to simulate molecular binding processes and recover their binding free energy surfaces. Using the free energy landscape of receptor/ligand complexes, we can recover sets of poses inside and outside the binding pocket as well as their relative populations. These can be used to estimate the binding free energy and rank the poses for one ligand or rank different ligands. As validation we used a host-guest cucurbituril system and as a case study an NBS molecular switch found in the plant resistosome. In conclusion, we present an accessible, open-source software that we developed, which offers a faster sampling of biomolecules' conformational space, which will become increasingly necessary as more structural data is generated. *The authors marked with an asterisk equally contributed to the work.

P-01-014
Understanding the structural dynamics of metavinculin's tail domain: insights from MD simulations

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Vinculin (VCL) acts as an adaptor protein that interacts with binding partners in cell-cell and cell-extracellular matrix adhesion complexes connected to the actin cytoskeleton. This interaction is crucial for regulating and stabilizing adhesion junctions, mechanotransduction, and cellular migration. Metavinculin, a splice variation of vinculin, is present in muscle tissues, and its expression is associated with contractile cell characteristics. This isoform has a 68-amino-acid insert in its C-terminal region (tail), which promotes a distinct actin filament organization differently from vinculin. To understand the structural impact of missense variants, we used all-atom MD simulations to study the tail domain of metavinculin (MVt) with three previously identified pathogenic variants (A934V, L954del, and R975W) and the wild-type (WT) MVt for over 700 ns. Our findings revealed notable differences in the dynamical behavior of the metavinculin-specific insert region among MVt variants. Specifically, the WT MVt exhibited a more compact and less flexible N-terminal domain compared to its variants. Among the variants, L954del displayed the highest flexibility in the insert region, followed by the R975W mutant. Simulations of WT MVt unveiled a persistent salt-bridge

interaction between residues D928 and R975, which played a pivotal role in stabilizing the N-terminal domain and restricting its mobility in simulations. Thereby, our analysis highlighted the significance of residue R975 in this critical intramolecular interaction, shedding light on a possible pathogenicity mechanism of the R795W variant. This variant, lacking a basic amino acid in the insert region, failed to maintain a compact structure akin to WT MVt. Overall, our results underscore the essential role of electrostatic interactions in the metavinculin-specific insert for conformational dynamics, emphasizing the utility of all-atom MD simulations in understanding the structural impact of missense variants.

P-01-015
Architectural role of the CTCF-CHD8 complex in shaping and organising the human genome

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The higher-order genome organization is intricately associated to gene expression and vice versa. Accordingly, disorders affecting spatial genome architecture and functional organization often result in genomic instability, a leading cause of cancer and several neurodevelopmental disorders. Specific "architectural" protein complexes shape the three-dimensional structure of the genome, which is packaged into a tight protein-DNA complex named chromatin. The fundamental functional unit of chromatin is the nucleosome, formed by 150 base pairs of DNA wrapped around a histone octamer. The mechanisms by how the chromatin is dynamically arranged and how DNA–protein interactions play a crucial role in this process remain poorly understood. The highly conserved zinc finger protein CCCTC-binding factor (CTCF) is one of the core genome architecture proteins and acts as a transcription regulator. CTCF regulates long-range chromatin loops and contributes to the establishment of topological-associating domains. Chromatin remodeling protein chromodomain helicase DNA binding protein 8 (CHD8) has been shown to colocalize extensively with CTCF at CTCF binding sites near chromatin loop anchors. Recent studies highlight the importance of the crosstalk between these CTCF and chromatin remodellers to establish specific chromatin structures, affecting genome architecture and function, but the mechanisms underlying this process are severely ill-defined. The proposed project aims at filling this gap by exploiting a multidisciplinary approach, involving biochemistry, biophysics (Single-molecule Optical tweezers), and structural biology (XL-MS and cryo-EM) to characterize the structural and functional role of the CTCF-CHD8 complex. Specifically, the main objective is to understand at the molecular level how CTCF and CHD8 interact and to study the function of this complex by cryo-electron microscopy in the context of chromatin to probe its role in genomic functional and spatial organization.

P-01-016
Structural studies of *Caenorhabditis* elegans septins through CryoEM

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Septins are highly conserved GTP-binding proteins that form filaments *in vivo* through repeating heterooligomers, in which the subunits alternate G and NC interfaces. *Caenorhabditis elegans*, an important animal model, has two septins named UNC-59 and UNC-61, that assemble into a tetramer. It is proposed that this tetramer is formed via a homodimeric G interface between two subunits of UNC-61 and a heterodimeric NC interface, with UNC-59's G domain exposed to the media [John CM et al. (2007) EMBO J., 3296-3307]. This organization is unlike other organisms, that usually present an exposed NC interface. To confirm the subunit organization, proteins were expressed in *E. coli Rosetta* and purified by affinity and size exclusion chromatography, then submitted to SEC-MALS, circular dichroism (CD) and nucleotide content experiments, and cryogenic electron microscopy (CryoEM) single particle analysis to obtain a 3D structure of the tetramer. UNC-59 and UNC-61 were purified as a tetramer, and nucleotide content analysis indicated GDP bound to both subunits. CD analysis for secondary structure confirmed proteins were properly folded. The tetramers were used for CryoEM grid preparation, and initial data collection has indicated a preferred orientation of the proteins on the grid, which has held back data processing. However, 2D classification in this orientation allowed visualization of the interfaces and visualization of the septins' C-domains, long coiled-coils formed at the NC interfaces that are not usually observed due to their high flexibility. The position of the interfaces is congruent with the proposed model of subunit organization, but the position of each septin subunit is still unclear without high-resolution data. Therefore, with further optimization of CryoEM grid preparation to obtain a 3D structure of the *C. elegans* septin tetramer, this study will contribute to understanding the structure of this unusual septin organization.

P-01-017
Elucidating structural dynamics of peroxisomal matrix protein import complex

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Peroxisomes are crucial organelles involved in cellular metabolic process. Defects in the normal functioning of these organelles may lead to various peroxisome biogenesis disorders (PBDs). There are peroxisomal membrane proteins and receptor proteins (peroxins) responsible for maintaining the influx of peroxisomal proteins from cytosol. Pex5 is a receptor protein that recognizes peroxisomal signals on cargo proteins and interacts with peroxisomal membrane proteins Pex14 to facilitate the transport of cargo proteins. In the present study the structural characterization and interaction dynamics between cargo protein, Pex5 receptor and Pex14 proteins were explored by the Cryo-EM analysis. Further the mutations were introduced in the interacting sites and their effect on binding affinity is measured by biophysical

method (ITC analysis). The present structural analysis of complex and *in-vitro* binding analysis reveals the crucial binding sites for interaction and the mechanism of peroxisomal protein import.

P-01-018
Investigation of the binding mechanism and molecular nature of human Sigma 1 receptor ligands

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The Human Sigma 1 Receptor (hS1-R) is an enigmatic endoplasmic reticulum resident transmembrane protein. hS1-R is implicated in neuroprotection and neuroplasticity, therefore it is considered a potential therapeutic target to cure neurodegenerative diseases, like amyotrophic lateral sclerosis (ALS), Alzheimer's disease, Parkinson's disease, Huntington's disease. Kruse and coworkers solved the X-ray structures of hS1-R bound to agonists and antagonists [1], but the molecular mechanisms of hS1-R activation remain unclear. In particular, two of the most important features required to fully understand hS1-R function, namely the receptor endogenous ligand(s) and the molecular mechanism of ligand access to the binding site, have not yet been unequivocally determined. To shed light on the nature of the endogenous hS1-R ligand(s), we used a combination of computational virtual screening (VS), electron density maps fitting and fluorescence titration assay to measure ligand binding to hS1-R *in vitro*. We found that the molecules with steroid-motif were the ligands with the highest affinity for the receptor, and that among them 16,17-didehydroprogesterone was shown by fluorescence titration to bind HS1-R, with significantly higher affinity than the prototypic hS1-R ligand pridopidine in the same assay [2]. To investigate the molecular mechanism of the ligand access to the binding site, we performed all atoms MD simulations of hS1-R embedded in a lipid bilayer. The MD trajectories suggested that ligands access the binding site through a cavity, opening on the protein surface in contact with the membrane. Furthermore, we started to investigate the structure of hS1-R in solution by cryogenic electron microscopy (Cryo-EM) to shed light on the molecular mechanism of protein polymerization/depolymerization induced by antagonists/agonists. References: 1: Schmidt HR et al. (2016) Nature 532, 527–30. 2: Pascarella G, Antonelli L, et al. (2023) Int J Mol Sci. 24, 6367.

P-01-019
Harnessing the AlphaFold protein structure database for human health and translational research

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Single amino acid substitutions, known as missense variants, are prevalent in human diseases and significantly impact protein function. However, accurately predicting their effects was previously hampered by the limited availability of protein structure data. Recent advancements in computational methods, particularly the development of high-accuracy protein structure prediction tools, offer a breakthrough. Public databases like AlphaFold DB now provide predicted structures for millions of proteins, including those linked to human diseases. These structures, accompanied by confidence scores, empower researchers to analyse missense variants within a 3D structural context. Recent advancements in computational methods, leveraging the wealth of AlphaFold structures, are enabling a paradigm shift in missense variant analysis. This presentation explores how these predicted structures, with their associated confidence scores, can be integrated with functional annotations from existing databases, such as PDBe-KB. This combined approach allows for a more accurate assessment of missense variants. By considering both the 3D protein structure and functional annotations, we can enhance variant effect prediction and develop a powerful strategy for prioritising variants for further investigation. *The authors marked with an asterisk equally contributed to the work.

P-01-020
Glacios 2 cryo-TEM and Smart EPU software accelerating cryo-EM enabled structure-based drug design: a perfect combination to streamline single-particle data acquisition through advanced automation

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Cryo-EM has revolutionized the field of structural biology due to its capabilities to resolve the three-dimensional structure of proteins, protein complexes and other biological macromolecules at high or even atomic resolutions. However, acquiring high-quality data still largely depends on the expertise of the microscope operator. This limits the speed of adoption as researchers must invest considerable time into understanding microscopy and the technicalities of the workflow. Here, we show how the new Glacios 2 cryo-electron microscope with low-energy-spread cold field emission gun (E-CFEG) combined with Smart EPU software enables users of all expertise levels to acquire high-quality Cryo-EM data. In collaboration with the Greber lab (Institute for Cancer Research) several high-resolution structures of the 85 kDa human CDK-activating kinase (CAK) were determined. CAK is

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a master regulator of cell growth and division and is a promising target for cancer therapeutics. Structures of CAK were rapidly determine in free and nucleotide-bound states as well as in complex with 14 inhibitors. In addition to achieving high resolution structures from large datasets, ~4 Å and ~3 Å-resolution structures of ligand-bound complexes were determined using from only 1 h and 4 h of data collection respectively. These results show the use of cryo-EM to enable structure-based drug design. Furthermore, in combination with an E-CFEG we have been able to generate a 1.5 Å reconstruction of apoferritin; the highest resolution 200kV structure to date.

P-01-021
Proteins that do interesting things with DNA

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Cells use duplex DNA (dsDNA) to secure their most precious information. As such, the duplex is closely guarded, and access is only granted to read, replicate, and repair information stored within. Here, we will report on our studies of one of these processes: the initiation of DNA replication. Our efforts to understand the initiation of DNA replication have focused on bacteria. An important component of DNA replication machinery is the replicative helicase, which travels ahead of the replication fork, melting the duplex to expose the Watson-Crick-Franklin base pairs for DNA synthesis. However, in *E. coli*, the DnaB replicative helicase is a closed protein ring that must be loaded onto single-stranded DNA, an infinitely long polymer with no free ends. Loading of the DnaB helicase requires a specialized protein known as a helicase loader. Multiple copies of the bacterial phage λP loader proteins bind to the closed planar ring of the DnaB replicative helicase and trigger its reconfiguration into a right-handed open spiral wherein an internal chamber becomes accessible to physiological single-stranded DNA at the replication origin. My presentation will describe and analyze the structure of the DnaB helicase bound to the λP loader (BP) at high resolution (2.64 Å) using cryogenic electronic microscopy. We find that the λP ensemble adopts a profoundly asymmetric configuration; one copy of λP, which is visualized in full, binds at the top and bottom of the open DnaB spiral; the presence of a single breach in the DnaB open spiral means that the remaining four copies of λP must adopt distinct, and currently unknown, conformations. Although DnaB's internal chamber remains accessible to the entry of ssDNA, the λP protomer whose binding site spans the breach effectively blocks the path into the inner chamber and gives rise to an autoinhibited configuration for the BP complex.

Integrative Structural Biology Approaches

P-02-001
Carbonyl–carbonyl interactions as key contributors to beta turn classification

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Beta turns, simple yet pivotal protein secondary structures, consist of four residues and play a crucial role in conferring flexibility to protein structures. Originally defined by Venkatachalam, these non-helical conformations involve a hydrogen bond formation between the carbonyl group of the first residue (i) and the nitrogen-bonded hydrogen of the fourth (i + 3) residue. Over the past five decades, the classification of beta turns expanded to encompass eight types and a miscellaneous category. This study explores the impact of weak intramolecular interactions, specifically n→π* interactions, in the stabilization of beta turns. By identifying repeated CO···CO interaction patterns between carbonyl groups in 19 beta-turn classes, as defined by Shapovalov and colleagues, we conducted an extensive analysis using high-resolution X-ray structural data encompassing 36,949 beta turns. The obtained results highlight the discriminatory potential of CO···CO interactions among beta turn classes. Additionally, distinct patterns for each beta turn type were identified, shedding light on the structural diversity within this secondary structure. Intriguingly, a novel Type II beta turn, denoted as pA in Dunbrack's notation, was discovered. This new beta turn type exhibits an alternative conformation to Pa, featuring opposite φ and ψ values and a direct carbonyl–carbonyl interaction between the second and third residues of the turn. The findings underscore the chemical significance of CO···CO interactions in explaining the diverse array of beta turn types. This work contributes to a deeper understanding of the structural nuances within beta turns, providing valuable insights that may impact future research in protein folding and design. References: Venkatachalam CM. Biopolymers. 1968;6(10):1425–36. Newberry RW, Raines RT. Acc Chem Res. 2017;50:1838–46. Shapovalov M, Vucetic S, Dunbrack RL Jr. PLoS Comput Biol. 2019;15(3): e1006844.

P-02-002
Interaction and activity modulation of two aldo-keto reductase 1 isoforms, C2 and C3

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The family of four aldo-keto reductase 1 (AKR1C) genes in humans share over 86% homology. All four enzymes are found in the liver, but they have different extrahepatic distributions. Whereas AKR1C4 is expressed predominantly in the liver, AKRs 1C2 and 1C3 are dominantly expressed in the prostate and mammary glands. In particular, AKR1C3 is a critical enzyme involved in the pre-receptor regulation of androgen action in the prostate and has been implicated in the pathogenesis of castrate-resistant prostate cancer (CRPC) [Previously published in: Pippione JT et al. (2017) Eur J Med Chem 25:150:930–945]. AKR1C3 is the most upregulated gene in CRPC and catalyses the reduction of weak androgen precursors to the potent testosterone and dihydrotestosterone. Because of its critical role in steroidogenesis, it represents an important target for the treatment of CRPC. Although much is known about the substrate specificity and tissue distribution of AKR1C enzymes, their physiological roles remain elusive. Also, the extent of redundancy in the function of AKR1C enzymes remains unclear. In this work, we examined the biochemical and biophysical aspects of the connection between AKR1C3 and its isoform 1C2, both of which are expressed in prostate glands. A combined approach of mutagenesis and spectrophotometer assays allowed the characterization

of the modulation in the activity in the presence of the two isoforms and different substrates. Our research revealed that the two enzymes' combined activity is modulated, indicating a potential function for their cooperation in androgen metabolism. Finally, we characterized the interaction between the two human proteins using microscale thermophoresis and co-crystallization. Collectively, our results support a model in which the interaction between the two isoforms might influence their activities. This outcome will impact the future strategy to develop more potent and drug-like inhibitors.

P-02-003
Structural basis for the recognition of the respiratory nitrate reductase NarG by the redox enzyme maturation protein NarJ

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In bacteria, nitrate reductase A, consisting of NarG, NarH, and NarI, plays an essential role in anaerobic respiration by transferring electrons to nitrate via heme, iron-sulfur clusters, and molybdenum cofactor (Moco). NarJ is required for the assembly of the functional NarGHI complex as a redox enzyme maturation protein. NarJ recognizes the N-terminal signal peptide of NarG and subsequently facilitates the incorporation of Moco into NarG. To elucidate the exact mechanism of NarG signal peptide recognition by NarJ, we solved the structure of NarJ in association with the NarG signal peptide through X-ray crystallography and analyzed the NarJ-NarG interaction via a mutational study. NarJ forms an α-helical single-domain structure with a U-shaped hydrophobic groove. NarJ uses the left and right parts of the hydrophobic groove to accommodate hydrophobic residues from the N- and C-terminal regions of the NarG signal peptide, respectively. Interestingly, the NarG signal peptide undergoes a substantial structural rearrangement from a helical conformation to an extended structure upon NarJ binding. Our further comparative structural analysis, combined with a modeling study, suggests that the complementary hydrophobic recognition mechanism of NarJ is ubiquitous among diverse NarJ subfamily proteins.

P-02-004
Purification of components of the *Mycobacterium tuberculosis* translation machinery to understand translation initiation control

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Mycobacterium tuberculosis is the causative agent of tuberculosis, one of humankind's deadliest diseases. During its infection cycle, this pathogen usually persists for prolonged periods in a non-replicating state and must adapt to a variety of harsh environments. Recent studies have revealed differences in translational control between *M. tuberculosis* and other organisms, which may contribute to its ability to adapt during infection. We have characterized that over 50% of its genes lack the canonical signals for translation initiation, and they are robustly translated during exponential growth and conditions that mimic persistence inside the host, suggesting the existence of alternative mechanisms for translation initiation. However, the molecular basis and the role of non-canonical translation during stress adaptation remain unknown. For this reason, we are interested in characterizing translation initiation in *M. tuberculosis*. To achieve this, we have acquired *M. tuberculosis* genes encoding translation factors to be recombinantly expressed and purified in *Escherichia coli*. We have purified all initiation factors (IF), including the full-length IF2 and a truncated IF2 version that lacks the N-terminal unstructured extension of the protein. We have also optimized protocols for isolating the ribosomal subunits and obtaining fMet-tRNA^{fMet} from *E. coli*. Together with the remaining components, we will establish an *in vitro* translation system to characterize variations in initiation mechanisms. Additionally, while several structures of mycobacterial ribosomes have been reported, none correspond to a functional translation initiation complex. Hence, we aim to obtain high-resolution structures of canonical and non-canonical initiation complexes using cryo-electron microscopy. We expect that these structures, together with our *in vitro* translation experiments, will help us identify crucial elements involved in translational control in *M. tuberculosis*.

P-02-005
The symmetric asymmetry of the ER stress response factor (ERSF: NF-Y/ATF6) bound to the ER-stress elements ERSE and ERSE II

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Physiological and environmental stress conditions can cause protein unfolding/misfolding in the endoplasmic reticulum (ER stress), where newly synthesized proteins fold and assemble. In eukaryotic cells one strategy to cope with the accumulation of unfolded proteins is the induction of molecular chaperones and folding enzymes. This homeostatic response is achieved through the transcription of specific genes by selected transcription factors (TFs). Among them, the ER stress response factor (ERSF) is a TF that binds to the ER stress response elements (ERSEs) present in the promoters of inducible genes. The typical ERSE sequence is CCAAT-9N-CACCG but there is also a second reverse configuration CCACG-1N-ATTGG (ERSE II). ERSF is a heterologous complex consisting of the constitutive component NF-Y, binding to the CCAAT box, and an inducible component, ATF6, which binds to the CCACG motif of ERSEs. NF-Y is a trimeric TF acting as a pioneer in opening chromatin domains. ATF6 is a dimeric basic leucine zipper-type (bZIP) TF, synthesized as ER transmembrane protein and proteolyzed in response to ER stress, allowing translocation of its bZIP portion into the nucleus. Here we report the cryo-EM structure of the ERSF complex, bound to the ERSE and ERSE II motifs (both complexes <85 kDa). Surprisingly, NF-Y and ATF6 bind similarly to both ERSE and ERSE II, despite the completely different configurations of the two ERSEs, generating a unique "symmetric asymmetry" in their molecular architecture. Our data shed first light on the molecular mechanism underlying the ER stress response, including the binding cooperativity of NF-Y and ATF6 on ERSEs.

P-02-006
SARS-CoV-2 BA.2.86 spike: insights into its structure, function, and implications

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Since its first appearance in China, SARS-CoV-2 has undergone significant molecular evolution, with the spike protein playing a key role in transmission and evasion of host immune surveillance. In this work, we analyzed the spike protein of BA.2.86 variant, commonly known as Pirola, and its earlier variants to identify structural differences related to virus adaptation and evolution. We evaluated the effects of mutations in N-terminal Domain (NTD) and Receptor Binding Domain (RBD) using homology modeling, binding energy prediction, surface electrostatic potential analysis and molecular dynamics. Furthermore, we analyzed in detail the effect of the Val483 deletion, detected in a loop near BA.2.86 RBD interface with ACE2 with a prevalence of about 70%. The results indicated an increase in the net charge of the RBD of BA.2.86 up to +6.6 compared to +5.5 of XBB lineage variants, while the NTD was found to have a more negative net charge (−1.7) compared to the NTDs of the earlier variants. Binding energy predictions suggest BA.2.86-ACE2 complexes are slightly more stable than those formed by the earlier variants. According to the results of molecular dynamics experiments, mutations in BA.2.86 alter the flexibility of loops in both RBD and NTD compared to the original wild-type virus. Furthermore, we observed an alteration in the dynamic trajectory of the interface loop in the presence of Val483 deletion, which may indirectly destabilize the interaction with the receptor ACE2. This observation could explain the RBD resistance to deletions. Examining the molecular evolutionary trajectory of SARS-CoV-2 may help unravel principles of broad interest in protein evolution and monitor the emergence of new potentially dangerous variants, with the aim of informing the development of adaptive strategies to mitigate the impact on global public health. *The authors marked with an asterisk equally contributed to the work.

P-02-007
Antibiotics discovery based on the structure of toxin–antitoxin system from *Klebsiella pneumoniae*

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The bacterial toxin–antitoxin (TA) system is a module that play a role in cell survival under stress conditions. Generally, toxin molecules act as negative regulators in cell survival and antitoxin molecules as positive regulators. Since TA systems are able to control the fate of bacteria, they are considered as potent targets for the development of new antimicrobial agents. TA systems are widely prevalent systems existing in bacteria: there are eight types of bacterial TA systems depending on the property of the antitoxin which binds either the protein toxin or mRNA coding the toxin protein. Knowledge on TA systems such as the individual characteristics of TA systems, integrative working mechanisms of various TA systems in bacteria, interactions between toxin molecules and cellular targets, and so on is currently limited due to their complexity. In this regard, it would be helpful to know the structural characteristics of TA modules for understanding TA systems in bacteria. Here, we present the structural information of TA systems by using NMR, X-ray crystallography and suggest antibiotics candidates which inhibit the interaction between toxin and antitoxin proteins. *Klebsiella pneumoniae* causes severe human diseases, but its resistance to current antibiotics is increasing. Therefore, new antibiotics to eradicate *K. pneumoniae* are urgently needed. By using the structural information, we could design the peptides and small-molecule compounds that can disrupt the binding between K. pneumoniae MazE and MazF, which release free MazF toxin. Because the MazEF system is closely implicated in programmed cell death, artificial activation of MazF can promote cell death of *K. pneumoniae*. The bacterial cell killing by small-molecule compound could be confirmed by using flow cytometry. Our findings can contribute to understanding the bacterial MazEF TA system and developing antimicrobial agents for treating drug-resistant *K. pneumoniae*.

P-02-008
The effect of interactions of the silver nanoparticles with the thiol groups presented in the protein surface: example of the EMAP II polypeptide

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Metal nanoparticles (NPs) are promising agents for biomedical applications, specifically for diagnostic and therapeutic purposes. Silver NPs (AgNPs) are frequently used as active components of antibacterial coatings in wound dressings, medical devices, implants, cosmetics, textiles, and food packaging. Nevertheless, since Ag^+ ions form potent bonds with thiol groups in proteins, applying AgNPs may harm human health. Endothelial monocyte-activating polypeptide II (EMAP II) is a cleaved C-terminal peptide of the intracellular aminoacyl-tRNA synthetase complex protein AIMP1. EMAP II, expressed in endothelial cells, plays an important role in cancer, diabetes, atherosclerosis, chronic myocardial infarction, and lung injury. EMAP II contains five cysteine residues (Cys) exposed at its surface. Due to a high affinity of Ag^+ ions to Cys thiol groups, the binding of some or all of these thiols to Ag^+ ions present at the surface of AgNPs or released from it due to the oxidative metabolism of the cell could cause the inactivation of EMAP II. Our studies aim to explore the relationship between EMAP II and AgNPs using UV-vis, fluorescence, and thermophoresis techniques. To further explore the structural and dynamic aspects of the EMAP II interaction with AgNPs, we also recorded NMR data for the 1H, 15N, and 19F resonances. The obtained results demonstrated the existence of dimerization/oligomerization processes that block the EMAP II active site(s) due to significant alterations of the 3D structure upon saturation with the AgNPs. This project was supported by EMBO Solidarity Grant SLG 5427-2023 (for L.K.), 2021/41/B/ST4/03807 National Centre for Science, Poland (for I.Z.), and 2021/43/O/ST4/02667 National Centre for Science, Poland (for P.S., W.B.).

P-02-009
Structural characterization of human RNA polymerase-III pre-initiation complex (PIC)

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RNA polymerase III is an essential multisubunit enzyme present in all eukaryotes responsible for the transcription of various short non-coding RNAs. It is recruited to genes via its assembly with general transcription factors (GTFs) forming a pre-initiation complex (PIC) at Class I, II and III promoters. Pol III-mediated transcription is tightly regulated in response to changes in cell growth, proliferation, and stress. Dysregulation of this process has been linked to diseases such as Alzheimer's, leukodystrophy, fragile X syndrome, and various cancers. Here we present cryo-EM structures of the U6 SnRNA hPol III PIC assembled with full length and mini SNAPc at a resolution range of 3.1–4.1 Å revealing three states: two open complexes (OC) with different clamp conformations and an intermediate melting complex (MC). TFIIIB:SNAPc engagement varied across states, impacting stability however the core SNAPc structure resembled previously reported miniSNAPC, suggesting dispensability of SNAP2/5 for PIC formation. Structural comparison of human and *S. cerevisae* PICs highlighted a common mechanism of transcription activation via Brf1 and Brf2 with conserved docking positions on Pol III. In contrast, the Brf1 and Brf2-associated transcription factor Bdp1 displays significant structural differences between the human and yeast PICs with additional differences in the polymerase active site, particularly in the 'rudder' and 'Switch III loop' suggesting a differential regulation of transcription initiation between two systems. Employing an integrative approach, the SNAPC2 and SNAPC5 subunits were localized, providing the most complete description of the SNAPc complex to date. Comparative structural analysis also revealed the structural basis of the specific recruitment of Brf2 to class III promoters and identified a novel double-sided interaction motif in SNAPC4 which facilitates the unique ability of SNAPc to engage both Pol II and Pol III PICs.

P-02-010
Kinetics and thermodynamics characterization of serum amyloid A (SAA) protein

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Serum amyloid A (SAA) protein is an inflammatory acute-phase apolipoprotein found as the major component of secondary amyloid deposits characterizing systemic (AA) amyloidosis, ensuing upon long-term complications of several chronic inflammatory disorders. The formation of amyloid fibrils in AA amyloidosis is due to the misfolding of circulating SAA after a significant increment of serum SAA1 level [1]. In this context, to better understand the unfolding mechanism of SAA, it is essential to characterize it in terms of structural and kinetic ensembles to determine its normal and aberrant behaviour. To achieve this, we attempt to describe the thermodynamic ensembles that are represented by the free-energy landscape giving information about the molecular structures of SAA and the populations of their metastable states using enhanced sampling techniques (i.e. metadynamics) [2]. Firstly, collective variables (CVs) that reveal much of the physics that underlies the folding/unfolding process can be constructed by using the supervised learning class classification paradigm. These identified CVs can be used in bias-exchange metadynamics (BE-META) simulations to identify different metastable states on the proposed transition pathway. Then, the kinetic ensembles can be constructed by exploiting the theory of stochastic processes using Markov state models analyses to gain new insights about the transition rates between the defined metastable states [3]. A deep understanding of the structural and kinetic properties of the monomeric SAA will be key to determining the effects of small-molecule drug candidates on the aggregation behaviour of this protein. References: 1. Sack GH Jr. (2018) J Mol Med 24, 46. 2. Granata D et al. (2015) J Sci Rep 5, 15449. 3. Löhr T et al. (2021) J Nat Comput Sci 1, 71–78. *The authors marked with an asterisk equally contributed to the work.

P-02-011
Structure-guided inhibition of the Ras1-activation virulence mechanism in *Candida albicans*

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Candida albicans, part of the commensal microbiota of most individuals, is a notorious opportunistic human pathogen. *C. albicans* causes more than 150 millions of mucosal infections/year, with high mortality rates in immunocompromised patients. Considering its significance, the WHO has included this pathogen in the highest priority group of human fungal pathogens to guide research, development and public health action, emphasizing the urgent need to identify new targets and antifungal drugs. The pathogenicity of *C. albicans* is linked to its ability to switch between yeast and hyphal forms. Ras1 has a crucial role in regulating the hyphal morphogenesis signaling pathway, namely through its interaction with Cdc25. Although this activation mechanism is a potential target for drug development, the high amino acid sequence conservation of Ras1 relative to human homologues and the scarce structural information on the Ras1/Cdc25 interaction, have so far impeded progresses in this direction. In this work, by combining structural, biophysical techniques and AI-based predictive modeling, we report structural and functional features for these important proteins, uncovering new antifungal drug targets to guide the inhibition of Ras1-activation. In particular, we found differences in the interaction surface in Cdc25 compared to humans, underscoring its potential for developing fungus-specific inhibitors [Manso JA et al. (2023) mBio 14, e0063823]. In this regard, we have designed a peptide

capable of inhibiting the Cdc25 activity, which is the first reported specific inhibitor of this mechanism. Furthermore, we have recently identified in Cdc25 three potential ligand-binding sites with druggable features, opening new perspectives for drug discovery studies (ongoing research, unpublished). Inhibiting this important virulence mechanism would reduce levels of the activated form of Ras1 in cells and, therefore, would prevent the virulent-associated hyphal morphogenesis pathway.

P-02-012
Exploring the interaction between galectin-3 and lipopolysaccharide from *Pseudomonas aeruginosa*

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Galectin-3 (Gal3), a β-galactose binding lectin protein, has emerged as a relevant therapeutic target in many disease conditions, such as cystic fibrosis, inflammation, respiratory infections, neuroinflammatory disorders and cancer [as previously published in Bouffette, S et al. (2023) Trends Pharmacol Sci 44 (8), 519–531]. Given the significant role in inflammation and the involvement in chronic infection displayed by Gal3/lipopolysaccharide (LPS) interaction [reported in Wang, F et al. (2024) Inflammation 47 (1), 454–468], the aim of the following work was to further study the interaction between the carbohydrate recognition domain of Gal3 ($Gal3^{CRD}$) with the LPS from *Pseudomonas aeruginosa* (LPS*pa*). To achieve this purpose, researchers actively employed several biophysical and structural techniques. To better explore the interaction and to investigate how $Gal3^{CRD}$ interacts with LPS*pa* and whether the interaction can lead to a disaggregating effect on LPS*pa*, circular dichroism, spectrofluorometric analyses and dynamic light scattering experiments were carried out. Furthermore, in consideration of the role of the bacterial cell surface in molecular binding, zeta potential measurements were conducted to monitor the effect of $Gal3^{CRD}$ on the membrane surface charge of the *Escherichia coli* and *Pseudomonas aeruginosa* cells. Finally, the interaction mode between $Gal3^{CRD}$ and LPS*pa* gains support from nuclear magnetic resonance data analysis and a binding model data was obtained by isothermal titration calorimetry. Recent results published by Pirone, L et al. (2024) Int J Mol Sci, 25, 2895, provide valuable indications for the development of LPS receptor-targeted agonists and/or antagonists such as galectins as adjunctive therapy for *Pseudomonas aeruginosa* infection.

P-02-013
Calcium influence on the human Nedd4-2 enzyme and its ability to form a complex with 14-3-3η

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Neuronal precursor cell-expressed developmentally down-regulated 4-2 (Nedd4-2) ubiquitin ligase is one of the nine human members of the Nedd4 HECT E3 family, characterized by three distinct domains: membrane- and calcium-binding C2, four WW and the catalytic HECT domain (in that order). As the last link in some ubiquitination cascades it plays an important role in choosing target molecules which will be ubiquitinated, altering their fate within the cell. So far, several mechanisms of its regulation were described: auto-inhibition by intramolecular binding of the N-terminal region to the HECT domain, activation by calcium ions and interaction with several binding partners. Our group performed a detailed structural characterization of the phosphorylated Nedd4-2 with 14-3-3η, a homodimer protein that interacts with phosphorylated Ser or Thr residues of this ubiquitin ligase [previously published in: Pohl P et al. (2021) Commun Biol 4, 899; Joshi R et al. (2022) Biophys J 121, 1299–1311]. Our focus since has shifted towards studying the effect calcium has on the Nedd4-2 ligase by itself and within a complex with 14-3-3η. We employed a number of techniques: analytical ultracentrifugation, functional assays (liposome-binding and ubiquitination assays) and X-ray crystallography of the C2 domain in the presence of calcium ions. Results show Nedd4-2 is a calcium dependent enzyme – it requires these ions in order to bind to membranes, but only alters the ability to perform ubiquitination. The affinity of Nedd4-2:14-3-3η complex wasn't reliant on the presence or absence of calcium – 14-3-3η maintained its inhibitory effect on Nedd4-2 function. Finally, we solved the crystal structure, illuminating which residues within the C2 domain bind calcium. This study was supported by the Czech Science Foundation (Project: 23-04686S), the Czech Academy of Sciences (RVO: 67985823) and the Visegrad Scholarship (Project: 52310440).

P-02-014
Crystal structures of human histone deacetylase 8 in complex with novel hydroxamic acid inhibitors

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Histone deacetylases (HDACs), also known as lysine deacetylases, are enzymes involved in the control of gene expression. They catalyze the removal of acetyl moieties from acetyl-lysine residues in histones and non-histone proteins and the hydrolysis

of amides of short and long fatty acids, playing a key role in the regulation of many biological processes as well as in the occurrence and progression of several diseases, including cancer. HDACs are validated targets for drug design and some HDAC inhibitors (HDIs) have been approved by the FDA for the treatment of cutaneous or peripheral T-cell lymphoma and multiple myeloma. Currently approved compounds lead to adverse effects and some of them are not selective. HDAC8, a Zn^{2+}-dependent HDAC, is an emerging anticancer target for structure-based drug design and exhibits unique structural features, including the high flexibility of the L1 loop of the active site that can adopt two different conformations, open and closed. Consequently, the size of the binding pocket can change, and this aspect can be exploited to develop selective inhibitors. As reported by Taha et al. (ACS Med Chem Lett. 8:824–829, 2017), novel tetrahydroisoquinoline (TIQ)-based HDAC8 inhibitors have shown an improvement in potency and selectivity for HDAC8 compared to other HDAC8 inhibitors studied. We solved several X-ray crystal structures of human HDAC8 in complex with TIQ-based HDAC8 inhibitors. The 3D structures show that the inhibitors are bound at the catalytic site, occupying a hydrophobic and narrow tunnel with their hydroxamic acids group chelating the Zn^{2+} ion. Unlike HDAC8 bound to the HDAC8 isoform-selective aromatic acid-based inhibitors, TIQ compound(s) bind to the "closed" conformation with the loop L1 that moves toward the active site, making it deep and narrow. These findings provide insights for further rational design of novel HDAC8-selective TIQ-based inhibitors.

P-02-015
Structural characterization of hypoxia inducible factor α – prolyl hydroxylases interaction through MD simulation

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The prolyl hydroxylases (PHDs) are an enzymatic family that regulates cell oxygen-sensing. Under normoxia condition, PHDs hydroxylate the hypoxia-inducible transcription factors α (HIFs-α), driving their proteasomal degradation. Hypoxia inhibits PHDs activity, stabilizing HIFs-α, which initiate the transcription of genes involved in cell metabolism adaptation to hypoxia. As a main hallmark of cancer, hypoxia promotes neo-angiogenesis, cell proliferation, and survival. The PHD isoforms are thought to have a variable and cell-dependent impact on tumor progression. All isoforms hydroxylate HIF-α (HIF-1,2,3α) with different affinities. However, what determines these differences and how they pair with tumor growth is poorly understood. Here, molecular dynamics simulations were used to identify and characterize the PHD2 [Previously published in: Camagni GF et al. (2023) Int J Mol Sci 24, 4710] and PHD3 isoforms binding properties in complexes with HIF-1α and HIF-2α. In parallel, conservation analysis and binding free energy calculations were performed to better understand PHDs substrate affinity. We observed that the β2β3-loop and the C-terminus show a significant conformational change in all systems due to disorder content of these portions. Furthermore, our data indicate a specific interaction between the PHDs C-terminus and HIF-2α that is not observed in the PHDs/HIF-1α complexes. We also observed a statistically significant difference in binding energy between complexes that pair with a number of inter-molecules interactions specific for each substrate and isoform. Identifying these sites may be relevant for cancer research as their mutations can interfere with the binding of a specific substrate without impacting the PHDs enzymatic activity. Collectively, our findings suggest that the PHDs C-terminus may act as a molecular regulator of PHD's activity, highlighting differences in the two isoforms substrate specificity.

P-02-016
Integrating molecular modeling, synthesis, and biological evaluation for the design of novel pyrido[2,3-d]pyrimidine-4(3H)-one derivatives as NEK6 kinase inhibitors

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The nima-related kinase-6 (NEK6) plays a crucial role in cell cycle regulation. Research has highlighted its overexpression in human ovarian cancer, establishing it as an adverse prognostic marker. This makes NEK6 an interesting target for anticancer therapy. Through computer-aided drug design, we identified a pyrido[2,3-d]pyrimidine-4(3H)-one derivative (21), as a potent NEK6 inhibitor with antiproliferative effects across various cancer cell lines. However, the limited water solubility of 21 raised concerns about its suitability as a drug candidate. In response to this issue, we synthesized three new derivatives of compound 21 through noninvasive structural modifications of the indenone ring system. The aim was to improve solubility while preserving the affinity for the NEK6 binding pocket. The synthetic approach was guided by molecular modeling studies and free energy perturbation (FEP) calculations. Furthermore, the LANCE Ultra TR-FRET kinase assay demonstrated that the new derivatives inhibited NEK6 at different levels. In particular, one of the three new compounds exhibited slightly higher activity in NEK6 inhibition experiments compared to the parent compound and also showed a better pharmacokinetic profile (measured through logP and ADME *in silico* calculations). These findings encourage further medicinal chemistry efforts, prompting ongoing modifications to the pyrido[2,3-d]pyrimidinone core to explore additional avenues for drug development. *The authors marked with an asterisk equally contributed to the work.

P-02-017
Elucidating flavonoid-mediated ADA2 inhibition through computational analysis: a novel strategy for enhancing immune response in glioma

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Brain tumors, especially gliomas, pose substantial obstacles to effective therapy owing to their intricate microenvironmental complexities and increased mortality rates. Tumor-associated macrophages (TAMs) prominently contribute to dismal prognoses, with adenosine deaminase 2 (ADA2) emerging as a central orchestrator of immunosuppression, tumor progression, and angiogenesis via the MAPK and PDGFβ pathways. Conversely, adenosine deaminase 1 (ADA1), vital for lymphocyte survival through purine metabolism, offers a contrasting role. Targeting ADA2 while preserving ADA1 function stands as a promising avenue for modulating macrophage polarization and stimulating anti-tumor immunity. Employing computational screening methods, we have identified Daidzin, a flavonoid compound, as a potential ADA2-specific inhibitor. Daidzin demonstrated remarkable ADA2 binding affinity while exhibiting minimal interaction with ADA1. Molecular dynamics simulations unveiled stable Daidzin-ADA2 complexes, characterized by lower binding energy and tighter clustering compared to Daidzin-ADA1 complexes. Remarkably, the secondary structural analysis revealed that Daidzin induced significant conformational alterations within the ADA2 dimerization domain, crucial for its activity, and reinforcing its selectivity. Moreover, Daidzin showcased markedly lower toxicity relative to the standard ADA inhibitor, Coformycin, amplifying its therapeutic promise. Our study presents compelling evidence endorsing Daidzin as a potent ADA2 inhibitor, offering promising prospects for modulating macrophage polarization within the glioma microenvironment. By highlighting the pivotal role of specificity in small molecule therapeutics, our findings underscore Daidzin's potential as a targeted therapeutic agent capable of re-educating the tumor microenvironment and revolutionizing glioma management.

P-02-018
Molecular reasons for aging: increased disbalance in the system oxidative stress – energy provision

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Earlier we found increased oxidative stress intensity and decreased antioxidant defense potency as mice transitioned from young to middle age whereas later with aging these parameters were changed negligibly. This work aimed to extend our previous findings by behavior tests and investigate if alpha-ketoglutarate (AKG) may impact metabolic markers of interest under basal conditions (physiological aging). Young and middle-aged mice showed no difference in overall mobility in the open field test, middle-aged mice exhibited heightened signs of anxious behavior and declined spatial memory, indicating age-related brain function deterioration. Generally, this work confirmed our previous findings on oxidative stress intensification and decreased antioxidant defense potency in the brains of middle-aged mice and showed activation of the pentose phosphate pathway probably to supply regenerative coenzyme NADPH to antioxidant systems. Mechanistic insights into these processes are detailed in our review paper (Bayliak et al., 2023), attributing the phenomenon to heightened reactive oxygen species production in aging brains, prompting energy resource redirection for protective molecule synthesis. Unexpectedly, AKG consumption under basal conditions induced physiological metabolic changes in young mice, triggering anxious behavior and altering enzyme activities and gene expressions in the brain, notably decreasing the activities glucose-6-phosphate dehydrogenase and hexokinase while leaving other antioxidant enzymes unaffected. AKG also enhanced levels of transcripts of autophagy markers in the brain, suggesting a hormetic effect under physiological conditions. Middle-aged mice fed AKG displayed enhanced antioxidant defense activation in the brain, further supporting AKG protective role. Our findings propose AKG mild prooxidant effect as a potential mechanism for its protective action under basal conditions. Previously published in: Bayliak MM et al. (2023) BBA Adv 3, 100077.

P-02-019
Structural study of European *Borrelia* decorin binding proteins – interspecific differences and interactions with glycosaminoglycans using NMR spectroscopy

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Decorin-binding proteins (Dbp) are adhesins that attach to glycosaminoglycans (GAGs) on the surface of cells and are expressed in DbpA and DbpB homologs. They have been identified as the main factors of virulence [Lin et al. 2014 PLoS Pathogens10 (7)]. Previous research has shown that Dbp-GAG binding mechanisms differ among various strains of *Borrelia* species [Morgan and Wang 2015 Biochemical J 467(3), 439–451]. Therefore, we aimed to investigate the structural and dynamic properties of Dbps and their interactions with GAGs using nuclear magnetic resonance (NMR) spectroscopy. We have made almost complete backbone and sidechain assignments of DbpA from *B. Afzelii* and *B. Bavariensis* (Hejduk et al.2021, Biomol. NMR Assign.), with assignments for other variants currently in progress. We compared secondary structure propensity based on chemical shifts, T_1 and T_2 spin relaxations, and heteronuclear NOE-based backbone dynamics with available NMR structures of North American *Borrelia* species. We used selective isotopic unlabeling to solve assignment bias. We performed initial studies on protein-GAG interactions of various Dbps and GAGs using NMR titration, protein dynamics assessment via heteronuclear NOE experiments, and hydrogen-deuterium exchange mass spectrometry (HDX-MS). We also measured the affinity and K_D of binding by microscale thermophoresis. We are currently studying unspecific saccharide interactions and optimizing experiments for future use of labeled synthetic GAGs. The synthetic GAG ligands with well-characterized structures will be crucial for a fine

structural description. Combined NMR and HDX analyses indicate interspecific differences in GAG binding, setting the starting point for detailed research on the influence of structural differences and their impact on tissue tropism.

P-02-020
Biochemical and structural study of closed conformation of the voltage-gated hERG potassium channel

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The human potassium channel hERG (Kv11.1) is a voltage-dependent channel. It has been found in cardiomyocytes and neuronal cells and has a role in repolarization of the cardiac action potential. Disfunction of hERG channel leads to the syndrome of prolonged QT interval on electrocardiogram and cardiac arrhythmias. We aimed to make an expression of double cysteine mutant hERG channel and perform subsequent biochemical, electrophysiological analysis to obtain a closed channel conformation in advance of a structural analysis. Cysteine mutations of hERG channel allow to obtain a closed conformation. The deletion of N- and C- terminus sequence facilitates to improve hERG channel stability during purification. Using techniques of culturing and transfection of the HEK Expi suspension cells, we selected optimal conditions for expression of hERG channel. Using a plasmid with multiple protein affinity purification tags, such as Strep-Tag and His-Tag, we improved the protein purification result. By applying several solubilization techniques, including testing different detergents for membrane protein release, we achieved a good solubilization and high purification of the target protein. Using negative staining and Cryo EM imaging, we obtained structural data of the target mutant protein complex. These biochemical studies are the first step to the experimental block of obtaining the closed conformation of the mutant hERG potassium channel. For the structural study of the closed channel, we expect to carry out investigations using cryo-electron microscopy techniques in the nearest future. This work was supported by Grant of Education bureau of Guangdong province "innovation team" №2022KCXTD034.

P-02-021
Electrophysiological study of mutant HERG channels and the influence of mutant β subunit on the activity of this channel

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Voltage-gated potassium channels are a large group of ion channels. There is a great number of diseases associated with mutations in ion channels; such diseases are called channelopathies. Of particular interest are diseases in voltage dependent channels of the EAG family and in particular channel Kv11.1. Dysfunction of voltage-dependent potassium channels has been implicated in various cardiac diseases like long QT syndrome and the lethal cardiac arrhythmia torsade de pointes. Investigating the structure, function, and regulation of these channels can provide insights into the pathophysiology of these disorders and potentially lead to the development of novel therapeutic strategies targeting these channels. Identifying point mutations associated with various diseases and studying their impact on the functioning of these channels is an important task both for the understanding of channel function and for the treatment of patients. In this work, we studied the effect of point mutations in the Kv11 channel located in different regions of the polypeptide chain using electrophysiological methods and immunochemical methods on standard cell lines. In addition, we studied the effects of mutation in an additional subunit of this channel and showed the possibility of LQT syndrome development in patients with such mutation. In addition, based on the available structural data on this channel family, we considered possible structural changes that lead to altered activity of the mutant channels. The study of mutant channels, as well as possible mechanisms of occurrence in channel malfunction, will help to select therapy and create more effective drugs for the treatment of such patients more effectively. This work was supported by Grant of Education bureau of Guangdong province "innovation team" №2022KCXTD034.

P-02-022
Elucidating the peroxisomal protein import machinery interactions

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Peroxisomes are essential organelles present in most eukaryotic cells, playing a crucial role in processes such as glycolysis, lipid breakdown, and detoxification. Despite their importance, peroxisomes do not contain their own DNA, necessitating the import of proteins synthesized in the cytoplasm into the peroxisomal matrix. The recognition and sorting of these proteins are facilitated by specific peroxisomal targeting signals (PTS), namely PTS1 and PTS2. Proteins with a PTS1 signal are recognized by the receptor protein Pex5, whereas those with a PTS2 signal engage with Pex7. Notably, alcohol oxidase (AOX), a key enzyme in methanol metabolism and detoxification within peroxisomes, exhibits an unconventional import mechanism. Although AOX typically harbours a PTS1 signal in its C-terminal domain facilitating peroxisomal import, it has been demonstrated that AOX can still bind with Pex5 even in the absence of this signal. Moreover, AOX, which usually exists as a homo-octamer, interacts with Pex5 in a monomeric form during the import process. This suggests the existence of an alternative peroxisomal protein targeting pathway that does not rely on the traditional PTS1 signal, the intricacies of which remain largely unexplored. Our research is centered on the peroxisomal targeting of AOX from *Pichia pastoris*, aiming to elucidate these alternative pathways and their implications. We have successfully isolated pure octameric AOX and are now focused on uncovering the structural basis of its interaction with Pex5. Currently, efforts are underway to obtain a monomeric form of AOX to examine its binding affinity to Pex5 using biochemical assays. Our ultimate objective is to determine the complex structure of the AOX monomer bound to Pex5, complemented by binding studies, to shed light on the molecular mechanism underpinning its peroxisomal import. This study holds the promise of broadening our understanding of the novel pathways utilized for AOX import.

P-02-023
Targeting fibroblast activation protein in cancer: structural characterization of a novel peptidomimetic

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Fibroblast activation protein (FAP) is a membrane-bound serine protease that has emerged as a promising tumor marker. FAP is overexpressed in the tumor stroma of many cancers, including most carcinomas, and has been linked to promoting angiogenesis, tumor cell invasion, and immunosuppression. Moreover, its expression is highly increased relative to healthy tissue, making it a robust target for cancer imaging and therapy. Small-molecule FAP inhibitors have been widely employed as a targeting moiety of FAP-targeted radiotracers, which have been successfully tested as theranostics in humans. However, limited structural information on FAP–inhibitor complexes has hampered further elaboration and refinement of inhibitor structures through rational design. In our recent work, we conducted a structure-activity relationship study to explore the chemical space in the P1′ and P2′ positions and developed a new class of peptidomimetic inhibitors bearing an α-ketoamide warhead. Besides other lead-like properties, the compound I22AP446 (IC_{50} = 89 pM) outperformed the most potent inhibitor published to that date. To gain insight into the binding mode of the α-ketoamide derivative, we determined a crystal structure of the FAP–I22AP446 complex at 1.75 Å resolution revealing key interaction features between the inhibitor and the enzyme. We thus present the first reported crystal structure of FAP bound to a peptidomimetic. Our findings provide a basis for structure-guided modifications of our lead compound and will fuel the development of selective peptidomimetics targeting FAP.

P-02-024
Dissecting the structural basis of LRRK2 interactions with AlphaFold and coevolution analysis

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Leucine-rich repeat kinase 2 (LRRK2) is a multi-domain protein implicated in both familial and idiopathic Parkinson's disease (PD). Besides its kinase and GTPase activities, LRRK2's function extends beyond its enzymatic role through its scaffolding domains, which play a critical role in maintaining LRRK2's auto-inhibited state and mediating protein-protein interactions (PPIs), thereby positioning LRRK2 as an important signaling hub. We leveraged a novel dataset of LRRK2 interactors, identified through a proximity labeling experiment, and used it to perform a comprehensive computational analysis of the LRRK2 PPI network. Our findings reveal that direct LRRK2 interactions exhibit a stronger coevolutionary relationship than interactions catalogued as associations in the IntAct database. Interestingly, LRRK2 proximity interactors display coevolutionary patterns similar to those of direct interactors. By clustering the interactors on the basis of their pairwise coevolution, we identified functional clusters within LRRK2 interactors, shedding light on LRRK2's roles in various molecular pathways. Further, we employed AlphaFold2-Multimer (AF2) to predict the structure of LRRK2 interaction complexes, enabling us to classify interactors based on their interaction interface fingerprints with distinct LRRK2 domain regions. For some complexes, i.e. RAB10, we were able to validate the predictions with mass spectrometry cross-linking (MS-XL) data, which are mostly satisfied by the models [Previously published in: Guaitoli et al. (2023), *BioRxiv*, 2023.08.11.549911]. This approach also allowed us to identify distinct LRRK2 conformations associated with different interactions, offering insights into the structural dynamics underpinning LRRK2's function. This study not only advances our understanding of LRRK2's structural and functional landscape but also shows the potential of integrating coevolutionary data with structural prediction methods to elucidate complex biological mechanisms.

P-02-025
Exploring P2X4 and 5-HT3A receptor crosstalking

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P2X4 and 5-HT_{3A} ionotropic receptors play significant roles in pain and nausea/vomiting transduction pathways. We have shown via atomic force microscopy (AFM) analysis that both receptors can physically interact each other in 1:1 stoichiometry [Previously published in: Soto P et al. (2020) Front Cell Neurosci 14:106]; nevertheless, the functional consequences of this interaction remained unknown. In this work, we explored the modulation of the receptors-mediated intracellular Ca^{2+} increase observed in response to the ligands ATP, 5-HT by each receptor alone or the combination of both in transfected tsA201 cells. Furthermore, using AFM imaging we evaluated whether the purified P2X4/5-HT_{3A} receptor complexes depend on ligand binding and through mass spectrometry-based metabolomics and lipidomics, we determined changes in transduction pathways after activation of P2X4/5-HT_{3A} receptor complexes. Preliminary results have shown a reciprocal negative modulation of the Ca^{2+} response activated by each receptor when forming complexes. Also, minor changes in the stability of the purified complexes can be observed depending upon the presence of receptor ligands. Pathway analysis and simulations have shown that networks of biochemical cascades can arise as targets following the activation of receptor complexes by ligands. Taken together, these results suggest a potential mechanism of crosstalking between P2X4 and

$5\text{-}HT_{3A}$ receptors, which paves the way to further explore the role of their structural interaction. Funded by ANID Fondecyt Regular 1211060 and ANID/ACT210057.

Proteomics and Metabolomics

P-03-001
Novel approaches for proteomic profiling of hereditary arrhythmias and gene therapy treatment

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The calcium release unit (CRU) is a group of proteins regulating Ca^{2+} release and homeostasis in the heart. This complex is harboured in the junctional sarcoplasmic reticulum (jSR), a specialized domain of the endoplasmic reticulum. Mutations in the protein components of the CRU lead to catecholaminergic polymorphic ventricular tachycardia (CPVT), an inherited disease characterized by lethal arrhythmias. It is known that lower expression or absence of any accessory protein of the CRU decreases the expression of the other members of the complex (Denegri *et al.*, 2012). What is not known is the linkage between the pathophysiological processes of CPVT and the alteration in expression of CRU proteins. Dissecting this mechanism will reveal important therapeutic targets for hereditary arrhythmias. Our group possesses an extensive experience in developing murine models for CPVT and pioneered the application of gene therapy in this pathological context. We have developed a cardiac-specific protocol for enrichment of jSR membrane vesicles from a single murine heart. Our mass spectrometry preliminary data confirm that we are now able to characterize, for the first time, the proteomic landscape of CRU machinery. By taking advantage of two in house mouse models of CPVT variants, we will perform quantitative mass spectrometry (qMS) experiments to evaluate and quantify the relative proteins' abundances and stoichiometries. The functional relevance of these results will be assessed in the same murine models treated with our DNA-based gene therapy (Denegri *et al.*, 2014).

P-03-002
The discovery of new inhibitors of adenosine deaminase

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Adenosine deaminase (ADA) is an enzyme involved in purine metabolism, playing a significant role in immune system development and maintenance. The enzyme activity changes in various pathologies, for instance, it increases upon different cancers, autoimmune diseases, chronic hepatitis and liver cirrhosis. The increased level of ADA activity has been recognized as a suitable, low-charge ADA-test in differential diagnosis of some diseases (tuberculosis, rheumatoid arthritis, etc.). The increasing of enzyme activity upon inflammation diminished the concentration of its substrate, adenosine, well-known as anti-inflammatory agent. This ubiquitous enzyme also can deaminase adenosine analogs widely used in chemotherapy, immunotherapy and in treatment of several other pathologies. Therefore, the discovery of new compounds with ability to inhibit or activate ADA is required in modern biochemistry and pharmacology. We studied the influence of more than 100 new synthesized derivatives of piperazine, pyridine, imidazole, etc. on ADA activity purified from bovine lung Some of studied compounds possessed the ability to inhibit ADA about 40%. The compound D011 182 demonstrated the higher effect with IC_{50} value equal 19.95 μg/ml. Besides the radical scavenging capacity of novel synthesized compounds D011 180, D011 181, D011 182 were estimated with ABTS assay and IC_{50} values of these compounds were determined 38.6 ± 0.66 mg/ml; 42.6 ± 0.499 mg/ml; 47.9 ± 1.53 mg/ml, respectively. The low activity was obtained for these compounds with DPPH radical scavenging method. The studied compound D011 182 exhibits both antioxidant and ADA inhibiting properties. Consequently, in the base of our studies we can recommend chemists to synthesize derivatives that could potentially demonstrate enhanced ADA inhibition and antioxidant capabilities for further promising application in medicine.

P-03-003
Temperature variation induces neurotoxicity in *Danio rerio*

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Global warming taking place on our planet is amplified by anthropic action, which causes the release of CO_2 and other greenhouse gases. Temperature plays a central role in ectothermic animals, influencing their physiology and behavior. We recently demonstrated that environmental temperature variation heavily alters locomotor activity but also complex behaviours such as anxiety, social behaviour, aggression, learning, and cognitive behaviours in *Danio rerio* [1, 2]. In summary, our data suggested that temperature variation can be counted among the factors that generate neurotoxicity. To confirm such hypothesis, the eight different protein datasets used in our previous studies (18°C, 26°C and 34°C) for 4 days (acute) or 21 days (chronic treatment), and 24 of BDNF+/- and BDNF-/-zebrafish kept at 26°C or 34°C for 21 days) were re-analysed by an integrated proteomic approach by means of the software Ingenuity Pathway Analysis (IPA). The output of our analysis provides three protein panels related to 18°C, 34°C and BDNF depletion that can be linked to anxiety-like or boldness behaviour upon these treatments and identifies proteins that can be used as hallmarks of neurotoxic processes common to all the treatments applied. To further characterize the impact of environmental changes on the zebrafish brain, we are now analyzing the lipid component of the brain using a targeted and untargeted lipidomic approach and we are studying the eye proteome, as the eye is anatomical extension of the brain which is often affected in neurological disorder. Previously published in: 1. Toni M et al. (2019) J Proteomics 30;204:103396. 2. Toni M et al. (2022) Int J Mol Sci 23(10):5606. 3. Maffioli E et al. (2023) Int J Mol Sci 24(21):15735.

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P-03-004
Exploring the role of important exoribonucleases in the foodborne pathogen *Campylobacter jejuni*

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Campylobacter jejuni is the most prevalent enteric pathogenic bacteria and the leading cause of human gastroenteritis worldwide. Extensively found in nature, *C. jejuni* has the ability to persist and grow at low temperatures, representing a major threat for food safety and public health. Moreover, infections caused by this pathogen are related with the development of dreadful secondary disorders like the Guillain-Barré syndrome. As an oral–fecal pathogen, *C. jejuni* has to cope with different stresses during the infection process. Therefore, rapid adjustments in cell metabolism and gene expression profile are essential for its survival. Ribonucleases, being involved in RNA metabolism and stability, are active players in that regulation. *C. jejuni* encodes an interesting set of endo- and exoribonucleases, but the knowledge on RNA degradation network of this pathogen is still limited. In this work, our aim is to explore this field focusing on two important *C. jejuni* exoribonucleases, PNPase and RNase R. Both proteins proved to play a major role in cell motility, adhesion and invasion mechanisms, observed in the first stages of host infection. In this work we have performed differential RNA sequencing analysis in *C. jejuni* wild-type and mutant strains deficient in PNPase and RNase R, and we have identified the genes which were more affected by the absence of these ribonucleases. Additionally, considering that it was already demonstrated in other organisms that there is a connection between PNPase and some metabolites, we have performed NMR-metabolomics analysis of both wild type and Δ*pnp* strains to investigate the impact of this ribonuclease in *C. jejuni* metabolome. Altogether, we intend to exploit the connection between PNPase, RNase R and their specific cellular targets and unravel new regulatory pathways that can be important for *C. jejuni* biology or/and pathogenicity.

P-03-005
Deciphering the metabolic footprint of ketosis-inducing diets in healthy mice

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Emerging evidence associates diet-induced ketosis with several beneficial effects on health and disease such as reduced inflammation, improved lipid profiles, weight loss, and improved diabetes management. However, little is known about the effect of different ketosis-inducing dietary approaches on the metabolome. Therefore, we aimed to characterize simultaneously the metabolic profile in plasma as well as the liver, spleen, white adipose tissue, duodenum, and feces of healthy, male C57BL/6 mice fed with different ketosis-inducing diets, including caloric restriction (CR), intermittent fasting (IF), fasting-mimicking diet (FMD), ketogenic diet (KD), and regular mouse chow. Samples were collected at a mouse age of 16 weeks for all dietary intervention groups. We performed a comprehensive targeted metabolomics analysis enabling the quantification of 26 biochemical compound classes. In the dietary groups, lipid and polar metabolite classes varied substantially in plasma and tissues. Multi-tissue metabolomics revealed that all dietary regimes induced significant changes in polar metabolites as well as lipid profiles. For instance, CR, IF, FMD, and KD reduced glycerolipid levels (di- and triacylglycerides) in plasma by 38%, 56%, 90%, and 49%, respectively, compared to regular chow. Moreover, two metabolites, namely proline betaine and trigonelline, were consistently reduced in plasma and in all organs, while 3-methylhistidine was consistently increased in all matrices except feces by the ketosis-inducing diets. Our analysis provides a comparable multi-tissue metabolic footprint for each of the diets as a basis for understanding the various effects attributed to ketosis-inducing diets on a metabolic level in healthy mice. Further analysis will unveil the association of specific metabolic changes with diet-induced ketosis and disease-related markers such as inflammation-related markers.

P-03-006
The role of flavonoids in antagonistic microbial interaction

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The plant microbiome, including bacteria and fungi that live inside or outside plant tissues, significantly influences plant life and can mitigate the effects of abiotic or biotic stress. Endophytic fungi, which live inside the host tissue and usually do not cause any signs of disease, are present in almost all higher plants. Previous reports demonstrated that *Acremonium* could play an important role in biological control against plant pests and pathogens. Our research focused on sucrose as a nutrient commonly present in the culture medium, which can directly influence plant growth and the plant–microbe interaction itself. It was confirmed that in a low-carbon medium, the presence of endophyte promotes an increase in leaf rosette area by almost 20% compared to the control. However, our data showed that high carbon presence in cultivation media can reverse plant growth promotion. A reduction of the leaf rosette area by almost 40% was observed. The mechanism of the Janus interaction was subsequently studied using multiomics approaches. Proteomic analysis indicated that proteins related to flavonoid biosynthesis and anthocyanin accumulation could play a significant role in the interaction. These results were further confirmed at the transcriptional level using RNA-seq, and metabolomic profiling confirmed changes in the levels of flavonoids. Validation of the *Arabidopsis* mutant in flavonoid biosynthesis showed that the observed growth inhibition could be partially reversed. The present study reveals the role of flavonoid compounds in the antagonistic interaction between plants and microbes. Our study should enable more efficient use of endophytes in organic farming and environmental biotechnology.

P-03-007
NMR based metabolomic workflow to unravel the molecular mechanisms involved in strawberry vernalization

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Metabolomics, a discipline focused on elucidating the metabolic profile of complex mixtures, provides valuable insights into the physiological status of organisms. While mass spectrometry (MS) coupled with chromatography is commonly used, nuclear magnetic resonance (NMR) is gaining interest due to its unique benefits. However, challenges in NMR data analysis makes its standardisation difficult. In this study, we applied an automated NMR-based metabolomic workflow to investigate metabolic changes during vernalization in strawberry leaves, a critical process in successful cultivation with elusive molecular mechanisms. This work aims to enhance our understanding of vernalization biochemistry and promote NMR with computational tools for metabolomic analysis. Despite its advantages, complexities in spectrum profiles and biological interpretation pose challenges, hindering its routine use. In parallel, computational advances address the complexities, making NMR increasingly popular, especially in food science and biomedicine. We propose its application in highly valued agri-food products analysis, presenting a novel application field for NMR. This study not only elucidates the metabolomic processes triggered during vernalization in strawberry crops but also lays the background for the automated utilization of NMR in agronomic analysis.

P-03-008
Neuropeptide Y and substance P regulate embryonic salivary gland branching through the FGF/FGFR pathway in aging Kl-/- mice

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Salivary gland branching morphogenesis is regulated by the functional integration of neuronal signaling, but the underlying mechanisms are not fully understood. The purpose of this study is to investigate whether neurotransmitters regulate the development and function of accelerated aging klotho deficient (Kl-/-) mouse salivary glands. First, we investigated whether the neurotransmitters, substance P (SP) and neuropeptide Y (NPY), affect the branching morphogenesis of embryonic salivary glands in klotho deficient (Kl-/-) mice. In the salivary glands of embryonic Kl-/- mice, morphological analysis and immunostaining revealed that epithelial bud formation, neuronal cell proliferation/differentiation, and the expression of the salivary gland functional marker, tight junction protein-1 (ZO-1) were decreased in ductal cells. Interestingly, incubation with SP/NPY for 48 h promoted branching morphogenesis, parasympathetic innervation and epithelial proliferation. The ERK inhibitor U0126 specifically inhibited neuronal substance-induced epithelial bud formation in the embryonic salivary gland. RNA-seq profiling analysis revealed that the expression of FGFs/FGFRs was significantly regulated by SP/NPY treatment in the embryonic salivary gland (E15). The FGFR inhibitor BGJ389 inhibited new branching formation induced by SP and NPY treatment and ERK1/2 expression. These results showed that the neuropeptides SP/NPY induced embryonic salivary gland development through FGFR/ERK1/2-mediated signaling. This work was supported by the National Research Foundation of Korea (NRF) grant funded by the Korea government (MSIT) (RS-2023-00222390).

P-03-009
Top-down proteomics of cerebrospinal fluid to investigate intact neuropeptides and proteins' post-translational modifications in the context of multiple sclerosis

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Multiple sclerosis (MS) is a chronic neuro-inflammatory disease whose diagnosis is based on clinical marks, brain imaging, and cerebrospinal fluid (CSF) analysis. Proteomics studies of CSF from MS patients are crucial to discover specific biomarkers and to observe molecular pathways disease related. Indeed, mass spectrometry-based techniques have been applied, mainly choosing bottom-up proteomic approaches, resulting in large proteome profiling but lacking in the detection of post-translational modifications (PTMs) and intact neuropeptides [previously published in Monokesh K. Sen et al. (2021) IJMS 22, 7377]. Proteoforms, as indicators of health/disease, have a long history in biomedical research, thus, a top-down proteomic approach was optimized to investigate CSF from 9 MS relapsing-remitting patients under no drug treatment. Samples were diluted 1:1 (v/v) in 0,2% aq. trifluoroacetic acid, centrifuged, and analyzed by reverse phase HPLC coupled with a high-resolution mass spectrometer (LTQ-Orbitrap-XL). Proteome Discoverer analysis identified 524 peptides deriving from the endogenous cleavage of 103 proteins, as neurosecretory protein VGF, secretogranin-1 and -3, chromogranin-A, Transthyretin, Fibrinogen alpha chain, Cystatin-C, Beta-casein, Alpha-1-antitrypsin, complement C3. The manual inspection of MS/MS spectra revealed small intact proteins and their PTMs: Transthyretin and its oxidative derivatives on Cys_{10} (sulfonation, sulfhydrylation, cysteinylation and cysteinyl-glycine adduct); Cystatin C and its derivatives (Met_{14} and Met_{110} oxidation); β2-microglobulin and its C-term oxidated; Secretogranin-1 and its phosphorylated isoform. The analysis of proteins PTMs and endogenous intact peptides may be of great interest to monitor the disease progression and therapy effects. Hence, top-down proteomic approaches applied to CSF may be a key factor to improve the understanding of MS.

P-03-010
A structural model to explain the role of MucR as histone-like protein

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MucR from *Brucella abortus* is a member of the family Ros/MucR, which comprises proteins regulating genes mostly involved in virulence. These proteins show a C-terminal DNA-binding domain (DBD) whose structure was solved by NMR in the homologous protein Ros from *A. tumefaciens*. MucR and other members of the family have been studied as classical transcription regulators for three decades. Recently, an oligomerization domain at the N-terminus of MucR has been identified and the ability of these proteins to oligomerize has been reported [1]. The DNA-binding activity of MucR showed that this protein recognizes preferentially AT-rich sequences containing TA-step and contacts the DNA minor groove [2]. These data led to hypothesize that MucR is a new histone-like nucleoid structuring (H-NS)-like protein, never recognized before in α-proteobacteria. H-NS-like proteins, already discovered in β- e γ-proteobacteria, are able to condense the bacterial genome repressing the expression of virulence genes when it is not required. Recent studies showed the ability of MucR to structure the genome of *Brucella*, definitely classifing MucR as a new H-NS-like protein [3]. Combining cryo-EM, NMR and structure prediction, here we report the first structural model of the MucR oligomer. Our structural data, together with functional studies, explain the modality adopted by MucR to condense DNA, which is different from that of the other H-NS-like proteins previously studied, uncovering a novel type of H-NS-like protein. References: 1. Pirone L et al. Sci. Rep. 2018, 8. 2. Baglivo et al. Sci. Rep. 2017, 7. 3. Barton et al.. mBio. 2023, 14.

P-03-011
Advances in thyroglobulin measurement: exploring dried blood spot sampling and mass spectrometry for enhanced clinical utility

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In contemporary medical practice, human thyroglobulin (Tg) represents the primary tumour biomarker for detecting the recurrence of differentiated thyroid carcinoma (DTC) in patients who have undergone thyroidectomy. Tg is a large and highly glycosylated tissue-specific protein exclusively produced by both healthy and tumour thyroid follicular cells in the thyroid gland. Different techniques, including immunometric assays (IMA) and radioimmunoassays (RIA), have been implemented in clinical settings to gauge Tg levels in blood samples collected through venipuncture. However, the reliability of these methods is compromised by the presence of antibodies, including anti-thyroglobulin antibodies (Tg-Abs) and heterophile antibodies (HAs), resulting in frequent inaccuracies in the quantification of T due to either the under- or overestimation of the actual values. In recent years, liquid chromatography tandem mass spectrometry (LC-MS/MS) has emerged as a distinctive and alternative tool aimed at overcoming the challenges posed by antibody interference. Despite its potential, the effectiveness of LC-MS/MS has yet to be fully explored and, if performed, could improve our knowledge regarding the potentiality of this tool for the detection of Tg. In this work, we present a workflow, based upon LC-MS/MS and SISCAPA technology, to quantify Tg in patients with thyroid cancer, indicating greater sensitivity and specificity with respect to the routinely available protocols. Moreover, this workflow is also currently being translated and tested for use with samples obtained with DBS (dried blood spot) devices, a simple, cost-effective, and minimally invasive alternative to those obtained by venipuncture. Based upon these findings, this LC-MS/MS and SISCAPA based approach not only shows the potential for improving the accuracy of Tg quantification but may also simplfy this process for patients living in remote areas who could independently collect DBS samples for Tg monitoring. *The authors marked with an asterisk equally contributed to the work.

P-03-012
Data analysis optimisation of a DIA-PASEF mass spectrometry approach for the study of idiopathic membranous nephropathy disease

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In membranous nephropathy (MN) about 75% of cases are classified as idiopathic (IMN) since they have no clear secondary cause. Therefore, new blood antigens could represent a crucial tool in helping patient stratification and therapy choice. In our work, a proteomic approach based on mass spectrometry was applied for the analysis of serum samples collected from IMN (n = 15) patients and from patients affected by other nephropathies (same presentation but different aetiology, PN, n = 15) with the aim of pinpointing disease-specific protein signatures. The novel data-independent acquisition-parallel accumulation serial fragmentation (DIA-PASEF) technique based on trapped ion separation (TIMS) was employed: DIA-PASEF allows a rise in performances and sensitivity, leading however to an increase in spectral complexity. To maximise the information gathered from our data, we implemented a pilot study to evaluate the different performances of a library-free or a library-based approach which was then applied to the whole cohort study. The optimization led to the identification of 467 protein groups eligible for a label-free quantitative evaluation [previously published in: Previtali P et al. (2023) Int J Mol Sci 24, 11756]. Considering an adjusted p-value < 0.05 and a fold change of ± 1.5, 57 proteins emerged as deregulated in IMN vs PN. Performing a classification tree MRC1 (macrophage mannose receptor 1) and BTD

(biotinidase) were able to separate IMN from PN; while the pathway investigation pointed out the critical role of serpins and lipoproteins in MN. Currently, to maximise our results both in terms of robustness/quality and in terms of biological implications, a comparative and integrative study of different software for proteomic DIA data processing is ongoing. To conclude, the application and the evaluation of customised data elaboration strategies in DIA-PASEF proteomics could have a relevant impact on the biological outcome to help overcome clinical challenges. *The authors marked with an asterisk equally contributed to the work.

P-03-013
Investigating the presence of eNAMPT/visfatin in human skimmed milk and milk fat globule membrane before and after Holder pasteurization

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Maternal milk is the perfect nutrition for infants of all species. Human milk proteins, a mixture of whey proteins including caseins, milk fat globules membrane proteins (MFGM), various peptides and their amino acids, play a crucial role in the growth and development of the child also as non-nutritional bioactive components. Extracellular nicotinamide phosphoribosyltransferase (eNAMPT/visfatin), is a conserved cytokine/enzyme released by many mammalian cells correlated with multiple metabolic and immune processes (Audrito et al., 2020). After visfatin detection in skimmed milk and the hypothesis of its potential role in regulating infant adiposity through maternal milk (Bienertová-Vašků et al., 2012), no other investigations have been reported. We have analyzed milk samples from human milk donated bank. After milk fractionation (2000 ***g*** for 30 min at 10°C) both skimmed milk and MFGM were analyzed by SDS-PAGE and MALDI-TOF mass spectrometry. In order to evaluate eNAMPT/visfatin content in skimmed milk ELISA assay was performed, while MFGMs were investigated through immunoblot. Data show a higher eNAMPT/visfatin content in raw skimmed milk if compared to pasteurized samples. In addition, we identify eNAMPT/visfatin associated with MFGM proteins, never described before, where their levels seem to be higher in MFGM proteins derived from pasteurized milk if compared to raw milk, an opposite result compared milk samples. Overall, these results may suggest an involvement of eNAMPT/visfatin in the reshuffling of MFGM induced by the Holder pasteurization process. References: Audrito V et al. (2020) Front. Oncol. 10, 358. doi: 10.3389/fonc.2020.00358. Bienertová-Vašku J et al. (2012) Diabetes Research and Clinical Practice, 96(3), 355–361. doi: 10.1016/j.diabres.2011.06.009.

P-03-014
Defining the molecular landscape of reparative cardiac progenitor cells by MS-imaging and advanced MS-proteomics approaches

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Understanding the epicardium role in cardiac repair has intensified interest in developing strategies to harness its regenerative potential for human therapies. Matrix-assisted laser-desorption ionization mass spectrometry imaging (MALDI-MSI) emerges as a powerful tool, providing spatial information on diverse biomolecules directly *in situ*. This study aims to unravel the proteomic response to novel compounds targeting epicardial cells using MS-based proteomic approaches. Epicardial slices were cultured and treated with novel pharmacological compounds. Additionally, nano-scale liquid chromatography tandem mass spectrometry (nLC-MS/MS) characterized the proteomic response. Epicardial slices proved to be an exceptional model for studying epicardium physiology and implementing pharmacological therapy. MALDI-MSI analysis distinguished epicardial from myocardial zones based on spectral profiles. Principal component analysis (PCA) differentiated average spectra, while a pairwise ROC approach highlighted distinguishing peaks (e.g., ions at m/z 976.50, 1019.64, and 2705.45). nLC-MS/MS revealed candidate proteins responding to drug assays, including collagen alpha-6 (COL6A), cadherin-13 (CDH13), gap junction alpha-1 protein (GJA1), vimentin (VIM), prostaglandin E synthase-3 (PTGES3), Moesin (MSN), and proliferating cell nuclear antigen (PCNA). Protein-protein interaction analysis identified pathways such as vascular endothelial growth factor A (VEGFA) and VEGFR2 signaling, cellular response to stress, fibroblast metabolic pathways, and hypoxia-induced factor signaling as crucial in these proteomic responses. In conclusion, epicardial slices emerged as a suitable organotypic model, offering insights into proteomic responses to pharmacological compounds targeting epicardium-driven heart remodeling through MALDI MSI and advanced proteomics. *The authors marked with an asterisk equally contributed to the work.

P-03-015
Chemogenetic split fluorescent reporters for super-resolution imaging of protein-protein interactions

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Mapping the subcellular organisation of protein-protein interactions (PPI) is essential to understand their role in cellular functions. Although super-resolution microscopy techniques can allow the visualisation of fluorescent molecules with enhanced spatial resolution, few strategies enable the observation of the organisation of PPI at subdiffraction resolution. In our approach, PPIs induce the reconstitution of the chemogenetic

PPI reporter splitFAST[1,2] which displays brightness and photostability suitable for stimulated emission depletion (STED) nanoscopy. Here we present a new strategy for imaging the organisation of protein-protein interactions in super-resolution in living cells. This approach allowed us to image the spatial organisation of chemically induced or constitutively present protein-protein interactions with enhanced spatial resolution, opening great prospects for studying the nanoscale organization of PPI in living cells. References: 1. Tebo, A.G., Gautier, A., 2019. Nat Commun 10(1), 2822. 2. Rakotoarison, L.-M. et al. 2024. ACS Chem. Biol. 19(2), 428–441.

P-03-016
Exploring tardigrade-derived protein Dsup as a novel strategy to mitigate ischemia-reperfusion injury: insights from proteomic analysis

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During ischemia, the lack of oxygen and nutrients leads to cellular dysfunction and metabolic alterations. Reperfusion paradoxically exacerbates tissue damage leading to the generation of reactive oxygen species, which triggers oxidative damage to lipids, proteins, and DNA. Uncover new pathways involved in cell protection during ischemia and reperfusion injury is essential to design new drugs able to preserve tissue integrity. To do that, we transfected HEK293T cells with the tardigrade *Ramazzottius varieornatus* damage suppressor protein (Dsup) which is known to confer to tardigrades characteristics of resistance to several stresses. Empty vector and Dsup+ cells were exposed to 250 μM O/N H_2O_2 and to 150 μM $CoCl_2$ (24 h, 72 h and 72 h + 24 h of reperfusion) and analyzed by functional proteomic. We identified 60 statistically significant differential proteins. Enrichment analysis by GO terms of biological processes showed that mRNA splicing processes and redox homeostasis are mainly represented by high abundant proteins in Dsup+, whereas ubiquitin-dependent proteasome-mediated degradation processes and metabolic processes are mainly represented by low abundant proteins in Dsup+. Enrichment analysis of cellular compartments highlights a different sub-cellular localization of differential proteins, as the high abundant ones in Dsup+ are mainly associated with ribonucleoproteins complexes, spliceosome and nucleoplasm. Pathway analysis highlights the involvement in transcriptional regulation mechanisms, oxidative stress response and energetic metabolism. Dsup+ cells showed an increased autophagy revealing the importance of this pathway for physiological processes. Transfection of HEK293T with Dsup, revealed that autophagy, ribonucleoproteins complexes and spliceosome must be restored to preserve tissue integrity during reperfusion. These results may be used to design new drugs targeting these processes to be used during reperfusion injury. *The authors marked with an asterisk equally contributed to the work.

P-03-017
Initial insight into the proline-rich region of the postsynaptic Shank3 protein

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Shank3 is a major scaffolding protein of the postsynaptic density. Shank3 is a modular protein containing globular domains and a long intrinsically disordered, proline-rich segment. The functional importance of this segment is suggested by the presence of a number of binding sites in the region including that of Homer protein. Mutations of both Shank and Homer proteins are reported to be associated with neurological disorders, such as autism spectrum disorder (ASD) and schizophrenia. However, we do not have any information about the exact mode of action, structural preferences or internal dynamics of the Shank proline-rich segment. The aim of this study was the biotechnological production of Shank3 proline-rich region segments (S3-PRO) for *in vitro* functional and structural investigation of its interaction with the EVH1 domain of Homer1 protein. After careful codon optimization, BL21(DE3) cells were able to express the protein constructs, which had excellent solubility, but were susceptible for proteolytic fragmentation. According to CD spectroscopy analysis and thermal shift assay, S3-PRO construct can be characterized by approximately 50% disordered, 20% turn and 25% beta sheet structural elements, and is largely temperature resistant. Preliminary interaction studies have shown that the strength and kinetics of Homer binding depends on the length of the S3-PRO constructs used. Our results demonstrate that recombinant bacterial expression of functional S3 PRO protein constructs is feasible. The expressed regions exhibit properties typical for disordered proteins. The length-dependence of the Homer interaction is largely unexpected and requires further investigations.

P-03-018
Caenorhabditis elegans strain expressing amyloid beta peptide as a model of fragile population in toxicology: behavioral and metabolic responses to atrazine exposure

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The use of *Caenorhabditis elegans* models in toxicology is well established and the availability of *C. elegans* strains representative of protein misfolding diseases allows to evaluate the effects of potentially harmful chemicals in a "fragile population", where

the presence of an underlying pathological condition could potentially amplify their toxicity. In this regard, we investigated whether the presence of amyloid β peptide, responsible for Alzheimer's disease, exacerbates the toxic effects of exposure to atrazine, an herbicide that may contaminate groundwater. The effect of the pollutant at different concentrations on unhealthy worms expressing amyloid β peptide and on the healthy counterparts are compared at different life stages of the nematodes. Larval growth, fertility, body length and width changes show that healthy worms are able to partially overcome the behavioral damage induced by atrazine exposure unlike the unhealthy ones. A targeted metabolomics approach is pivotal in highlighting changes in amino-acids, carbohydrates and energy metabolism. Tangible alterations can be observed already at day 1 of adulthood of the worms. Dose/response analysis and the metabolism of toxic agents are typically assessed using models that represent the healthy population. However, it's possible that a concentration that has no effect in a healthy individual could become toxic in the presence of an underlying pathological condition like Alzheimer's disease. Exposure to a seemingly "safe" concentration of pollutants in the healthy population could significantly harm patients with chronic diseases substantially altering their metabolism and disease progression.

P-03-019
Comparison of metabolomic information between dried blood spot and serum

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Dried blood spots (DBS) are used for the screening of neonatal diseases and monitoring adults suffering from certain diseases in clinical context. DBS is a self-sampling device which is less invasive and requires less sample than a blood test. They can be sent by mail to the hospital, allowing everyone to get access easily to biological analysis, even in remote area or for elderly patients with mobility issues. Their use in new contexts has been widespread: carrying out anti-doping tests, the research of biomarkers of galactosemia or detection of cancer. In this work, we propose to compare quantitative data and exploratory metabolomic data between DSB and serum. For quantitative data, we quantified 6 short-chain fatty acids (SCFA), 20 bile acids, 20 tryptophan intermediates and 8 organic acids from TCA cycle. Two trends emerge. The first one is that the majority of the serum information is found in DBS. The second one is that DBS brings complementary information not found in serum. Indeed, an overlay of the metabolic cards of serum and DBS highlights a wider metabolic coverage for DBS. These results make it possible to envisage the use of DBS in both quantitative and exploratory metabolomic analyses. However, building up a cohort can last several months or years, so it will be necessary to clearly define the impact of storage conditions (temperature, hygrometry and light exposure) as well as its lasting.

P-03-020
Proteome and metabolome remodeling in Caenorhabditis *elegans* strains expressing different isoforms of amyloidogenic human β2-microglobulin

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β2-microglobulin (β2-m), the light chain of the class I major histocompatibility complex, is a well-known amyloidogenic protein in humans. The wild type (WT) form of β2-m generates amyloid deposits in long term hemodialyzed patients, when its serum concentrations reach values higher than normal; otherwise, β2-m genetic variants, such as D76N β2-m, can cause amyloid deposition although plasma levels are within the normal range. Despite the progress achieved in elucidating the general mechanism of β2-m amyloidogenesis, a detailed understanding of proteotoxic pathways remains a challenging issue. We have therefore exploited two *Caenorhabditis elegans* (*C. elegans*) transgenic strains: the first one expressing human WT β2-m at high concentrations, mimicking the condition that underlies dialysis related amyloidosis and the other one expressing the D76N β2-m variant at lower concentrations [1]. Both strains exhibit pathological phenotypes and show a significant remodeling of proteome and metabolome profiles, being more pronounced in the presence of higher levels of WT β2-m. The organism proteostasis is challenged by the expression of β2-m, inducing higher levels of molecular chaperones and various proteins involved in protein degradation. A redox imbalance and a strong alteration in amino acids metabolism have emerged. Furthermore, alterations in oxidative phosphorylation, fatty acids degradation and Krebs cycle were observed, thus suggesting an impairment of the mitochondrial aerobic metabolism and a shift to anaerobic metabolism. This first characterization of proteomic and metabolomic alterations linked to the expression in *C. elegans* of proteins responsible for systemic amyloidosis in humans, provides important clues on the molecular basis of amyloid cytotoxicity. Furthermore, the exposure of worms to hypoxic condition reveals how lack of oxygen could enhance amyloid toxicity. Reference 1. Previously published in: Raimondi S et al. (2023) FASEB Bioadv 5:484–505.

P-03-021
Phospho-enrichment optimization of *Stenotrophomonas maltophilia* K279a

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Phosphorylation at serine, threonine and tyrosine in eukaryotes as a post-translational modification (PTM) is well-known as its essential role in regulatory and signaling functions. Reported phosphoproteomic analyses revealed that phosphorylation is dramatically lower in bacteria than in eukaryotes. Phosphorylation at Ser/Thr/Tyr affects metabolism development, pathogenicity, virulence, sporulation and antibiotic resistance. Histidine is also phosphorylated and even it is the most abundant phosphorylation in prokaryote, however, it is difficult to detect because of its reduced phosphohistidine half-life under the acidic pH conditions used in LC-MS/MS analysis. The characterisation of Ser/Thr/Tyr and His phosphorylations will improve our understanding of prokaryotic physiology. We have worked on the optimization of the phospho-enrichment of bacterial samples using three different methods. The bacterium used as a model is *Stenotrophomonas maltophilia* (strain K279a), a multidrug-resistant and opportunistic pathogen that results in nosocomial infections. The first method consisted in using commercial affinity enrichment protocols with TiO_2 columns, the second one was phosphopeptide preenrichment with calcium phosphate precipitation followed with TiO_2 columns and the third one in only calcium phosphate precipitation. We detected phosphopeptides after performing the first and third method but not after the second method. In this proof-of-concept study, we observed that calcium phosphate precipitation step alone, without further phosphopeptide enrichment, resulted in a higher number of detected phosphopeptides than using TiO_2 columns. In fact, the phospho-enrichment performed better with calcium phosphate precipitation than using TiO_2 columns in terms of the number of phospho-PSMs (3858 *vs* 905), phosphopeptides (563 *vs* 119) and phosphoproteins identified (289 *vs* 104). The optimization of this method will allow phosphoproteomic studies in these and related bacteria to better understand their physiology and metabolism.

P-03-022
Modeling ApoE/TREM2 complex using cross-linking mass spectrometry and protein-protein docking

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Alzheimer's disease (AD) is a devastating neurodegenerative disease with few disease-modifying therapies. Human genetics studies have identified two major genetic risk factors for late-onset AD–apolipoprotein E (*APOE*) and triggering receptor expressed on myeloid cells 2 (*TREM2*). ApoE binds to the low-density lipoprotein receptor (LDLR) to facilitate the uptake of ApoE-lipoprotein particles, while TREM2 is a cell surface receptor expressed on microglia in the brain. The activation of TREM2 is essential for microglial survival, proliferation, and phagocytosis in order to carry out their protective functions against AD pathology. Recently, several studies have shown the activation of TREM2 signaling through the direct interaction between TREM2 and ApoE. In addition to the important role of ApoE/TREM2 interaction in AD pathogenesis, this interaction has been shown to induce immunosuppression of neutrophils within the tumor microenvironment. Therefore, a detailed understanding of this interaction could lead to novel therapeutic strategies targeting ApoE and TREM2. Although biophysical studies have been carried out to characterize this interaction, there is still no detailed structural model. Here we carried out chemical cross-linking of the ApoE/TREM2 complex followed by bottom-up mass spectrometry analysis to identify inter-protein cross-links, which were used as distance restraints to guide protein-protein docking using Haddock. We obtained the first structure model of the ApoE/TREM2 complex and identified key residues important for the binding interaction. We believe this structure model will facilitate future research of designing probe molecules to better understand the physiological functions of the ApoE/TREM2 interaction.

P-03-023
Prolonged thermal stress leads to different metabolic adaptations in *Stylophora pistillata* and *Pocillopora damicornis* coral species

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Coral bleaching occurs when the symbiotic relationship between the corals and their algal endosymbionts (zooxanthellae) is disrupted. This phenomenon, which is intensifying worldwide, leads to the loss of color and the death of coral reefs. This event may be triggered by several stresses, such as UV radiation and low salinity, although the seawater temperature increase seems to be the main reason. In this work, we evaluated the metabolic effect of thermal stress in two coral species: *Stylophora pistillata* (SP) and *Pocillopora damicornis* (PD). Preliminary metabolomics analyses aimed to compare the basal metabolism of the two species in normal growth conditions (at 2526°C) reveal significant differences: glycolysis, TCA cycle and pyrimidine metabolism are upregulated in SP compared to PD, while in PD there is an increase of metabolites implicated in the pentose phosphate pathway (PPP) and purine metabolism. Thermal stress conditions were replicated by growing the corals in tanks containing seawater at a temperature of around 31°C and collecting them at different times (24, 72 and 240 h). In both species, the shortest thermal expositions (24 and 72 h) result in a slighter metabolic rewiring compared to control than the one showed by the longest exposition (240 h). However, the metabolic adaptation to prolonged thermal stress (240 h) is different in the two species. In SP corals, thermal stress leads to an upregulation of pyrimidine and amino-sugar metabolism, and a down-regulation of PPP, glycolysis and

TCA cycle. Conversely, in PD samples, we demonstrated an increase in one-carbon metabolism, ammonia recycling and amino acids metabolism, while a reduction in the nucleotide metabolism is observed. In conclusion, our data provide insights into the different adaptation strategies to prolonged thermal stress that corals develop and pave the way to the use of the metabolomics tool for the identification of early coral bleaching biomarkers. *The authors marked with an asterisk equally contributed to the work.

P-03-024
The recombinant human Paraoxonase 2: optimization of protein purification procedure, stabilization and proteomics approaches

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Paraoxonase 2 (PON2) is the oldest and least studied member of a small family of human enzymes endowed with arylesterase and lactonase activity[1]. PON2 has a major role in the development of diseases associated with high levels of ROS, such as cancer, cardiovascular diseases, neurodegeneration, and diabetes, thus it represents a prognostic marker and a pharmacological target[2]. Some post-translational modifications (PTMs) cluster nearby two polymorphic sites (SNPs) at opposite sides of the molecule, impacting its catalytic activity[3].The link between those PTMs, the consequent modulation of catalytic activity and the SNPs can most likely only be fully understood if a high amount of stable PON2 protein is available to perform more in-depth biochemical studies. Thus, in the present study we focused on improving the procedure of recombinant PON2 (rPON2) purification from *E. coli* inclusion bodies, to obtain a protein more suitable for biochemical studies. We tested several compounds that might help to stabilise the active monomeric form of the enzyme. The following order of stabilizing activity was detected: Trehalose > Triton X-100 > Ethylene Glycol > Glycerol > NaCl. The active enzyme was used to determine the catalytic parameters towards the substrate 3-Oxo-dodecanoyl-homoserine lactone (3Oxo-C12-HSL). The enzyme was also tested for its ability to interfere with the biofilm formation of *Pseudomonas aeruginosa* (PAO1) and it was found more efficient than reported before. Finally, we used the purified rPON2 to detect by direct molecular fishing (DMF) method new putative PON2 interactors from soluble extracts of HeLa cells. The identified interactors will be discussed considering of PON2 functions and available literature. References: 1. Carusone, T.M. et al. (2020) Cell Death Dis 11, 324. 2. Mandrich L et al. (2015) PLOS One 10(12) e0144579. 3. Manco G. et al.(2021) Antioxidants 10(2):256 *The authors marked with an asterisk equally contributed to the work.

P-03-025
Degradomics study of the neprilysin protease: novel targets, mitochondrial localization, and a possible role in neurodegeneration

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Neprilysin is an endopeptidase ubiquitously distributed. It exists in both transmembrane and soluble catalytically active forms, and it is responsible for the cleavage of several substrates: angiotensin, bradykinin, substance P, glucagon, insulin β-chain, oxytocin, enkephalins, and amyloid-beta. Recently, by using the mitochondrial dimethylation-TAILS (terminal amine isotopic labeling of substrates) degradomics approach in a cellular model of dopamine (DA) dyshomeostasis in neuroblastoma SH-SY5Y cells, we demonstrated that neprilysin is also a candidate protease involved in mitochondrial dysfunction related to Parkinson's disease (PD) [previously published in: Lualdi M et al. (2019) Front Aging Neurosci 2019, 11, 195]. To gain more insight into the role of neprilysin as a mitochondria-localized protease, we first used mitochondrial sub-fractionation to assess its presence and localization inside the organelle. Neprilysin resulted to be a soluble mitochondrial protein. Moreover, a marked co-localization between neprilysin and mitochondria was observed by IF, which was significantly increased by the induction of DA imbalance in SH-SY5Y cells. Then, to obtain the complete repertoire of neprilysin substrates, we applied the dimethylation-TAILS approach on the total proteome of SH-SY5Y cells. A list of 155 candidate substrates emerged; 20 of them resulted to be mitochondria-associated proteins, including some ribosomal proteins, heat shock proteins, membrane transporters, and metabolic enzymes. The functional enrichment analysis highlighted "protein translation", "ribosomal function", "processing of RNAs and proteins", and "neurodegeneration" as significantly enriched pathways in the whole substrates list. In conclusion, we demonstrated that neprilysin exists as a mitochondrial protease and may play a role in mitochondrial dysfunction linked to DA imbalance in PD.

P-03-026
Target metabolomics analysis of plant origin objects: opportunity of NMR spectroscopy

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Various samples of living systems can be objects for metabolomic analysis. However, such objects are completely different in terms of metabolites number. For example, the plant metabolome contains more than 200 000 metabolites. Animals and humans have many fewer. Therefore, targeted metabolomic analysis of plant objects is a more complex task. In addition, it is very important to examine primary substances in metabolomic studies. Most substances of plant origin are glycosides. Glycosidic bonds can easily be destroyed during sample preparation or analysis.

Therefore, minimal impact on the object during sample preparation and non-destructive methods of analysis are necessary. Mass-spectrometry and NMR spectroscopy are the main instrumental methods of analysis for profiling samples of human, animal and plant origin. Both methods have their advantages and disadvantages. But NMR spectroscopy is non-destructive method and needs simple sample preparation. In addition, quantitative studies using NMR spectroscopy do not require authentic standard samples or their isotopic analogues. We have developed and validated various techniques for targeted metabolomic analysis of biological origin samples using NMR spectroscopy. For example, determination of anthraquinone derivatives in *Rubia tinctorum* L. roots and rhizomes extracts analyzing aromatic region of the ^{1}H NMR spectra (6.50–8.00 ppm); determination of flavonolignans in *Silybum marianum* L. seeds extracts analyzing 11.50–12.50 ppm region of the ^{1}H NMR spectra; flavonoids in *Ginkgo biloba* L. leaves extracts analyzing 12.40–13.00 region of the ^{1}H NMR spectra. These methods require minimal sample preparation, which includes sample grinding, extraction by deuterated solvent, and signal selection for quantitative measurements. The deuterated solvent residual protons signal can also be used for this purpose. *The authors marked with an asterisk equally contributed to the work.

P-03-027
Lipidomic changes in human plasma differentiating schizophrenia from affective disorders

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Schizophrenia (Sch) and depression (Dep) are severe mental disorders characterized by unclear pathogenesis and partially overlapping symptoms. Recent research has highlighted alterations in the lipidome composition of blood plasma associated with these disorders. However, the specificity of lipidomic changes between the diseases remains unexplored. To address this gap, we analyzed blood plasma lipids from two independent cohorts collected at urban locations, comprising 280 participants (85 Sch, 35 Dep, 160 controls (Cnt)) and 297 participants (100 Sch, 85 Dep, 112 Cnt), respectively. Using direct infusion mass spectrometry, we detected 155 lipid species across 13 lipid classes. Of these, 138 lipids showing significant alterations in either Sch or Dep across both cohorts underwent clustering analysis. The analysis identified two lipid groups: "shared," indicating consistent changes in both Sch and Dep, and "distinctive," where lipid alterations differed between the disorders ($p < 0.005$). Notably, distinctive lipid clusters included triacylglycerols (TG) and phosphatidylcholines (PC), crucial components of plasma lipoproteins. Specifically, four polyunsaturated fatty acids (PUFAs)-containing TG and one PC were reduced in Dep compared to Sch, with this difference correlating well between cohorts ($r = 0.64$, $p < 0.05$). This finding suggests a heightened inflammatory process in depression, leading to the oxidation and destruction of certain PUFAs-containing lipids in lipoproteins. Then, we extended our analysis to include other schizophrenia spectrum disorders (schizoaffective and schizotypal disorders) and conducted principal component analysis (PCA) using the five Sch-Dep distinctive lipids. The PCA results demonstrated a clear separation between affective and schizophrenia-like disorders, mirroring symptom distribution. Our findings offer potential insights into the molecular underpinnings of Sch and Dep and may contribute to improving the diagnostics of these disorders.

P-03-028

Abstract moved to Talks.

P-03-029
Combined targeted and non-targeted metabolomics-based approaches for analyzing the profile of oxylipins in the ATP-stimulated astrocytes

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Over the last decade, we observed a significant breakthrough in the understanding molecular mechanisms of neuropathological processes. It largely arose from studying numerous metabolites by the targeted and non-targeted (metabolic fingerprinting) metabolomics-based approaches. The ATP-dependent activation of the astrocyte P2-purinergic receptors is crucial for inflammation and the development of neuropathologies. Oxylipins, the oxidized derivatives of polyunsaturated fatty acids (PUFAs), mediate inflammatory processes. However, the profile of oxylipins upon the ATP-induced cell activation has not been analyzed yet. Employing the non-targeted (GC/MS) and targeted (UPLC-MS/MS) metabolomics-based approaches, we obtained the oxylipin profiles after the 15-min stimulation with 100 μM ATP (the acute response) and evaluated the ability of metformin, considered as a potential anti-inflammatory drug for treating CNS pathologies, to alter the profile of oxylipins. We assessed 50 compounds involved in the enzymatic pathways of oxylipin biosynthesis dependent on the lipoxygenase (LOX), cyclooxygenase (COX), and epoxygenase (CYP). ATP induced a significant activation of the COX pathway reflected by elevated levels of TXB2, PGD2, 12-HHT, but not of PGE2, indicating the activation of the thromboxane synthase and PGD synthase upon P2 receptor stimulation. Treating cells with metformin prior to the stimulation with ATP had no effect on TXB2 and PGD2, whereas it promoted the synthesis of 12-HHT and 11-HETE metabolites. In contrast to the derivatives of arachidonic acid, the profile of oxylipins, the metabolites of other PUFAs, was not altered. The ability of metformin to induce the synthesis of 12-HHT,an endogenous ligand of the BLT2 receptor, reveals novel mechanisms underlying its anti-inflammatory activity, although the biological role of 12-HHT in the CNS is yet to be clarified. This work was supported by the RUDN University Scientific Projects Grant System №214853-2-000. *The authors marked with an asterisk equally contributed to the work.

P-03-030
Modulating homeostasis: clozapine's influence on neuronal signaling pathways

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Numerous clinical studies have indicated that clozapine is more effective in treatment of schizophrenia than other available antipsychotic drugs and that clozapine is the only effective medication in treatment-resistant schizophrenia. However, clozapine's usage is limited due to its severe side effects, and despite over five decades of research into its mechanism of action, the reasons behind its unique effectiveness remain unclear. This study adopts an unbiased proteomic approach to identify key regulatory pathways influenced by antipsychotic treatments. Human neuronal cell line LUHMES was treated with antipsychotic drugs clozapine and risperidone, which is the most commonly prescribed antipsychotic drug. Proteomic alterations triggered by clozapine were compared with a control and risperidone to unveil unique effects of clozapine treatment. Mass spectrometry based proteomic analysis enabled identification of over 5000 proteins including neuron specific transcription factors and synaptic proteins. Differential expression analysis revealed that clozapine treatment induces changes in protein metabolism and reduction of activity of insulin receptor signalling pathways and P2Y purigenic receptor signaling pathway.

P-03-031
A proteomic approach identified TFEB as a key player in the neuroprotective action of novel CB2R bitopic ligand FD22a

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Neurodegenerative diseases (NDDs) are progressive multifactorial disorders of the nervous system sharing common pathogenic features, including intracellular misfolded protein aggregation, mitochondrial deficit, and inflammation. Taking into consideration the multifaceted nature of NDDs, development of multitarget-directed ligands (MTDLs) has evolved as an attractive therapeutic strategy. Compounds that target the cannabinoid receptor type II (CB2R) are rapidly emerging as novel effective MTDLs against common NDDs, such as Alzheimer's disease (AD). We recently developed the first CB2R bitopic/dualsteric ligand, namely FD22a, which revealed the ability to induce neuroprotection with fewer side effects. To explore the potential of FD22a as multitarget neuroprotective drug, we investigated here its ability to prevent the toxic effect of β-amyloid ($A\beta_{25\text{-}35}$ peptide) on human cellular models of neurodegeneration, such as microglia (HMC3) and glioblastoma (U87MG) cell lines. Our results displayed that FD22a efficiently prevented $A\beta_{25\text{-}35}$ cytotoxic and pro-inflammatory effects in both cell lines, and counteracted β-amyloid induced depression of autophagy in U87MG cells. A quantitative proteomic analysis of U87MG cells was performed. Sixty-seven and 41 protein spots were differentially expressed by the treatment with FD22a and $A\beta_{25\text{-}35}$, respectively. whereas pretreatment with FD22a reduced protein spot changes, as suggested by FD22a + $A\beta_{25\text{-}35}$ vs $A\beta_{25\text{-}35}$ comparison. In particular, we found 20 protein spots modified in FD22a + $A\beta_{25\text{-}35}$ compared to $A\beta_{25\text{-}35}$. Protein spots of interest were identified by LC/MS/MS. Ingenuity pathway analysis (IPA) of differentially expressed proteins revealed that FD22a was able to potently stimulate the autophagy–lysosomal pathway (ALP) by activating its master transcriptional regulator TFEB, ultimately increasing the potential of this novel CB2R bitopic/dualsteric ligand as a multitarget neuroprotective drug. *The authors marked with an asterisk equally contributed to the work.

P-03-032
SARS-CoV-2 spike glycoprotein role investigation in the shift from aerobic to anaerobic metabolism

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SARS-CoV-2 leads to coronavirus disease 2019 (Covid19). Among the 4 structural proteins, the Spike glycoprotein is mainly responsible for viral attachment by recognizing the specific receptor present on the host cell surface, leading to viral entry into the host cells. Beyond receptor recognition, other roles of Spike have been reported, been related to pro-inflammatory mediators release, but also to a leukocyte adhesion function, barrier dysfunction [Previously published in: Biering SB, et al. Nat Commun. 2022 Dec 9;13(1):7630] and endothelial injury [Previously published in: Bhargavan B, et al. Int J Mol Sci. 2023 Aug 9;24 (16):12585]. In this study, we aim to better characterize the role and protein partners of Spike in cellular metabolism. We performed an AP-MS experiment on HEK293 cell lines and compared the interactome obtained with other Spike interactomes previously investigated in different cell lines [Previously published in: Iacobucci I, el al. Front Mol Biosci. 2022 Sep 26;9:975570], to highlight common processes in which the protein is involved. 58 shared putative interactors were identified belonging to HEK293 interactome and at least one other of the three used for the comparison. The shared putative partners were classified according to their function, and, of these proteins, a large number fall within the energetic metabolism process. Therefore, we further investigate the interaction between Spike and the lactate dehydrogenase, B isoform (LDHB), since it was a partner shared by all the interactomes. The interaction between the two proteins was validated by co-immunoprecipitation and immunofluorescence experiments. Moreover, we found that Spike inhibits LDHB catalytic activity with an increasing of lactate concentration in vivo in cells transfected with the S1 subunit of the protein. For the first time a hypothesis of the biochemical mechanism exploited by Spike to induce the shift from aerobic to anaerobic energetic metabolism has been proposed.

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P-03-033
Discovering a novel function of protein kinase CK2: regulation of extracellular matrix composition

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The protein kinase CK2 is widely recognized for its involvement in diverse cellular processes, but its role in controlling the cell secretome and extracellular matrix (ECM) composition remains relatively unexplored. This study uses a triple SILAC quantitative proteomic approach to investigate how the absence of CK2 affects the cell secretome in C2C12 cells. We compared the secretome composition between WT C2C12 cells and C2C12 cells knocked out for the two catalytic subunits (CK2α and CK2α') or the regulatory subunit (CK2β) of the protein kinase CK2. Analysis of proteins with significant expression changes (fold change ≥1.5 or ≤−1.5) revealed that CK2 plays a critical role in cell secretion and ECM production. Some of the proteomic results were confirmed by western blotting and replicated with a CK2-specific inhibitor. Functional experiments showed that CK2-dependent changes in the secretome significantly affect cell migration, as evidenced by impaired migration properties in wild-type C2C12 cells exposed to conditioned medium from CK2-deficient cells. This phenomenon was further validated in a breast cancer cell model. MDA-MB-231 cells incubated with conditioned medium from MDA-MB-231 cell lines depleted of CK2α or CK2α' did indeed show impaired cell migration, highlighting the importance of targeting CK2 in pharmacological cancer treatments to affect not only intracellular CK2 signaling but also the extracellular environment. Overall, this research contributes to our understanding of the involvement of CK2 in ECM generation and adds a new puzzle to the complete understanding of the cellular functions of CK2.

P-03-034
Enhanced untargeted metabolomics in a respiratory-deficient yeast cell model through LC-coupled high-resolution mass spectrometry

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Untargeted metabolomics analysis allows the comprehensive analysis of all measured compounds in a sample and, in principle, the determination of the metabotype (metabolic phenotype) of a biological system. Despite the increased availability of high-resolution mass spectrometry (HRMS) analysis systems, structure identification of all detected compounds is still a challenge. We used "petite yeasts" (ρ0), characterized by a complete lack of mitochondrial DNA (mtDNA), as a model to study mitochondrial dysfunction, which has a key role in the molecular etiology of human diseases. To acquire a comprehensive knowledge of metabolic reprogramming due to mtDNA depletion, we performed a comparative untargeted metabolomics study of ρ0 and ρ+ *Saccharomyces cerevisiae* prototrophic cells using a UHPLC coupled to Orbitrap Fusion™ Tribrid™ HRMS platform, which allows MS^n analysis of crucial importance for the determination of the greatest possible number of metabolites simultaneously. We used a single extraction method, aimed at obtaining as much information as possible from our samples without performing specific extractions for each class of metabolites. By implementing HILIC chromatography and RP C18, both in positive and negative mode, for each sample analysis, we determined roughly 3000 molecular features after background subtraction. Through Fragment Ion Search (FISh) tool, which provides fast screening of structurally similar compounds based on the fragmentation pattern of the parent compound acquired either by theoretical fragment prediction or experimental MSn data, we exceeded the annotation threshold of 10% by 4.5% additional structural identifications. We obtained new insights into the metabolic reprogramming of respiratory-deficient yeast cells. This work was performed at CNRBiOmics Center of Excellence of ELIXIR IIB Infrastructure at IBIOM-CNR, Bari, Italy, and funded by ELIXIR Infrastructure. F.M. is funded by ELIXIRxNextGenerationIT PNRR Project (IR0000010).

P-03-035
Towards the *in situ* localization of glycopeptides by MALDI imaging: a proof of concept in thyroid oncology

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Glycosylation has been added as a new hallmark of cancer. Glycoconjugates indeed mediate cell surfaces features and are responsible for the plasticity of the protein functions. To address their complexity, the development of accurate methods for their characterization and quantification becomes crucial. Until now, mass spectrometry imaging technologies have allowed direct spatial resolution of glycan patterns in many clinical samples for oncology as formalin-fixed paraffin-embedded (FFPE) tissue blocks and microarrays. However, no MS proteomic strategy able to simultaneously explore proteomic hallmarks and signatures related to N-glycopeptides has been applied. Thus, an innovative sample preparation protocol has been designed and applied on FFPE Thyroid cancer tissues finalized to gain new insights for the direct *in situ* recognition and mapping of glycopeptides. Enzymes and matrix deposition was performed with optimized methods based on HTX TM-Sprayer. 6-Aza-2-thiothymine matrix solution was used. PNGase F was deposited for the deglycosylation step after trypsin digestion. MALDI-MS images were acquired using a rapifleX MALDI Tissuetyper mass spectrometer. Peptide identification was performed on MALDI matrix extracts using a data-independent acquisition-parallel accumulation serial fragmentation mass spectrometry strategy by trapped ion mobility separation. A considerable increase in the

sensitivity of MALDI images without losing spatial distribution, and in the number of the deglycosylated identified peptides in PNGase treated samples are like to confirm the feasibility of the approach. Some of the candidate identified glycopeptides are interestingly recognized also in MALDI-MS images suggesting a possible role related to their specific localization. The development of tailored strategies in IMS can pay the way to the understanding of glycosylation-regulated biosystems holding a promise for the study of challenging malignancies.

P-03-036
Tenocytes exposed to IL-1 beta undergo proteomic alterations that impact molecular and metabolic pathways involved in tendon homeostasis

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Interleukin-1 beta (IL-1β) is involved in the onset and progression of tendinopathy, as well as in the modulation of tendon repair and regeneration. However, the mechanisms underlying the effects of this cytokine on tendon tissue remain unclear[1]. In this study, in order to provide a comprehensive assessment of tenocyte cellular activities affected by IL-1β, a quantitative label-free proteomic analysis has been performed. Tenocytes represent approximately 97% of tendon cell populations. They are mainly dedicated to extracellular matrix turnover and control of tissue homeostasis. Stressors impacting their metabolism and functions predispose to tendon lesions and compromise the regenerative processes[2]. Results revealed 199 differential abundant proteins. Most of them play a role in various pathways already linked to tendinopathy including fibrillogenesis, ECM organization and degradations, oxidative stress, hypoxia response and apoptosis. Furthermore, protein enrichment analysis showed that IL-1β is able to exert in tenocytes changes in proteins involved in ferroptosis, cytoskeleton organization, fatty acid and glucose metabolism. To confirm and validate proteomic results, qPCR and western blotting have been done, while the putative alteration of fatty acid metabolism has been explored by using lipidomic analysis. This study could clarify the role of IL-1β in the disturbance of tenocyte homeostasis, elucidating some molecular processes underlying the etiopathogenesis of tendon disorders. The cellular pathways involving the deregulated proteins may represent potential targets for new therapeutic approaches. References: 1. Jiang L et al. (2023) Open Life Sci 18: 20220729; 2. Mohindra R et al. (2022) 390: 131–140.

P-03-037
Plasma fractionation unused intermediates proteome as a potential source of new therapies for ultra-rare diseases

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Human plasma, with its complex proteome, is a valuable source for developing therapeutics to address rare diseases caused by protein deficiencies[1]. Despite the complexity, only around 20 plasma protein therapeutics are utilized for treating life-threatening conditions[1]. Current plasma fractionation processes generate significant unused intermediates, providing an opportunity for novel therapy development. At Kedrion's manufacturing sites (Bolognana, IT; Melville, US; Godollo, HU; Elstree, UK), diverse unused intermediates are generated, recently characterized in the case of the Bolognana plant[2]. This project aims to map their protein content for discovering therapies for rare diseases without diverting plasma from other manufacturing processes. In particular the main purpose is to detect and characterize a specific protein among unused intermediates, namely plasminogen (PLG) which plays a crucial role in fibrinolysis, wound healing, and other processes[3]. Main PLG indication is related to protein Type I deficiency, for which a therapy (derived from whole plasma) is already available. The use of waste fractionation intermediates could expand its availability for alternative conditions, such as diabetic wounds and burns[3]. To achieve this goal, the following workflow is being carried out: proteomic analysis on unused intermediates to map PLG presence, followed by validation by western blot and antigen determination techniques. Selection of the proper intermediate and solubilization conditions, including buffer and pH, will be then based on PLG characterization in terms of antigen level, protein fragmentation pattern, and functionality by chromogenic assay. This will be the initial step for the development of a new PLG experimental prototype from unused fractions. References: 1. Strengers PF (2023) Transfus Med Hemother, 50(2):p. 116–122. 2. Zanardi A, Nardini I et al (2024) Commun Biol.; 7(1):140. 3. Al Kayal T et al (2022) Pharmaceutics 14(2):251.

P-03-038
Development of MRM-based assay for the absolute quantitation of eIF4E isoforms in potato leaf tissue

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Potato and other *Solanaceae* have a small family of eukaryotic translation initiation factors 4E (eIF4E) consisting of four isoforms: eIF4E-1 and its paralog eIF4E-2, eIF(iso)4E and nCBP (novel cap-binding protein). In addition to their cap-binding function eIF4E isoforms also act as susceptibility factors for potyviruses. The purpose of this work is to develop a method for quantifying eIF4E isoforms in potato lysates to assess involvement of different isoforms in adaptation mechanisms to various stress conditions. First, we performed bioinformatic analysis of the amino acid sequences of potato eIF4E, revealed and then synthesized several unique tryptic peptides for each isoform. Then, we optimized a cytoplasmic protein isolation protocol from potato leaf tissue based on differential centrifugation. To quantify potato eIF4Es we developed a mass spectrometry-based protocol for detecting isoforms in the proteome isolated from potato *S. tuberosum* leaves using a MRM (multiple reactions monitoring) approach. The choosen technique allowed us to extract selected peptides and to estimate their quantities by using synthetic peptides as an internal standard. The isolated proteins were digested with trypsin, the resulting peptides were analyzed by HPLC-MS. To obtain the abundances of all four eIF4E isoforms, received data was processed using Skyline (MacCoss Lab Software). The ratio of MRM peak areas between control and spiked samples was used to estimate the absolute abundance of each isoform. As a result, we report that our method allows us to detect all four eIF4Es from the most abundant eIF4E-1 to the least nCBP. The study is supported by the RSF project №21-76-10050.

Long ncRNA and microRNA Networks

P-04-001
Investigation of mir-128 expression at the tissue level in bladder cancer patients in Türkiye

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Cancer is the second leading cause of death after cardiovascular disease, and among cancers, bladder cancer (BC) is the 10th leading cause. Studies suggest that microRNAs (miRNAs) may serve as important biomarkers for BC, potentially opening new horizons in cancer diagnosis and treatment. Based on this rationale, our aim is to investigate the expression of miRNA-128 (mir-128) in patients with BC and to compare its levels in tumour and normal tissues. The aim is to elucidate the potential significance of mir-128 in the diagnosis and treatment of BC. The study included 50 patients diagnosed with non-muscle invasive BC (NMIBC) between May 2022 and April 2023. Tumour and non-tumour tissue samples collected during transurethral bladder tumour resection (TURBT) procedure were evaluated. mir-128 expression was measured by simultaneous qRT-PCR. In tissue samples from BC patients, mir-128 expression was not significantly different in tumour tissue compared to non-tumour tissue ($p = 0.281$). In addition, mir-128 expression levels were found to be significantly higher in patients with low-grade tumours compared to patients with high-grade tumours ($p = 0.005$). In relation to mir-128 expression levels, there was no statistically significant difference in other significant difference in other pathological parameters, including tumour stage (Ta vs T1), presence of carcinoma *in situ* (CIS) and pathological variant. ROC analysis shows that when the cut-off point for the mir-128 value was 0.86, the sensitivity and specificity for indicating high-grade tumour in NMIBC were 55% and 95%, respectively (area under the curve 0.735). In this study, mir-128 was evaluated and expressed at the tissue level and correlated with some histopathological parameters of BC. Despite the low sensitivity of this tissue marker, the data obtained from this study may help in the development of urinary biomarkers in the future.

P-04-002
miR-27b-5p: a novel player in the biology of head and neck squamous cell carcinoma

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Head and neck squamous cell carcinoma (HNSCC) is one of the most common and fatal cancers worldwide. Recently, non-coding RNAs were described as molecules with therapeutic or diagnostic potential in different malignancies. One of them, miR-27b-5p, is a mature strand of a well-known epithelial-to-mesenchymal transition (EMT) modulator whose aberrant expression was correlated with tumor development in many cancers. Expression and patients' clinicopathological data were downloaded from the UCSC, the cBioPortal, and the GEO database. The miR-27b-5p level, survival curves, patients' immune profiles, and gene enrichment were studied. Said microRNA (miRNA) potential targets were investigated utilizing online databases and tools. Finally, miR-27b-5p expression was studied in FFPE tissue samples and cancer cell lines. Two cell lines were modified with a miR-27b expressing plasmid or control one and subjected to functional tests. Irradiation and chemotherapy response were assessed with apoptosis and proliferation assay, respectively. In patients, miR-27b-5p higher expression was linked with absent perineural invasion, HPV(+) status, and better overall survival. Analysis of patients' immune profiles indicated substantial differences in tumor infiltration degree, the composition of the infiltrating fraction, and other immune features. Low miRNA levels were associated with overexpression of genes implicated in processes promoting cancer growth: EMT, angiogenesis, or hypoxia. Induced overexpression of miR-27b in cancer cell lines negatively affected their growth and migration ability. Moreover, it was linked with a lower proliferation rate after the cisplatin administration and, interestingly, was also associated with a worse response to applied irradiation and doxorubicin therapy. Based on the above data, we emphasize the complex and relevant impact of miR-27b-5p on HNSCC biology. This research was supported by the National Science Centre, Poland, grant: 2021/41/N/NZ5/02966.

P-04-003
Unveiling small noncoding RNA dynamics and their impact in a model of cardiac organoid

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Heart failure (HF) involves substantial changes in cardiac structure and function, including hypertrophy, sarcomere assembly alterations, cellular proteostasis disruptions, and a metabolic shift due to fetal cardiac gene reactivation. Intracellular signaling and transcriptional mediators play crucial roles. Next-generation sequencing reveals numerous noncoding RNAs (ncRNAs) as epigenetic regulators. Our study explores the roles of these ncRNAs in HF using an *in vitro* disease model. Human induced pluripotent stem cells (iPSCs) were differentiated into self-assembling cardiac organoids and exposed to endothelin-1 (ET1) to induce HF-like changes. Our study focused on ncRNAs, particularly microRNAs (miRNAs) and tRNA fragments (tRFs), using paired-sequencing analysis. ET1 treatment resulted in a significant downregulation of various miRNAs (e.g. hsa-miR-145-5p, hsa-miR-30c-5p, hsa-miR-455-3p) and an upregulation of many others including hsa-miR-129-5p and hsa-miR-92b-5p. The downregulated miRNAs play pivotal roles in endothelial cell survival, apoptosis inhibition, and cardiac/endothelial cell proliferation, while the upregulated ones serve as markers in diverse cardiovascular pathologies. Panther DB statistical overrepresentation analysis of the genes regulated by these miRNAs underscored their significant correlation with these molecular pathways. Notably, regarding tRNA fragments (tRFs), a novel class of RNA implicated in biological functions in different pathological contexts, including cardiac hypertrophy, organoids exhibited modified expression of several tRFs after ET1 treatment. Sequencing data were also processed using MINTmap, a software package that was developed specifically for the quick, deterministic, and exhaustive identification of tRFs in short RNA-seq datasets. Currently ongoing in silico and in vitro analyses aim to unravel and validate the roles of these ncRNAs in molecular changes related to HF. Supported by INRC and Fondazione Carisbo.

P-04-004
circ-BCL2L12-92, a novel circular RNA derived from BCL2L12, is able to regulate the mRNA levels of its parental gene

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Circular RNAs (circRNAs) have emerged as a class of non-coding RNAs implicated in diverse regulatory processes, including microRNA (miRNA) sponging and modulation of gene expression. In this study, we focus on circ-BCL2L12-92, a novel circRNA originating from the BCL2L12 gene, which encodes a proline-rich protein with anti-apoptotic properties. After identifying a plethora of novel BCL2L12 circRNAs using an innovative method based on long-read sequencing, we employed a comprehensive experimental approach to elucidate the functional significance of circ-BCL2L12-92 in colorectal cancer (CRC). Utilizing HCT 116 CRC cells, we performed RNA extraction followed by RNase R treatment to enrich circular transcripts. Subsequent reverse transcription and semi-nested quantitative PCR (qPCR), targeting the back-splice junction (BSJ) of circ-BCL2L12-92, confirmed its presence in CRC cells. Bioinformatics analysis was employed to predict potential miRNA binding sites on circ-BCL2L12-92, common with BCL2L12 mRNA. Furthermore, we constructed a reporter plasmid comprising the BSJ of interest and designed a siRNA targeting this BSJ. A dual luciferase assay confirmed the efficient binding of the designed siRNA to the BSJ of circ-BCL2L12-92 while avoiding the BCL2L12 3′-untranslated region (3′-UTR). siRNA transfection, total RNA extraction, reverse transcription, and qPCR were conducted to evaluate the

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downstream effects of circ-BCL2L12-92 level manipulation. Our results demonstrate that downregulation of circ-BCL2L12-92 (~75%) led to a circa 50% reduction in BCL2L12 mRNA expression, revealing the role of circ-BCL2L12-92 in regulating the expression of its gene of origin. These findings underscore the importance of elucidating circRNA-mediated regulatory mechanisms, particularly in the context of apoptosis-related genes. Further exploration of circ-BCL2L12-92 and its interactions may yield insights into novel therapeutic strategies targeting apoptotic pathways in CRC and beyond.

P-04-005
Non-invasive detection of markers of embryo compatibility and maternal readiness in the IVF process

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Infertility currently affects 17.5% of the population. It is estimated that there will be a 2-fold increase by 2050. The success rate of *in vitro* fertilization, despite progress, cannot overcome the threshold of 40%. Therefore, it is necessary to develop diagnostic procedures that would rapidly increase the sensitivity of the test of maternal readiness and compatibility of the embryo with the mother. Short non-coding RNAs (ncRNAs) prove to be suitable adepts for their analysis in maternal blood serum and in the waste material of IVF embryo culture. Our selected ncRNAs (miR-16-5p, -92a-3p; piR-28263, -18682) in combination with other piRNAs have proven 95% sensitivity, 100% specificity, and 86% accuracy in predicting the success of the IVF process. Analyzing the expression of free ncRNAs is currently time, instrumentally, and financially demanding, and therefore we propose an ON-OFF-ON fluorescence assay based on the principle of biometal coordination compounds with the dipyridophenazine ligand. Significant autofluorescence of complexes is quenched by binding to ssDNA primer. The subsequent binding of complementary target ncRNA and the formation of the ssDNA-ncRNA hybrid has a lower affinity to the complex, therefore the complex is released from the hybrid, and its fluorescence increases. Non-complementary ncRNA with ssDNA primer should not form a stable hybrid structure which does not lead to the recovery of the fluorescence intensity. Our preliminary results show sufficient selectivity of ncRNA detection in relatively small sample volumes. Although further studies are required, this approach has a high potential in rapid diagnostics, so to speak "on the table" and without the need for RNA isolation, and its expensive and complicated determination. This work was supported by APVV-22-0357.

P-04-006
Targeting the MALAT1 long non-coding RNA with an LNA-modified deoxyribozyme

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While 75% of the human genome is non-coding (i.e. transcribed to RNAs but not translated), most loss-of-function studies and pharmacological treatments rely on targeting proteins. In contrast, the RNA world (especially non-coding RNAs) remains largely unexplored. Given the increasing roles that non-coding RNAs are playing in human disease, we envision them as promising targets for loss-of-function and pharmacological strategies. Antisense oligonucleotides targeting mRNAs have been developed, but they require additional mechanisms in place (such as RISC) to be effective, hence alternative approaches are required. Here we report the design and use of deoxyribozymes (or DNAzymes), which are artificial single-stranded DNA oligonucleotides with autonomous catalytic activity that can be designed to recognize and cleave any target RNA by Watson-Crick interactions. With a specific type of DNAzyme (named 10–23), we aimed to specifically degrade the long non-coding RNA MALAT1, one of the most over-expressed transcripts in human cancer cells. *In vitro* RNA-cleaving assays were performed at 37°C against a 20-nt segment of MALAT1 in single-turnover conditions (DNAzyme:RNA = 1:10). We used different concentrations of the required divalent metal cofactor, Mg^{2+} and Ca^{2+}. The reaction rate constant (k_{obs}) for a non-modified DNAzyme ranged from 0.02 to 0.8 min^{-1}, at 2 mM and 10 mM metal concentration, respectively. When DNAzyme nucleotides were modified with locked nucleic acids (LNA) modifications at the 3' and 5' end, k_{obs} values increased, ranging from 0.15 to 1.5 min^{-1}. Finally, we incubated human cells with LNA-modified deoxyribozymes, finding median effective concentrations for RNA degradation (EC50) between 125 and 250 nM. Thus, we offer an alternative approach for loss-of-function experiments centered on RNAs. This autonomous system works in human cell contexts and holds potential for application in other biological models. (ANID FONDECYT Regular 1240853) *The authors marked with an asterisk equally contributed to the work.

P-04-007
The Sense/Antisense double-stranded RNA (SensR) viewer is a tool for generating hypotheses about mechanisms of gene regulation in *Saccharomyces cerevisiae*

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Strand-specific RNA profiling studies have identified numerous co-expressed overlapping sense and antisense transcripts in both prokaryotic and eukaryotic cells. Subsequently, it was discovered that these transcript pairs can form stable double-stranded RNAs (dsRNAs). To shed light on the extent of dsRNA formation by sense/antisense transcripts during growth and development, we used a *Saccharomyces cerevisiae* strain expressing the ribonuclease DCR1 and the RNA binding protein AGO1 from *Naumovozyma castellii* in combination with a small RNA sequencing protocol for genome-wide dsRNA profiling. We introduce the Sense/Antisense double-stranded RNA (SensR) expression viewer. Users can retrieve various graphical representations of dsRNA data using genome coordinates and systematic or standard names for mRNAs and different types of stable or cryptic long non-coding RNAs (lncRNAs). Our data are a useful resource for work on the annotation of the yeast genome in general, and for generating hypotheses about regulatory mechanisms controlling the budding yeast life cycle in particular. In addition, the results are also interesting from an evolutionary perspective, since natural antisense transcripts that form stable dsRNAs have been detected in many species from bacteria to humans. The viewer can be accessed at https://sensr.genouest.org. *The authors marked with an asterisk equally contributed to the work.

P-04-008
Functional dissection of PRC2-dependent dysregulation in Weaver syndrome through cortical brain organoids reveals cell migration defects and impaired differentiation trajectories

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Weaver syndrome (WVS) is a rare multisystem disorder characterized by macrocephaly and intellectual disability. As of today, WVS remains a clinical unmet need due to the experimental challenge of accessing patient biological samples. WVS is caused by heterozygous mutations in polycomb repressive complex 2 (PRC2), which catalyzes the tri-methylation of Lysine 27 on histone 3 (H3K27me3), promoting transcriptional repression during corticogenesis. Nevertheless, our knowledge on the impact of such mutations on the landscape of H3K27me3 and on the transcriptome is still incomplete and several questions remain unsolved. To gain insight in molecular circuits underpinning the WVS phenotype, we profiled patient-derived cortical brain organoids (CBOs). The intersection of differentially expressed genes between WVS-CBOs and control CBOs, single cell RNA-seq data, H3K27me3 genomic distribution, PRC2 occupancy and DNA methylation profiles, revealed a dysregulation in neuronal differentiation timings and neuronal migration processes at relevant developmental stages. This finding is consistent with clinical data from WVS patients, whose MRI profiles show an alteration of the cortical development due to defects in neuronal migration. Moreover, from the intersection of omics data, AJAP1 resulted one of the most significantly and strongly upregulated genes in WVS-CBOs. AJAP1 is an adherens junction associated protein involved in cell migration and associated with intellectual disability. Therefore, to elucidate the role of AJAP1 in WVS, we established a CRISPR-based synthetic system to perturbate the expression of AJAP1 to revert the phenotype in WVS-CBOs and recapitulate it in controls, mimicking the disorder. In conclusion, our findings support impaired differentiation trajectories and cell migration defects throughout corticogenesis due to PRC2 misfunction in CBOs and shed light on cellular biology processes that were not known to be tightly regulated by PRC2. *The authors marked with an asterisk equally contributed to the work.

P-04-009
Role of miR-802 in brain insulin signaling in Down syndrome

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Down syndrome (DS) individuals are characterized by a variety of pathological phenotypes that manifest with wide variability in the different tissues. At the level of the central nervous system, the accelerated aging phenotype is associated with the risk to develop Alzheimer-like dementia. A central aspect of neurodegeneration is the close association between metabolic disorders and cognitive decline. Several studies have suggested a link between metabolic disorder and microRNAs (miR), small non-coding RNAs acting as post-transcriptional regulators of a plethora of genes. Among triplicated miRNAs on chromosome 21, we focus on miR-802 because recent studies demonstrated its association with development of insulin resistance in obesity and diabetes. Considering these findings and based on the "gene dosage hypothesis" of DS, the goal of the study is to decipher how miR-802 may contribute to aberrant insulin signaling (IS) and, in parallel to the risk to develop dementia early in life in DS. The miR-802 expression, protein levels and activation state of main components of the IS were evaluated (i) in the brain of autoptic cases from DS, DS with Alzhemer disease (DSAD) and age-matched controls and (ii) in the brain of euploid and Ts65Dn mice (a model of DS). Further, using bioinformatic tools we identified miR-802 predicted target genes that are involved in the IS (PTEN and GSK-3β). The IS alterations worsen in the

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transition from DS to DSAD and similar findings were collected inTs65Dn mice where IS dysregulation persists with aging where neurodegeneration becomes significant. Intriguingly, these latter changes were driven by the over-expression of miR-802, which negatively regulates PTEN and GSK-3β mRNA in the brain. In this picture, the identification of specific targets modulated by miR-802 and involved in IS pathway, will provide molecular basis to develop novel therapeutic strategies to prevent/delay the onset of brain insulin resistance in DS.

P-04-010
VDAC1P8 pseudogene is a lncRNA involved in acute myeloid leukaemia (AML)

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Voltage-dependent selective anion channels (VDACs) are the most abundant mitochondrial outer membrane proteins, encoded in mammals by three genes, VDAC1, 2 and 3, mostly ubiquitously expressed [1]. As 'mitochondrial gatekeepers', VDACs control organelle and cell metabolism and are involved in many diseases [2]. Despite the presence of numerous VDAC pseudogenes in the human genome, their significance and possible role in VDAC protein expression have not yet been considered. We investigated the relevance of processed pseudogenes of human VDAC genes in both physiological and pathological contexts. Using high-throughput tools and interrogating numerous genomic and transcriptomic databases, we showed that some VDAC pseudogenes are transcribed in specific tissues and pathological contexts. Experimental data obtained in several AML cell lines confirm the association of the VDAC1P8 pseudogene with acute myeloid leukemia [3]. Further analyses, the results of which have not yet been published, seem to indicate the involvement of the VDAC1P8 pseudogene also in other blood cancers, such as myelofibrosis, multiple myeloma and chronic myelogenous leukemia. Overall, our *in-silico* comparative analysis between the VDAC1 gene and its pseudogene VDAC1P8, together with experimental data produced in cellular models of AML, indicate a specific overexpression of the VDAC1P8 pseudogene in AML, correlated with a downregulation of the parental VDAC1 gene [3]. More recent unpublished data would suggest an involvement of the VDAC1/VDAC1P8 gene pair in leukaemias in general. References: 1. Messina A. et al. (2012) BBA – Biomembranes. 1818(6) 1466–76. 2. Shoshan-Barmatz V. et al. (2010) Mol Aspects Med. 227–85. 3. Pappalardo XG et al. Biol Res. (2023) Jun 22;56 (1):33.

P-04-011
Epigenetic profiling of male infertility: investigating the expressions of HAR1B, MEG3, MALAT1, and EZH2

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Male infertility is a rising health concern, with sperm counts decreasing by 50–60% in past decades. Despite efforts to identify the cause, at least 44% of cases remain idiopathic. Long noncoding RNAs although involved in cellular processes like proliferation, differentiation and apoptosis, their role in male infertility is largely unexplored. The purpose of this study was to evaluate the expression of HAR1B, MEG3 and MALAT1 along with the EZH2 gene, an epigenetic regulator that acts as a histone methyltransferase associated with gene silencing and a repressed chromatin state. Seminal liquid samples were obtained from infertile men (n = 40, range 20–55 years) and controls (n-10 range 20–45 years). The infertile group was divided in the following subgroups according to their sperm characteristics: azoospermia (AZO; n = 10), oligoasthenospermia (OAS; n = 10), oligoasthenoteratospermia (OATS; n = 10) and severe oligoasthenoteratospermia (OATSS; n = 10). RNA was obtained using TRIzol reagent and cDNA was generated using High-Capacity cDNA Reverse Transcription Kit (AppliedBiosystems). In order to evaluate the expression levels, qRT-PCR was performed and GAPDH was used as a housekeeping gene. Statistical analysis using GraphPad Prism 9.3.0 was performed. Significant diffferences in expression levels were found for HAR1B ($p = 0.0018$), its levels being significantly decreased in AZO samples ($p = 0.0159$) and slightly elevated in OAS samples ($p = 0.0317$). EZH2 expression levels were elevated in AZO ($p = 0.0317$) and also in OAS ($p = 0.0079$) compared to the control group. MEG3 displayed a similar profile in all group types, while MALAT1 had considerably lower levels in AZO and OAS groups, but statistical relevance was found only in the case of OAS ($p = 0.0159$). This study revealed distinctive lncRNA and EZH2 expression patterns, especially in the AZO and OAS groups, emphasizing the role of epigenetic modifications in the rising male infertility issue. Study supported by PN-III-P2-2.1-PED-2019-4402.

P-04-012
Illuminating stemness-regulating lncRNAs in head and neck squamous cell carcinoma through tumor heterogeneity-adjusted association analysis

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Numerous public RNA-sequencing data provide an obvious opportunity for exploring the regulatory network in cancer. Recent development of RNA therapy suggests that the regulatory lncRNAs for stemness genes might serve as potential therapy targets in cancer. However, the tumor samples are composed with more than cancer cells and tumor purity, the proportion of cancer cells present in a tumor sample, could vary a lot across different samples and cancer types. Here we propose to utilize methods that adjusts for tumor purity to investigate the regulatory relationships of lncRNAs in cancers. As a demonstration, we used partial correlation and liquid association to identify the regulatory lncRNAs for stemness, the Yamanaka factors, i.e. SOX2, MYC, KLF4 and OCT4 in head and neck cancer. Furthermore, we validate the regulatory role of one of the candidates lncRNA in oral cancer cell lines. These results suggest that the proposed tumor-purity-adjusted strategy will help to identify the crucial regulatory relationships within cancer cells and can lead to valuable perspectives as well as avenues for treatment. *The authors marked with an asterisk equally contributed to the work.

P-04-013

Abstract withdrawn.

P-04-014
Structural architecture of human long noncoding RNA as a novel target for anti-influenza drug development

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Changes in lncRNA levels have been observed in the course of respiratory diseases. LncRNAs are also induced by influenza virus infection; some are biologically important for virus replication. Interferon-independent human lncRNAs have recently been revealed and confirmed to interact with influenza virus proteins. These lncRNAs were proven to be specifically produced during influenza virus infection. In the present study, we considered that the structure of the lncRNA-PAAN (PA-associated noncoding RNA) necessary for influenza virus proliferation is still poorly understood and that the structural motifs present in lncRNAs may play an essential role in virus biology. ScanFold software was used to predict functional RNA motifs in lncRNA-PAAN. RNA mapping was performed *in vitro* and in a cellular environment to determine the RNA secondary structure of the lncRNA. Bioinformatic analysis revealed regions of lncRNA-PAAN with a high propensity for functional RNA structure. The functional and structural RNA motifs of the lncRNA-PAAN were identified and confirmed *in vitro* and in the cellular environment. Confirmed RNA motifs of lncRNA are extremely thermodynamically stable and probable to perform a biological function. For understanding the role of lncRNA in the virus's life cycle and designing a new drug, information about RNA secondary structure is critically important. Due to the variability of the influenza virus and the probability of the emergence of new strains, in the present study, the interest was directed towards selected long noncoding RNAs of the host (human), which are much less variable than the RNA and proteins of the influenza virus. It, therefore, may represent a new universal target for anti-influenza therapy.

P-04-015
Profiling array of human long non-coding RNA molecules between meningioma and glioblastoma tissues

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Despite the use of adjuvant radio- and chemotherapy as well as recent promising advancements in surgical techniques, the overall survival of patients with glioblastoma multiforme (GBM) has not improved significantly over the last decades. This emphasizes the need for exploring alternative therapeutic strategies. Temozolomide (TMZ) is a small lipophilic alkylating chemotherapeutic drug presently used to treat brain tumors. Growing evidence suggests that TMZ-induced epigenetic changes play a crucial role in the emergence of adaptive drug resistance in GBM since the effect of TMZ depends on cancer cells' DNA-repairing abilities. Recent findings indicate the importance of long non-coding RNA (lncRNA) dysregulations in the resistance of GBM cells to therapy. These noncoding transcripts have recently emerged as major players in different biological processes, including cancer development. In our study, a total of 84 lncRNA genes associated with cancer, disease, cell differentiation, inflammatory response, or autoimmunity were analyzed using the RT^2 lncRNA PCR array Human lncFinder (Qiagen). Amplification was observed in half of the products, 32 of which had higher expression levels. Most often they displayed increased expression in glioblastoma patients (n = 12) compared to meningioma patients (n = 10). Significant up-regulation was discovered for genes H19, HOTAIRM1, JPX, MIAT, SPRY4-IT4, and XIST. To investigate the potential of lncRNAs as biomarkers, we examined lncRNA dysregulation dependent on the IDH mutation status. We learned that several lncRNAs (H19, MALAT1, TUG1, and XIST) may function as risk factors for patients with glioma. The dysregulation of lncRNAs contributes significantly to the development of GBM. Thus, identifying lncRNAs associated with adaptive resistance to therapy is essential for further understanding the mechanism of tumorigenesis, which could lead to better prognosis and the development of new therapeutic targets. *The authors marked with an asterisk equally contributed to the work.

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P-04-016
MicroRNAs as prognostic markers in clear cell renal cell carcinoma

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Clear cell renal cell carcinoma (ccRCC) accounts for 75–80% of all renal cancers and ranks as one of the most prevalent urological malignancies worldwide. The treatment of patients with advanced ccRCC is based on prognosis assessment, which stratifies patients into groups of favourable, intermediate, and poor prognosis. The aim of the study was to analyse the expression of microRNAs in ccRCC and identify those with prognostic significance. The study group consisted of 20 patients with advanced ccRCC treated with receptor tyrosine kinase inhibitors (sunitinib or sorafenib). Total RNA was isolated from histologically verified sets of matched formalin-fixed, paraffin-embedded (FFPE) tissue samples (normal renal tissue, primary tumour, metastasis). The tissue expression of 2549 microRNAs was analysed using the SurePrint G3 Human miRNA microarray kit (Agilent Technologies). Based on analytic criteria, 13 up-regulated and 6 down-regulated microRNAs were identified in ccRCC tumour tissues compared to control tissues. Prognostic significance of deregulated microRNAs was further evaluated by the use of microRNA expression and clinical data of 475 patients available from TCGA Kidney Clear Cell Carcinoma (KIRC) database. Patients with high tumour tissue expression of miR-21, miR-27a, miR-34a, miR-106b, miR-210, and miR-342 showed significantly shorter overall survival. The opposite was observed for miR-30e, where patients with low expression had significantly shorter survival. In conclusion, the identified microRNAs (miR-21, miR-27a, miR-34a, miR-106b, miR-210, miR-342, and miR-30e) significantly associated with the prognosis of ccRCC patients could be included in prognostic expression panels to refine stratification scoring systems on which the treatment of ccRCC patients is based.

P-04-017
Integrative bioinformatics analysis reveals the LSD1-ATF4 axis governing microRNA regulatory networks in glioblastoma

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Glioblastoma (GBM) is an aggressive malignant tumor characterized by heterogeneity and resistance to treatment due to the presence of glioblastoma tumor initiating cells (GBM TICs), which possess stem-like properties. LSD1 has been identified as a significant epigenetic regulator in GBM TICs and possible molecular mediators of LSD1 are the miRNAs, a class of short non-coding RNAs with great importance and regulatory roles in tumors. Recently, a pivotal role for the ATF4 transcription factor as cofactor of LSD1 in GBM was also demonstrated (Faletti et al., 2021). We developed a bioinformatic pipeline grounded on the integration of omics datasets to define, as final output, a miRNA-mediated regulatory network sustained by LSD1 and ATF4 in a model of GBM TICs (BT165 cells). A LSD1 ChIP-Seq performed on the BT165 was overlaid with the miRNAs Transcription Start Sites (TSS) genomic coordinates in the human genome. Regions ranging from -2500 to +2500 bps around a TSS were considered as promoters for miRNA genes. The presence of binding sites for the LSD1-cofactor ATF4 was investigated with the JASPAR database. Through transcriptomic analysis, we identified 98 differentially expressed genes (DEGs) between LSD1-silenced and control BT165. The DEGs were used as input for the Ingenuity Pathway Analysis, thus allowing the prediction of the miR-124-3p as upstream regulator, characterized by the presence of LSD1 and ATF4 peaks around its promoter region. We cross-referenced miR-124-3p target genes obtained from MiRTarBase and TargetScan databases with the 98 DEGs, resulting in 12 potential target genes showing LSD1 and ATF4 binding at the promoter region. We finally graphically generated with Cytoscape an LSD1-ATF4-miRNAs mediated regulatory network of the miR-124-3p. Our results show the importance of non-coding RNAs in the regulatory network underlying the onset and progression of GBM. MiR-124-3p and the set of genes under its control could be explored for potential therapies.

P-04-018
Study of the role of lncRNA PHOX2B-AS1 in the pathogenesis of congenital central hypoventilation syndrome

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Paired-like homeobox 2B (PHOX2B) gene encodes for a transcription factor responsible for the development of the autonomic nervous system (ANS) and the neural structures involved in breathing control. Heterozygous mutation in *PHOX2B* cause Congenital central hypoventilation syndrome (CCHS), a rare genetic neurodevelopmental disorder that affects the ANS and the central chemosensitivity. *In vivo* and *in vitro* studies suggest that a loss of function mechanism, combined with a dominant-negative effect and/or toxic gain of function of the mutated proteins, is responsible for the entire disease spectrum. No effective pharmacological ventilatory stimulant is currently available. *PHOX2B-AS1*, a natural antisense lncRNA mapping in the *PHOX2B* locus, was recently identified. To characterize and study the function of *PHOX2B-AS1*, we took advantage of neuroblastoma cell lines and of recently generated control and patient-derived (20/25 genotype) induced pluripotent stem cell

(iPSC) lines [Previously published in: Cuadros Gamboa et al. (2022) Stem Cell Res 61, 102781]. RNAscope experiments showed that both lncRNA *PHOX2B-AS1* and *PHOX2B* mRNA are mainly cytoplasmic. Performing western blot analysis, we observed that a 50% reduction of *PHOX2B-AS1*, using a pool of three gapmers, does not affect the *PHOX2B* mRNA level, however there is a reduction in the protein level. Therefore, our data suggest that *PHOX2B-AS1* acts at favouring PHOX2B translation. Furthermore, the qPCR analysis has shown an ectopic expression of *PHOX2B* and *PHOX2B-AS1* in a patient-derived iPSC line, despite the expression of the pluripotency markers. Overall, these data suggest that dysregulated *PHOX2B-AS1* and *PHOX2B* transcription at earlier developmental stages may be involved in CCHS pathogenesis and the possibility to modulate *PHOX2B-AS1* expression as a new therapeutic strategy aimed at reducing the expression of mutant PHOX2B proteins.

P-04-019
miRNAs and lncRNAs as the potential modifiers and biomarkers of response to ionization radiation: *in silico* and *in vitro* analysis

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Head and neck squamous cell carcinomas (HNSCC) are still one of the most challenging types of cancer for treatment and it is characterized by high ability to lymph node invasion and metastasis. Patients are typically 50–80 years old, both men and women, but a rising number of young persons diagnosed is also observed. This type of cancer is mainly caused by tobacco use, alcohol consumption and HPV infections. In spite of a development in the treatments approach, surgery and radiotherapy, and/or chemotherapy, no dramatic changes in patients' survival were achieved. One of the promising improvement in HNSCC treatment is using the epigenetic biomarkers such as lncRNAs (long noncoding RNAs) and miRNAs, which could help in personalization of radiotherapy. Using the TCGA-based model, the protein-coding and non-coding RNAs (ncRNAs) were selected with the highest differences between the group of patients with response (RG) and no response (NRG) to radiotherapy. Next, selected ncRNAs were validated using *in vitro* models. The RG group of patients displayed a different lncRNAs and miRNAs expression pattern compared to the NRG group. We observed a dose dependent, stronger changes in the expression of ncRNAs than in protein coding genes. Dysregulated lncRNAs and miRNA were associated with cell cycle regulation, the p53 pathway, DNA damage and repairs mechanisms, cell death and autophagy, regulation of reactive oxygen species. Differences in ncRNAs are strictly associated with DNA damage pathway and response to this cellular stress. Further analysis of ncRNAs as biomarkers could help in the prediction of patients' response to radiotherapy. This work was supported by Greater Poland Cancer Centre — grant no.: 5/12/2022/PGN/WCO/011.

P-04-020
RBCs as circulating repositories of miRNAs

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MicroRNAs (miRNAs) are small noncoding RNA involved in many physiological and pathological conditions by negatively regulating gene expression. In blood, miRNAs are usually carried by blood cells and extracellular vesicles and/or associated with Ago2. Although red blood cells (RBCs) were considered to lack nucleic acids, their miRNA content has been recently demonstrated. Due to RBCs abundance in blood, they represent the major source of the same. RBCs have already been used into the clinics as drug delivery system, suggesting their role also in miRNAs delivery. In addition, so-called "storage lesions" occur in stored RBCs, in which miR-451 and miR-196a levels may be dysregulated. Here we demonstrate that RBCs are particularly rich in stable miRNAs exploring the reasons of this stability. RBCs purified from fresh blood went through total RNA extraction and RT-PCR to detect miRNAs expression levels followed by miRNAs short-term stability studies. Afterwards, RBCs were subjected to a loading procedure without adding any molecule, obtaining unloaded (UL) RBCs to be compared with untreated (NT) ones. Moreover, RNA immunoprecipitation (RIP) method was optimised and performed to isolate and then quantify Ago2-bounded RNA. First, we found that miR-451 is highly expressed in RBCs, while miR-196a is less abundant. In the following analyses, miR-106b and U6 snRNA were initially chosen as endogenous controls, but they showed high variability. U6 snRNA demonstrated a higher loss in stability compared to other miRNAs. Thus, absolute quantification my means of standard curves were used instead. Despite a little initial loss, miRNAs were retained in UL and resulted stable over time. Further analysis suggested that miRNA stability is related to their binding with Ago2 proteins, which protect them from degradation or release. Therefore, this finding may significantly change the RBCs exploitation as miRNAs delivery system, both in transfusion medicine and RBCs-based cell therapies.

P-04-021
The changes of miRNA profiles of airway produced extracellular vesicles upon viral infection

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Recent evidence suggests that peripheral infections can cause irreversible genetic and epigenetic changes, resulting in immune memory formation in the brain. Extracellular vesicles (EVs) from virus-mimetic poly(I:C)-primed airway cells can enter the brain and activate microglia via mitochondrial activity[1]. This study analysed whether changes in miRNA profiles of healthy and poly(I:C)-primed airway epithelial cell-produced EVs might be associated with this phenomenon. EVs from cultured poly(I:C)-treated human airway epithelial cells (poly(I:C)-EVs) were isolated by polymer precipitation, characterised by morphology (TEM), size (NTA) and specific markers (Luminex). Total RNA was extracted from EV suspension and used to prepare small RNA next-generation sequencing (NGS) libraries with commercial kits. Illumina NextSeq 550 was used for 75-cycle NGS. Reads were preprocessed using cutadapt tool, aligned to miRbase v22 using miRaligner tool. Annotated reads were collapsed and subjected to differential expression analysis using DeSeq2. Target enrichment and gene ontology analysis were performed using Tarbase v9 (microT Score cutoff 0.9), followed by Gorilla. Airway epithelial cell EVs mainly were 120-140 nm in diameter and contained endosomal origin markers. NGS did not identify unique miRNAs expressed at stable levels (expression >50% of samples, read number >5) between control and poly(I:C)-EVs samples. However, 6 differentially expressed miRNAs were found in poly(I:C)-EVs (upregulated miR-2110, miR-193b-5p, miR-423-5p, miR-1306-5p, downregulated miR-181a-5p, miR-221-3p). Target enrichment analysis revealed that these miRNAs have 809 possible unique target mRNAs, mainly involved in metabolic and inflammatory processes. EVs originating from poly(I:C)- treated cells are enriched with or depleted of functionally active miRNAs, which can result in the modulation of metabolic and inflammatory processes in microglia. Reference: [1]Kulakauskienė et al. (2021) Biology 10(12):1359.

Protein Post-Translational Modifications and Turnover

P-05-001
Extracellular nicotinamide phosphoribosyltrasferase (eNAMPT) and physiological roles of post-translational modifications

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Nicotinamide phosphoribosyltrasferase (NAMPT) is an essential enzyme involved in cellular metabolism which exists in two different forms. The intracellular form (iNAMPT) is involved in and NAD^+ synthesis, the extracellular protein (eNAMPT) acts as a pro-inflammatory cytokine (1). In the literature, just few post-translational modifications of NAMPT have been described: the H247 autophosphorylation (2) and the K53 deacetylation (3). No data are available on other sites. The aim of our work was to identify and characterize putative NAMPT phosphorylation sites involved in protein activity. We have generated a murine cell line (B16) over-expressing GFP-NAMPT which was then pulled-down with GFP-trap beads, precipitated using the TCA method and digested with Lys-C and analyzed by LC-MS/MS. We generated a mutated form of the protein in murine cells: serine residues 199, 200 and 472 have been replaced with alanine. We checked the enzymatic activity, the oligomeric state (Native PAGE and SEC), the viability, the localization (fluorescence microscopy) and the ability to bind TLR4 (SPR) of mutated proteins. By shot-gun LC-MS/MS proteomics we identified 8 phosphorylated residues but focused on: S199, S200 and S472. Recombinant mutated proteins NAMPTS199A and NAMPTS200A are enzymatically inactive. Moreover, S200A mutation altered NAMPT cellular localization. Interestingly, NAMPTwt, NAMPTS199A and NAMPTS200A demonstrated to be active as pro-inflammatory cytokine, whereas S472 mutation into alanine compromised NAMPTS472A pro-inflammatory activity. Here, for the first time, we have found novel phosphoresidues controlling NAMPT dimerization, activity, cellular localization, and its cytokine pro-inflammatory function and that could be essential in the understanding of NAMPT regulation mechanisms. References: 1. Gholinejad Z et al. (2017) Peptides; 92:9–15. 2. Burgos ES et al. (2009) Proc Natl Acad Sci USA; 106 (33):13748–53. 3 Sociali G et al. (2019) FASEB J.; 33(3):3704–3717.

P-05-002
In vitro study of the effects of protein arginine methyltransferase 5 mutations on enzyme activity

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Protein arginine methyltransferase 5 (PRMT5) is responsible for symmetric dimethylation modifications of histone substrates (H2A, H3, H4). PRMT5 interacts with the methylosome protein complex 50 (MEP50), and its activation is dependent on the phosphorylation of Thr 80 residue, on which the Rho-dependent kinase (ROK) and myosin phosphatase (MP) acts as antagonists. Since PRMT5 is responsible for the expression of proto-oncogenes, the upstream elements of the signaling pathway have a tumour suppressor role. Based on the COSMIC human tumour database, mutations of PRMT5 at the Thr80 regulatory site has oncogenic potential. Our aim was to investigate the role of PRMT5 in tumorigenesis, with focus on its mutations. By site-directed mutagenesis we generated the PRMT5 T80A and T80M mutant forms and overexpressed them in tsA201 cell line to conduct *in vitro* binding assays. By immunoprecipitation we isolated the recombinant proteins and performed Rho-kinase assay to phosphorylate the PRMT5. Western blot analysis was used to gain information about the changes in the quantity of MEP50 complex and regulatory role of ROK upon the mutations. To measure the activity of the enzyme, we analysed the level of dimethylation of H2A. We have also conducted molecular dynamic analysis to predict the conformational changes of the methylosome complex in the mutant form of PRMT5. Based on our results, it can be established that tumour-related mutations in PRMT5 increased the activity of the enzyme not through the shift in the methylosome complex formation, but by the elevated amount of histone substrate bound by the complex. This is caused by spatial structure changes by mutations and the exposition of new activating phosphorylation site.

P-05-003
Human mitochondrial protease OMA1 eliminates arrested protein import intermediates upon depolarization of the inner mitochondrial membrane

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Most mitochondrial proteins are synthesized as precursors in the cytosol and require an effective import into the organelle. Such precursors must be unfolded to pass through translocation channels in mitochondrial membranes. Misfolded proteins can become arrested in translocases, impairing protein import. Both cytosolic and mitochondrial quality control mechanisms can act on the stalled precursors. The toxicity of misfolded proteins and mitochondrial dysfunction are pivotal factors in many pathological conditions constituting hallmarks of aging-related degenerative diseases. We designed a fusion protein to stall at an intermediate step of import to study mechanisms that respond to import failure. We fused an inner mitochondrial membrane-directed domain and a fluorescent tag, including a superfolder GFP, which does not allow the completion of the fusion's translocation. The N-terminal part is built into the inner mitochondrial membrane, resulting in a stable arrest. Our results revealed that depolarisation of the mitochondrial inner membrane activates the proteolytic processing of the model protein by mitochondrial proteases. Our experiments provide evidence for the role of protease OMA1 in releasing stalled protein from the translocase. We have observed that OMA1 cleaves model protein inside the mitochondria, allowing the retrotranslocation of its arrested fragment to the cytosol. We have confirmed the participation of cytosolic factors VCP and proteasome in its further degradation. The mechanism we found differs significantly from these described in fungi, where ATPase-driven extraction of blocked protein is directly coupled with proteasomal processing. Upon evaluation of mitochondrial morphology, we observed that arrested protein strongly altered cristae organization by tethering outer and inner mitochondrial membranes. Notably, activating proteolysis by an uncoupler of the membrane potential resulted in significant restoration of the mitochondrial ultrastructure.

P-05-004
Modulating sialylation as therapeutic approach for preventing age-associated cardiac dysfunction

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Voltage-gated sodium channel Nav1.5 plays a pivotal role in the proper functioning of cardiac muscle cells. Sialylation, a post-translational modification involving the addition of negatively charged sialic acid moieties, is essential for Nav1.5 functionality. Altered sialylation has been observed in various cardiovascular diseases, including Chagas disease and congenital disorders of glycosylation affecting the heart. Furthermore, sialylation status has been shown to change during human adult life, impacting brain and muscle structural properties. Cardiac arrhythmias increase in prevalence with age, contributing to higher morbidity and mortality in older people. Using an aging mouse model, we observed a decline in cardiac sialic acid levels and NaV1.5 associated with the aging process. In compelling in-vitro experiments on HEK293A cells and induced pluripotent stem cell-derived cardiomyocytes (iPSC-CM), diminished external sialic acid induces Nav1.5 channel internalization, activating endocytic pathways and leading to proteasome-mediated degradation. Intriguingly, proteomic analysis revealed that introducing a specific sialic acid precursor increased membrane protein trafficking, restoring Nav1.5 protein levels and the sodium current. These findings are consistent with the observed rise in exogenously metabolized deuterated-sialic acid as detected by LC-MS. Electrophysiological experiments demonstrated functional recovery and highlighted the rapid response, observable within just 2 h of sialic acid stimulation. In summary, this study provides novel insights into the complex relationship between sialic acid and NaV1.5 channel trafficking, offering new perspectives and promising therapeutic strategies to prevent cardiac dysfunction in the elderly.

P-05-005
Mechanisms of autoregulation of Snf1/AMPK in budding yeast and its nucleocytoplasmic dynamics

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Mechanisms that control the balance between nutrient availability and metabolism are well conserved in eukaryotic organisms and the Snf1/AMPK kinases are the key regulators of energy homeostasis. The activation state of Snf1 in yeast is determined by the modulation of its phosphorylation on a conserved Thr, which in turn is accompanied by a variation of the nucleocytoplasmic distribution of the kinase. Snf1 phosphorylation is regulated by Sak1, one of the main activating kinases of Snf1 along with Tos3 and Elm1, and by the Reg1/Glc7 phosphatase complex [Coccetti et al., (2018). Microbial cell, 5(11), 482–494]. We recently identified several Snf1-dependent phosphosites on Sak1 and Reg1, leading to the intriguing hypothesis of an autoregulation mechanism of Snf1 activity [Caligaris et al., (2023). eLife, 12, e84319]. The aim of this study is to unravel the mechanisms underlying this regulation, by characterizing the loss of Snf1-dependent phosphosites on these two proteins. We therefore decided to study the effects of 10 Snf1-dependent phosphosites on Sak1 and 16 Snf1-dependent phosphosites on Reg1 by mutating all of them to Ala. By using these engineered strains, we were able to observe a loss of Snf1 autoinhibition following a nutritional shift down. To further investigate the spatial dynamics of Snf1, we set up a new live single-cell imaging method, to study the nucleocytoplasmic dynamics of Snf1 localization using microfluidics systems. Time-lapse imaging experiments at different time-points further support the notion of a negative feedback regulation. Altogether, our findings provide evidence indicating that Snf1 is capable of negatively regulating its own long-term activation state, by phosphorylating both its activating kinase and phosphatase.

P-05-006
The source of GDP-fucose does not influence fucosyltransferase expressions

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Two GDP-fucose synthesis pathways exist in mammals. The first one, *de novo*, uses GDP-mannose: enzymes GDP-D-mannose-4,6-dehydratase (GMDS) and GDP-L-fucose synthase convert GDP-mannose to GDP-fucose. The second, salvage, uses fucose from the lysosomal degradation of glycans or the environment: fucokinase (FUK) and GDP-fucose pyrophosphorylase transform fucose into GDP-fucose. The latest research showed that GDP-fucose might reside in distinct pools according to its origin, which is fucose- or mannose-derived. Moreover, the origin could determine which bond GDP-fucose would be incorporated into oligosaccharides. GDP-fucose is added to glycans by fucosyltransferases (FUTs). Thirteen fucosyltransferases have been detected in mammals. They are divided into groups: α-1,2, α-1,3/4, and α-1,6 fucosyltransferases and protein O-fucosyltransferases. Based on the theory of selective usage of GDP-fucose by FUTs, we wondered if distinct GDP-fucose pools may influence the production of fucosyltransferases upon the availability of nucleotide sugar from various sources. For this purpose, we employed HEK293T cell line wild-type and lacking GMDS or FUK. We also treated cells lacking GMDS in fucose as the external source of GDP-fucose. We checked glycan structures in all cell lines. We examined the diversity of fucosyl bonds by lectin blotting. Finally, we checked the mRNA and protein expression levels of FUTs. We observed differences in glycan synthesis and fucosyl bonds. Moreover, we found changes in the mRNA levels of FUTs, which were not in line with their protein level. However, variations in protein levels were relatively small and not significant. We believe that alterations in the fucosylation of glycans resulted from supplying FUTs via different routes of passing Golgi apparatus rather than enhancing the action of fucosyltransferases. Acknowledgements: This work was supported by PRELUDIUM grant no. 2022/45/N/NZ3/00093 from National Science Centre (NCN) Kraków, Poland

P-05-007
SARS-CoV-2 N protein activates protein kinase CK2

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The COVID-19 causing virus SARS-CoV-2, similarly to other viruses, exploits protein kinases of the host cell for its life cycle. CK2 has been proposed as a candidate among the protein kinases activated by SARS-CoV-2. CK2 is a constitutively active tetrameric Ser/Thr kinase, composed of two catalytic (α or α′) and two regulatory (β) subunits, but is also active as a monomer. Its activity is abnormally high in many human diseases, while it is defective in two recently discovered rare diseases, the Okur-Chung (OCNDS) and Poirier-Bienvenu (POBINDS) neurodevelopmental syndromes, due to mutations of the CK2α (CSNK2A) or CK2β (CSNK2B) genes, respectively. The cellular overexpression of the SARS-CoV-2 nucleocapsid protein (N protein, NP) was reported to enhance a number of CK2-dependent phosphosites. However, the direct evidence of CK2 activation by SARS-CoV-2 virus proteins, and in particular by NP, has never been provided. Here we show that NP strongly activates CK2 *in vitro*, with a more evident effect towards the monomeric CK2. We identified the NP region responsible for the activation, whose sequence, reproduced in a short peptide, recapitulates the effect of the whole protein and, once conjugated to a cell-permeable sequence, gives rise to a peptide usable to stimulate cellular CK2 activity. Our results pave the way for strategies aimed at activating CK2, that can be relevant in cells characterized by defective CK2 activity, as in OCDNS and POBINDS patients.

P-05-008
Hydroxylated collagen-like otolin-1 from *Danio rerio* and *Homo sapiens*

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Otolin-1 is a short collagen-like protein from the C1q family involved in the formation of fish otoliths and higher vertebrate otoconia. These calcium carbonate biomineral structures

responsible for the perception of gravity and acceleration, are formed in the inner ear of fish and humans. Otolin-1 serves as a scaffolding protein that connects otoliths and otoconia to the sensory epithelium of the inner ear and interacts with other matrix proteins that play a role in control of biomineralization. Otolin-1 is composed of a non-collagenous N-terminal domain, a central collagen-like domain, and a globular C-terminal C1q domain liable for protein molecule trimerization. Bioinformatic analysis of the otolin-1 sequence revealed that the central fragment of the protein contains repeats of three amino acids (Gly-X-Y) characteristic for collagen proteins, where Y is most often hydroxyproline, though the number of potential hydroxylation sites for otolin-1 compared to full-length collagens is limited. The presence of hydroxyl groups significantly affects the stability of the collagen triple helix. This raises the question what effect the introduction of hydroxyl groups would have on the conformation and stability of otolin-1. To discern the effect of hydroxylation on the biochemical and structural properties of otolin-1 we developed a method to produce hydroxylated recombinant otolin-1 from *Danio rerio* and *Homo sapiens*. The co-expression of both homological recombinant otolin-1 together with mimivirus prolyl and lysyl hydroxylases were performed in *Escherichia coli* cells. Acknowledgments: This work was supported by the National Science Center (Poland) [UMO-2020/39/B/ST10/01253] and in a part by statutory activity subsidy from the Polish Ministry of Science and High Education for the Faculty of Chemistry of Wroclaw University of Science and Technology.

P-05-009
The distribution and composition of γ-tubulin ring complexes during Drosophila spermatogenesis

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The gamma tubulin ring complex (γ-TuRC) is recognized as the primary contributor to microtubule nucleation in the microtubule organizing center (MTOC). Proteins involved in MTOC formation and development in Drosophila are conserved across eukaryotes, and mutations in many of these proteins are associated with human diseases. Centrosomes undergo significant transformations during spermatogenesis, transitioning into basal bodies that nucleate and stabilize microtubules of the axoneme. Our study aimed to investigate the distribution and role of γ-TuRC during the late stages of Drosophila spermatogenesis. In Drosophila, γ-tubulin exists in two complexes: the γ-tubulin small complex (γ-TuSC) and the γ-TuRC. Through phylogenetic analysis, we identified three testis-specific γ-TuRC proteins, suggesting the existence of a testis-specific γ-TuRC (t-γ-TuRC). These proteins include t-Grip84 and t-Grip91, paralogues of Grip84 and Grip91 respectively, along with t-Grip128, a paralogue of Grip128. Analysis of t-γ-TuRC mutants revealed that the t-Grip84 mutant is male sterile, the t-Grip91 mutant is male semi-sterile, while the fertility of the t-Grip128 mutant remains normal. We generated transgenic lines to assess the localization of t-γ-TuRC proteins, revealing their presence at the centriole adjunct post-meiosis, at the nuclear tip during nuclear elongation, and at the surface of mitochondria during cyst elongation. Biochemical assays confirmed the binding of t-γ-TuRC to γ-Tubulin, Mzt1, and each other. Additionally, we elucidated the interaction of several basal body components with various γ-TuRC proteins and demonstrated the presence of ubiquitous γ-TuRC in alternative MTOCs of post-meiotic spermatids. Our findings provide insights into the molecular composition of different MTOCs during late spermatogenesis and in mature sperm. This research was supported by the following grants: NKFIH_OTKA 132155 and 137914 (RS), 2022-2.1.1-NL-2022-00008 and LP2017-7/2017 (ZL). *The authors marked with an asterisk equally contributed to the work.

P-05-010
Confirmation of endogenous sialic acid production in *Ixodes ricinus* ticks and tick cell lines by Click chemistry

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Sialic acid (Sia) commonly decorates the surface of cells and secreted proteins of Eukaryotes. It is important in cell signalling, adhesion, and neurogenesis, and affects immune reactions. While sialic acid is widespread in high abundance in vertebrates, minor representation was reported in invertebrates and among medically important parasites as well. Thus, among the arthropods, the presence and its possible roles remain unclear. *Ixodes ricinus* tick is a significant vector of pathogens causing severe infections in humans and livestock. Indirect evidence suggested that sialylated glycoproteins are present in tick tissues. As a blood-feeding parasite, host blood full of sialylated molecules is its only source of nutrients. To overcome the detection of host sialylated proteins, we used an azide-modified precursor of sialic acid which can be metabolically incorporated into tick cells/tick tissues in the case that the corresponding biosynthetic pathway is present. The main goal of this project was to detect tick endogenous sialylated glycoproteins in a tick cell line and *Ixodes ricinus* ticks. Using *in vitro* feeding in combination with Click chemistry, we traced sialic acid from fed females through eggs to larvae. Using fluorescence microscopy and western blotting, we visualized sialylated molecules of tick origin in the IRE/CTVM 20 tick cell line and in tissues of fed tick females. After egg laying, we detected Sia in eggs with the highest concentration being present after oviposition and in specific tissues of the larvae that hatched from the eggs. Searching the *Ixodes ricinus* genome revealed at least one active sialyltransferase. Based on the results presented, we can conclude that *Ixodes ricinus* ticks as well as the IRE/CTVM 20 cell line produce sialylated molecules that we assume to be important during the development.

P-05-011
Molecular characterization of collagen lysine post-translational modification enzymes

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Collagen lysine residues may undergo multiple post-translational modifications: firstly, hydroxylation mediated by LH/PLOD enzymes introduces a 5-OH moiety on the amino acid side chain. Three distinct LH/PLOD isoforms act on different lysine residues: LH2/PLOD2 is a specific telopeptide hydroxylase, whereas LH1/PLOD1 and LH3/PLOD3 act on lysine residues located within the collagen triple-helical segments. Hydroxylysines (Hyl) can undergo further galactosylation (generating Gal-Hyl), through a process mediated by GLT25D transferases. Additional glycosylation of Gal-Hyl is mediated by LH/PLOD enzymes through their N-terminal glycosyltransferase domains (generating the final Glc-Gal-Hyl product). Here, we present new results on the structural and functional characterizations of full-length human LH/PLOD and GLT25D enzymes. Structural characterizations revealed unexpected features impacting on the processivity and molecular recognition abilities of the different enzyme isoforms, as well as opportunities to map the molecular significance of pathogenic mutations affecting the genes encoding for these enzymes. Biochemical characterizations and site-directed mutagenesis rationalize the structural insights and offer new opportunities for the detailed investigation of the Lys_to_Glc-Gal-Hyl pathway in collagen biosynthesis. *The authors marked with an asterisk equally contributed to the work.

Protein Phase Separation and New Organelles

P-06-001
Diffusion-based analysis of phase-separating PSD proteins: combining microfluidics with fluorescent microscopy techniques

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The postsynaptic density (PSD) is a complex, multi-layered protein network that is largely situated on the internal surface of the postsynaptic membrane, with receptor proteins extending it across the membrane. Changes in synaptic strength as well as plasticity have been shown to correlate strongly with the dynamic structural changes of this network which, in turn, have been associated with multivalent interactions between its components, referred to as protein phase separation. This phenomenon is often defined as the reversible formation of so-called membraneless organelles (MLOs), that constitute a protein-rich phase distinct from the "bulk solution" of the cytosol. We aim to develop a reliable, cost-efficient experimental method that can monitor the formation of MLOs via their diffusion. This method requires a microfluidic device that can achieve laminar flow for a fluorescent protein solution, a microscope that can record the flow within the device, and an array of custom scripts that can process the experimental data and convert them into approximate particle sizes. The main challenge of this technique comes from the dynamic range of particle size that varies from a few nanometres to several microns. With the design of the microfluidic device already determined, we turn our focus onto the evaluation of diffusion data coming from fluorescent samples of known composition. We will present the results of some preliminary experiments, as well as the challenges that revealed themselves with these results.

P-06-002
Role of RNP granules in regulating gene expression during plant germline differentiation

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Regulation of gene expression often involves RNP granules, dynamic structures formed by multivalent protein-nucleic acid interactions that exhibit properties of liquid-liquid phase condensates. We have uncovered an important role for RNP granules in the regulation of meiotic genes in Arabidopsis. Meiosis generates haploid spores from diploid progenitors, and the meiotic mode of chromosome segregation is imposed by the expression of specialized genes. We have previously discovered a mechanism that terminates the meiotic programme by inhibiting translation through sequestering the translation initiation factor eIF4F in P bodies [Cairo A et al. (2022) Science 377, 629–634]. We will present unpublished findings that further highlight the regulation of late meiotic genes by specialized RNP granules. Meiosis can last several days and most of the time the chromatin is in a highly condensed state, suggesting limited transcription, which is supported by the observation that major changes in the transcriptome occur at the onset of meiosis. This raises the question of how late meiotic genes are regulated. Through a genetic screen aimed at identifying genes that control meiotic progression, we uncovered a zinc finger-containing protein, CDM1. CDM1 forms prominent and highly dynamic cytoplasmic speckles in early meiosis, but curiously, its inactivation only leads to defects in late meiosis, namely cytokinesis and microspore formation. We hypothesized that CDM1 is an RNA binding protein that sequesters late meiotic transcripts synthesized early in meiosis in RNP granules and releases them for expression at the end of meiosis. RIP-seq analysis identified putative targets of CDM1 regulation, at least one of which was validated by analysis of its expression through meiosis using a YFP reporter line. Experiments including smRNA-FISH and *in vitro* RNA binding assays are underway to confirm that the candidate gene is a bona fide target of CDM1 regulation.

P-06-003
Acidic otolithic regulatory proteins are involved in liquid-liquid phase separation

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Some animal body parts consist of mineralized tissues. Fish otoliths are biominerals that contain both inorganic and organic matter. The inorganic matter is made up of calcium carbonate crystals, while the organic matter is primarily composed of proteins that regulate the size, shape, and polymorphism of the biominerals. Examples of otolithic proteins include Starmaker (Stm) from *Danio rerio* and Otolith matrix macromolecule—4 (OMM64) from *Oncorhynchus mykiss*. Regulatory otolithic proteins share a common feature of lacking a stable tertiary structure in their physiological state. This is due to their unusual amino acid composition, which is devoid of hydrophobic, order-promoting residues. In addition, they contain a number of acidic residues. The uncompensated charges prevent them from folding. The formation of biologically active condensates is a fundamental, thermodynamically driven process resulting from liquid-liquid phase separation (LLPS). Physiochemical changes in the microenvironment induce it. Our data indicated that otolithic proteins can undergo LLPS induced by metal ions. Liquid condensates may further solidify into biominerals after reorganization and maturation. However, this hypothesis requires further investigation. This work was supported by the National Science Center (Poland) [UMO-2020/39/B/ST10/01253] and in a part by statutory activity subsidy from the Polish Ministry of Science and High Education for the Faculty of Chemistry of Wroclaw University of Science and Technology.

P-06-004
Strategies for the generation of synthetic biocondensates resistant to organic solvents

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Liquid-liquid phase separation (LLPS), often involving intrinsically disordered proteins (IDPs), underlies the formation of liquid condensates. In the cell, LLPS is responsible for the formation of membraneless organelles (MLOs), which are crucial for dynamic regulation of biochemical reactions. Although biocondensation is still at an early stage of study, it holds potential as a system for concentrating enzymes and substrates within *in-vitro* catalytic microreactors. Little is known about the stability of biocondensates under many operating conditions typical of industrial biocatalysis (*e.g.* organic solvents). Our project aims at the synthesis of protein condensates formed by an engineered IDP capable of encapsulating enzymes of interest. As a proof-of-concept, we have chosen the green fluorescent protein (GFP) as a model globular protein. GFP is easily detectable in fluorescence microscopy and is easy to produce and manipulate. As a "scaffolding" IDR capable of promoting GFP condensation, we designed a synthetic tag, MK, inspired by the composition of human topoisomerase I N-terminal domain[1]. We applied two strategies to achieve GFP condensation: MK-GFP fusion and co-condensation of MK and GFP, expressed independently. Both strategies yield condensates incorporating GFP, with superior efficiency in the fusion construct. Notably, MK-GFP condensates exhibit resistance up to 20% v/v methanol. These results pave the way for utilizing MK in the development of catalytic condensates, whose activity will be evaluated in the presence of various organic solvents and will contribute to elucidate the benefits conferred by condensates to biocatalysis. Reference: [1]Previously published in: Bianchi et al. (2024), *IJBM*, 254, 127754.

P-06-005
Selective phase separation of transcription factors is driven by orthogonal molecular grammar

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Protein production is critically dependent on gene transcription rates, which are regulated by RNA polymerase and a large collection of transcription factors (TFs). Previous studies identified the formation of super-enhancer regions where increased transcriptional activity is observed. This has been linked to phase separation, in which the differential condensation behaviour of separate TF families has been hypothesised to cause the selectivity in gene expression. The underlying molecular forces that are responsible for this selectivity, however, are unknown. Here, we conduct phase separation studies on six TFs (FUS, EWS, TAF15, SP1, SP2, and HNF1A) from three different TF families by carrying out residue-scale coarse-grained molecular dynamics simulations. Our exploration of ternary TF phase systems revealed several orthogonal driving forces: electrostatic and cation-π interactions (TAF15), aromatic interactions (FUS, EWS and TAF15) and aliphatic interactions (SP1, SP2, and HNF1A). The contribution of these driving forces to the homotypic and heterotypic intermolecular strengths dictate the resultant condensate morphology. These results point to sequence-dependent orthogonal grammar as a generic mechanism responsible for selective transcriptional condensation in gene expression. Interestingly, our results also show how RNA polymerase is able to overcome this orthogonality to enter any TF condensate.

Cutting Edge Approaches for Sustainable and Environmental Biotechnology

P-07-001
Tapping the potential of *Dehalococcoides mccartyi's* respiratory complex as a 'power plant' to supply production strains with ATP

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The transition from conventional synthesis of chemicals, which relies on linear processes and fossil resources, towards microbial bio-catalysis using whole cells as 'microbial factories' or isolated enzymes holds great potential. However, cell maintenance and most biosynthesis require energy input in the form of ATP. Our hypothesis centers on utilizing the quinone-independent

organohalide respiratory (OHR) complex of *Dehalococcoides mccartyi* as a 'power plant' to supply production strains with ATP. By applying an external voltage to the OHR complex, electrons from the cathode flow through several metallocofactors within the complex, inducing proton translocation across the membrane and generating a proton motive force, which ultimately leads to ATP synthesis through ATPase. While hydrogen can be used as an electron mediator between the cathode and the entry point of the OHR complex, specific anode mediators shuttling electrons between the output module and the anode are needed. To identify such anode mediators, we conducted methyl viologen-based photometric activity assays and inhibition experiments, revealing electron transfer from the output module of the protein complex to different cobalt-chelates. Electrochemical analyses of these metal complexes *via* cyclic voltammetry showed reversible electron exchange with an electrode, indicating their potential suitability as anode mediators. Bio-electrochemical cultivation of *D. mccartyi* was established to validate *in-vivo* electron transfer from a cathode *via* hydrogen to the input module of the OHR complex, through the electron-conducting subunits of the OHR complex and *via* the identified anode mediators to the anode. This project serves as a cornerstone in engineering electro-active chassis production strains for electro-biosynthesis of valuable chemicals or fuels from renewable sources, using electric current as energy, electrons from an electrode as reducing agents, and the OHR complex as the energy-charging module.

P-07-002
Amplicon selection in comparative studies of *Tussilago farfara* microbiomes from mercury-contaminated and uncontaminated sites

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In an environment heavily contaminated with mercury, we identified the presence of the known medicinal herb *Tussilago farfara* (coltsfoot). Additionally, this species displayed no signs of disease and exhibited larger size compared to those plants from unpolluted habitats. Recognizing the potential significance of the plant microbiome in plants growth and survival, we embarked on an investigation of coltsfoot's leaves and roots metagenome from Hg-contaminated and uncontaminated sites. This approach encounters a challenge due to the undesired presence of prokaryotic host DNA, overshadowing relevant microbiome data, prompting a comparative sequencing analysis of V3–V4 and V5–V7 amplicons to select the most suitable one for further analysis. We obtained twice as many ASVs (amplicon sequence variant) for samples from V3-V4 amplicon than from V5–V7, but 96% or more of these reads were comprised of host DNA. In contrast, the V5–V7 amplicon showed only 60% of host DNA for underground uncontaminated samples and 14% for Hg-contaminated roots samples. Moreover, the use of the V5–V7 amplicon led to a reduction of mitochondrial DNA to 0.7% of the reads. Excluding host DNA, number of identified species were notably higher for the V5–V7 amplicon, as were the shannon and simpson parameters corresponding to biodiversity and evenness. Additionally, the V5–V7 amplicon revealed a higher number of ASVs categorized as "others". Our research highlights the importance of selecting the right amplicon for research. Using V3–V4 amplicon resulted in data loss, while opting for the V5–V7 amplicon proves to be a much more favorable choice for metagenome analyses of *Tussilago farfara*, primarily due to its lower proportion of host DNA, increased biodiversity, and a higher number of assignments and its abundance. This approach illuminates coltsfoot's ecological dynamics in mercury-contaminated areas, offering insights into its potential role in environmental remediation, such as phytobioremediation

P-07-003
Investigation of the microbial community from marine sediments and polynya in Terra Nova Bay, Rossa Sea, in Antarctica

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Extremophiles are bacterial species that thrive in conditions of pH, temperature, pressure and radiation that are incompatible with life as we know it. For decades, this group of microorganisms has been an object of interest in relation to the biomolecules that they produce that are the basis of their survival strategies of adaptation. Several extremophilic species have been isolated from different environments in Antarctica, and many of them have been shown to produce biotechnologically useful biomolecules. The marine Antarctic bacterial communities isolated from Terra Nova Bay have been the object of previous studies that identified as main phylogenetic groups Gammaproteobacteria, Deltaproteobacteria, CF group of Bacteroidetes, Actinobacteria. Here we describe the preliminary results of our sampling activities in the Antarctic Specially Protected Area (ASPA) and in the Terra Nova Bay (TNB) polynya. Polynyas are dynamic stretches of open water surrounded by ice, whose impact for both physical and ecological aspects on the polar oceans, is known to be relevant. Microbial communities inhabiting these extreme marine ecosystems are relevant for several issues form monitoring of the impact of climate change to the search of biotechnologically relevant species. Indeed, these areas host either psychrophilic or halophilic species that produce biotechnologically interesting molecules like enzymes, pigments, lipids, osmolytes. The latter, thanks to their peculiar features, find manifold applications in the biotechnology field with special regard to the sustainable production of chemicals and energy. The investigation of the marine microbial community of this area is therefore aimed at identifying new species and their biomolecules to be tested for new potential biotechnology applications. This research was supported by PNRA19_00073 (TENORE), PNRA18_00232 (AMICI), the PNRR European Commission – NextGenerationEU, Project "SUS-MIRRI" n. IR0000005.

P-07-004
Biotechnology-based approach for environmental bioremediation from toxic aldehydes using an aldehyde dehydrogenase endowed with broad substrate specificity

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Aldehydes are a large class of electrophilic carbonyl compounds present within the environment to which mammalian are exposed, several are considered human carcinogens. The need of detoxifying the environment could rely on the use of enzymes as the most efficient tools for bioremediation[1]. In this context, we identified the Archaeon enzymatic class of aldehyde dehydrogenases (ALDHs) as the best candidate for our purpose. ALDHs catalyze the NAD^+-dependent oxidation of toxic and insoluble aldehydes into the corresponding carboxylic acids which can be easily neutralized and removed from the environment. Starting from the hyperthermophilic ALDH from *S. todokaii*, the Lactaldehyde DH from *M. jannaschii* and the halophilic ALDH from *H. Salinarum*, we performed a structure-based analysis to investigate the interactions between such enzymes and the most environmentally present toxic aldehydes. Through an enzymological and structural approach combined with molecular biology it was possible to design an unnatural *Archaea*-derived ALDH. Specifically, we relied on molecular docking and dynamics simulations to predict possible binding modes and residence time of toxic aldehydes to ALDHs and to find specific residues within the catalytic site to be mutated in terms of chemical and steric features. The modified ALDH will be expressed, purified, and tested for its kinetic activity towards the selected toxic aldehydes. To complete our bioremediation tool, we plan to express and purify the NADH oxidase from *T. kodakarensis* and test it for its capacity to oxidase NADH to regenerate NAD^+ cofactor, as it is essential for ALDHs activity. In the end, we will prepare alginate capsules with the new ALDH in pair with *T. kodakarensis* NADH oxidase and the resulting solid system will be tested for its ability to detoxify all the aldehydes under investigation[2]. Previously published in: [1]Mousavi SM et al (2021) Biochem Res Int, 2021:5599204. [2]Elçin YM et al. (1995) Biomaterials, 16(15):1157–1161.

P-07-005
Tussilago farfara – a plant that withstands mercury pollution: soil and plant metagenome analysis from a Hg-contaminated area

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Tussilago farfara (coltsfoot), known medicinal herb, emerged as the predominant species in heavily mercury-contaminated area and remarkably was larger in size, showed no signs of disease and differences in the maximum quantum efficiency of photosystem II (Fv/Fm ~ 0.8) in comparison to this species in uncontaminated habitats. Building upon our prior *in vitro* investigations, which showed plant-growth promoting features of several bacterial isolates (identified as *Pseudomonas*) from this specific plant, we conducted a comprehensive analysis of the entire metagenome of coltsfoot, considering both above- and underground parts, along with the Hg-contaminated soil, applying the V5-V7 amplicon. The number of ASVs (amplicon sequence variant), diversity and evenness were assigned in the following order: soil > roots > leaves. Nevertheless, each group had a distinct microbiome, since only 7 common ASVs were found for the 3 groups simultaneously. Soil and roots shared 180 sequences, roots and leaves – 27, soil and leaves – 7. In addition, a large individual intra-group dependency was noticed – the metagenome of 5 repeats differed in each group. Most important, for plant samples we distinguished 3 families: *Rhizobiaceae*, *Comamonadaceae*, *Flavobacteriaceae* with higher abundance that showed significant differences among remaining groups. These taxa can be treated as biomarkers of the coltsfoot microbiota in Hg-contaminated habitats and may be important for the plant survival. Our holistic approach highlights the unique ecological dynamics of coltsfoot in mercury-contaminated areas, offering valuable insights into the potential applications of this plant and its associated microbiota in environmental remediation strategies like phytobioremediation.

P-07-006
Investigation of DNA methylation mechanism of cyfluthrin pesticide in zebrafish (*Danio rerio*, Hamilton, 1822) by Sanger and next generation sequence analysis

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Environmental pollution is considered one of the critical problems in modern human life. Excessive use of pesticides in the world negatively affects mainly aquatic organisms. This study aimed to investigate the DNA methylation mechanism of cyfluthrin, a synthetic pyrethroid insecticide known as an endocrine disruptor, in zebrafish (*Danio rerio*, Hamilton, 1822), an aquatic model organism. Zebrafish with an average length of 2.51 ± 0.49 cm and a weight of 0.14 ± 0.06 g were used, adapted to the laboratory, and distributed equally among aquariums. Care was taken to ensure the mortality rate was less than 5% during the adaptation process. In the control and experimental groups, dosing was done by taking 1/10 and 1/100 of the LC50 value of the test chemical (cyfluthrin 3.61 μg/l). Dosing was repeated by changing the aquarium water at the 96th hour and on the 7th day to remove metabolic residues from the aquatic environment. At the end of the 7th day, the head and tail parts of the fish were cut off and stored at −80°C. Sanger and next-generation sequence (NGS) analyses were conducted to comment on DNA methylation after DNA isolation, bisulfite conversion, agarose gel imaging, PCR, and library preparation. The working genes were *CYP1A* and *HSP70*; the reference genes were *ACTB, B2M*, and *EF1A*. As a result, Sanger sequencing methylation percentages were calculated as follows: Group 1 (Control) was 28.36; Group 2 (96th-hour low dose) was 27.26; Group 3 (96th-hour high dose) was 28.31; Group 4 (day 7 low dose) was 28.26 and Group 5 (day 7 high dose) was calculated as 28.22. According to the statistical results of the next-generation sequence analysis, the average differences in the CG, CHG, and CHH regions were significant at 0.05 levels, and a significant relationship was determined between the genes. These findings provide evidence for the

epigenetic mechanisms of cyfluthrin in a vertebrate model and argue for its reduction in the environment.

P-07-007
DNA methylation mechanism of fipronil pesticide in zebrafish (*Danio rerio*, Hamilton, 1822) by Sanger and next generation sequence analysis

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Aquatic pollution from pesticides has become a global concern due to their increasing use and presence in aquatic systems worldwide. Fipronil is a broad-spectrum insecticide from the phenylpyrazole group that acts as a blocker of chloride ion channels regulated by gamma-aminobutyric acid receptors in insects. This study aimed to investigate the DNA methylation mechanism of fipronil in zebrafish (*Danio rerio*, Hamilton,1822), an aquatic model organism. Zebrafish were used, adapted to the laboratory, and distributed equally among aquariums. Care was taken to ensure the mortality rate was less than 5% during the adaptation process. In the control and experimental groups, dosing was done by taking 1/10 and 1/100 of the LC50 value of the test chemical (fipronil 1.81 μg/L). Dosing was repeated by changing the aquarium water on the 7th day to remove metabolic residues from the aquatic environment. At the end of the 7th day, the head and tail parts of the fish were cut off and stored at −80°C. Sanger and next-generation sequence (NGS) analyses were conducted to comment on DNA methylation after DNA isolation, bisulfite conversion, agarose gel imaging, PCR, and library preparation. The working genes were CYP1A and HSP70; the reference genes were ACTB, B2M, and EF1A. As a result, the fipronil control group Sanger sequencing methylation percentage was 28.36; the fipronil day 7 low dose experiment group percentage was 26.73; and the high dose percentage on day 7 was determined as 27.65. According to the new generation sequence analysis statistics, the average differences of the CG, CHG, and CHH regions and a significant relationship between the genes were observed. The hypomethylation results need to be discussed from the environmental epigenetics point of view. Fipronil can be considered an endocrine disruptor chemical and an ecological pollutant for aquatic organisms. Keywords: DNA methylation, fipronil, zebrafish *The authors marked with an asterisk equally contributed to the work.

P-07-008
Unveiling the biodegradative proficiency of terrestrial mud volcano microbiomes for elevated bioremediation of chlorinated volatile organic compound contaminated soil and groundwater ecosystems

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The remediation of chlorinated volatile organic compound (CVOC) contamination in soil and groundwater poses considerable challenges, primarily due to the lack of specific microorganisms and electron donors in the groundwater ecosystem. Mud volcanoes, characterized by the emission of substantial quantities of methane, carbon dioxide, and hydrocarbons, coupled with abundant electron acceptors, foster diverse microbial ecosystems displaying remarkable metabolic versatility and genetic diversity. This study delves into the microbiome of mud volcanoes, elucidating its unique functions in the biodegradation of CVOCs. Results indicate a consistent reduction in oxidation-reduction potential (ORP) across samples, ranging from −52 to −170. Full-length 16S rRNA analysis identifies *Pseudomonas* as the predominant CVOC degrader at the genus level, closely followed by *Hydrogenophaga* and *Desulfomicrobium* in most samples. Methanotrophic bacteria, such as *Methylomicrobium* and *Methylophaga*, are exclusive to samples MV2-2, MV3-1, and MV4-1. Community physiological profiling demonstrates more extensive utilization of carbohydrates, carboxylic acids, polymers, amines, and phenols in the MV4-1 soil, followed by MV2-2. Functional analysis reveals prevalent microbial metabolic functions, including chemoheterotrophy and aerobic chemoheterotrophy in MV1-1 and MV2-1. Similarly, methane oxidation-related metabolic functions, such as methanotrophy, methanol oxidation, and methylotrophy, are more abundant in MV3-1 and MV4-1. This study underscores the biodegradation potential of mud volcano microbial communities and their metabolic capabilities, suggesting their promising role in enhanced bioremediation of chlorinated volatile organic compounds.

P-07-009
The cross-linking activity of polyphenolic extracts derived from winemaking by-products: an opportunity to valorize winery waste

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The most abundant solid by-products generated during winemaking is grape marc (or pomace), which is rich in bioactive compounds, dietary fiber, minerals, and polyphenols. Interestingly, some polyphenols have been shown lysyl oxidase-like activity and can mediate oxidative deamination of lysine residues in proteins. We characterized the enzymatic activity of polyphenolic extracts from grapes pomace using the recombinant human lysyl oxidase like 2 (LOXL2) against two substrates (cadaverine and collagen) through an HRP-coupled fluorometric assay. Moreover, inhibition of LOXL2 activity by β- aminopropionitrile (β-APN), which has been previously shown to be a competitive inhibitor of LOXL2, was used as a specific inhibitor to distinguish the enzymatic from non-enzymatic process. In this work, we identified a polyphenol-rich extract from grapes pomace that promote substrates modification by an amine (lysyl) oxidase-like reaction. In addition, human dermic fibroblasts were cultured with non-toxic concentrations of polyphenolic extracts, that was compatible with long-term exposure necessary for extracellular matrix production. Our results indicate that the treatment of cells with specific concentrations of extracts from grape pomace improve the collagen cross-linking in human dermal extracellular matrix. These finding

establish that the oxidative modification in collagen can be mediated by non-enzymatic systems to form oxidation products with carbonyl functionality.

Bio-Based Polymers for Engineered "Green" Materials

P-08-001
Customized fibrillar collagen scaffolds by 3D printing for cartilage regeneration

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Customized native porcine collagen/chitosan scaffolds were prepared by 3D printing, resulting in porous and biocompatible materials with good mechanical properties. The collagen/chitosan gels were rheologically analyzed to select the optimal formulation that could guarantee that the gel printability at room temperature and with good recovery once printed. Scaffolds of 14 mm in diameter and 5 mm in height were printed using a 14G conical needle and a printing speed of 10 mm/s. The scaffolds were subjected to repetitive 20% compression test cycles showing an excellent shape recovery. The cell density obtained by chondrogenesis showed a cell viability of 99%, with a high chondrogenic capacity and an excellent adhesion of the chondrocyte cells onto the surface and proliferation through the scaffold. Therefore, the customized collagen/chitosan scaffolds prepared in this work have excellent mechanical and biological properties for their use in cartilage regeneration.

P-08-002
Engineered *Bacillus subtilis* for enhancing poly-γ-glutamic acid production finalized to its application as potential wound healing agent

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Poly-γ-glutamic acid (γ-PGA) is a biodegradable and nontoxic homopolypeptide synthesized by *Bacillus* species. For improving γ-PGA production, generally the main routes involve identifying novel producer strains and optimizing fermentation conditions. However, a less explored approach involves controlling the degradation of γ-PGA by inhibiting specific enzymes. Engineered *Bacillus subtilis* strains, PB5390 and PB5523, were herein used to enhance γ-PGA production that was further exploited for preparing a crosslinked hydrogel, after blending with chitosan, for its potential in wound healing. Strain PB5390 possesses a mutation within the degS gene1, responsible for the complete activation of γ-PGA synthesis. The other strain, PB5523, was identical to the previous one but, in addition, it underwent the knockout strain of the genes pgdS and ggt, which play a role in γ-PGA degradation[1]. The biopolymer characterization showed that γ-PGA from PB5523 exhibited a higher molecular weight (135 kDa) compared to the one from PB5390 (105 kDa), with similar yields based on freeze-dried weight and NMR analyses. Zeta potential of γ-PGA from PB5390 was -22.2 ± 1.5 mV, whereas it was -28.0 ± 0.8 mV for PB5523 γ-PGA. Analysis of size distribution revealed variations in particle size and polydispersity index for γ-PGA produced by PB5390 and PB5523, with values of 3105 ± 320 and 3376 ± 392 nm, respectively. The successful formulation of hydrogels through the blending of γ-PGA variants with chitosan[2] showcased favorable attributes. These hydrogels underwent a notable transition from a hydrated state, characterized by compact and bonded behavior, to a rigid, light-pink form upon drying, closely resembling the texture and appearance of human skin. This study highlights the potential of the obtained hydrogel blends for wound healing, emphasizing the need for further evaluations in clinical applications. References: 1. Ermoli, et al. 2021, Res Microbiol 172(6), 103877. 2. Hejazi et al. 2023, Int J Mol Sci 24, 12495.

P-08-003
Methanolic extract of *Marrubium peregrinum* L. induce changes in cytokine levels in a model of experimental wounds in rats

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Marrubium species have been used since ancient times as food additives and herbal treatment. Their phytochemical composition and pharmacological activities have been the focus of scientific investigations recently. Skin restoration process consists of four stages: hemostasis, inflammation, proliferation, and wound remodelling. High levels of pro-inflammatory cytokines (TNF-α and IL-6) lead to delayed onset of the healing phase. Lowering the levels of these mediators could stimulate the healing process of skin wounds, therefore it is a good therapeutic target (1). The aim of the research was to investigate the cytokine levels in an experimental rat skin wound model treated with polyelectrolyte multilayer films with *Marrubium peregrinum* plant extracts. Twenty-eight white Wistar rats were divided into 4 groups of 7 – control group, positive control group (cream-treated), a group treated with a low dose of methanol extract (100 mg/kg) of the

plant and one with high dose (200 mg/kg). Statistical analysis showed a significant decrease in TNF-α ($p = 0.017$) and IL-6 ($p = 0.003$) levels in group 4 treated with extract at a high concentration compared to the blank film control group. Hydroxyproline, an amino acid that is used as a biochemical marker to assess collagen content in tissues during wound healing, was also increased in the skin homogenate in animals treated with a high dose of the extract (0.53 ± 0.26 pg/mg) compared to the control group (0.37 ± 0.18 pg/mg). Because of the mentioned benefits of the plant, more scientific research and well-designed clinical trials are needed to establish its wider use in wound treating medicaments. References: Gunasekaran, S., Nayagam, A. A. J., & Natarajan, R. (2020). Clinical Phytoscience, 6, 1–8. *The authors marked with an asterisk equally contributed to the work.

P-08-004
Genomic and chemical insights into extracellular polymeric substances produced by thermophilic *Parageobacillus toebii* strain H-70

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Extreme environments, such as geothermal springs, offer valuable habitats for extracellular polymeric substances (EPS) producing thermophilic microorganisms, which possess significant advantages in biotechnology and hold potential as tools in circular bioeconomy. This study aimed to characterize the pathways involved in EPS production in the genome of *Parageobacillus toebii* strain H-70 together with its partial chemical composition. The draft genome of strain H-70 was sequenced using Illumina paired-end technology and assembled using the CLC Genomics Workbench 8.5.1 resulting in 3.26 Mb of unique sequence data distributed into 158 contigs. The genome analysis was performed using the RAST and BV-BRC servers. Predicted enzymes were studied through the KEGG databases. DNA-DNA hybridization (90.9%) and ANI values (ANIb 98.05% and ANIm 98.85%) compared with type strain *P. toebii* NBRC 107807 confirmed that the strain H-70 belongs to the *P. toebii* species. For EPS production, the strain was cultivated in minimal media supplemented with glucose 2% (w/v) as a main carbon source (temperature 55°C, pH 7–7.5). EPS was isolated using cold absolute ethanol precipitation (v/v). Monomer composition was determined by high-performance anion-exchange chromatography (HPAE-PAD Dionex ICS 5000+ DC) with a CARBOPAC PA1 revealing the presence of glucosamine and mannose (1/0.3). Genome analysis revealed the presence of ABC and PTS transporter systems which are involved in the transport of monosaccharides and glucosamine. The genome also contained the genes encoding for EpsC and EpsD proteins, which play a direct role in EPS biosynthesis. The work was supported by the Science Committee of RA, in the frames of the research projects 21T-1F191 and Bilateral Agreement MESCS RA- CNR 23SC-CNR-1F010.

P-08-005
3D printable inks based on renewable biopolymers for the manufacture of scaffolds useful for *in vitro* models and organoids

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3D printing technique can create structures for a wide range of applications, such as biomedical scaffolds and functional materials; however, most 3D printing methods use non-biodegradable plastic products and pose a serious threat to the environment. Thus, a sustainable, ecological, and low-cost alternative is needed. In this context, the present activity would aim to take a step forward, developing, and functionalizing scaffolds based on poly(aspartic acid) (pAsp) which is a biobased poly(amino acid) deriving from aspartic acid that has plant origin. Crosslinked pAsp hydrogels have been evaluated in various applications (*e.g.* drug and gene delivery) due to their biocompatibility and biodegradability. Several crosslinking mechanisms have been investigated; but research focusing on functionalization of pAsp and its biological activity on human cell models is still scarce. Here, pAsp was modified comparing norbornene and methacrylate functionalities (De Grave et al. Eur. Polym. J.), and the influence of the crosslinking mechanism on the photo-crosslinking kinetics, mechanical properties and biocompatibility of the resulting hydrogels was studied. The biological effect of these materials was tested on HEK 293T cells, and both hydrogels were non-cytotoxic. Finally, the applicability of the hydrogels to serve as materials for digital light processing and two-photon polymerization was elucidated. pAsp-AEMA resulted better processable via 3D printing, showing higher precision and CAD-CAM mimicry, offering possibilities to create constructs such as organoids serving future biomedical applications. To this end, it was combined with other biopolymers (glycosaminoglycans and methacrylated gelatin) to print 3D porous scaffolds, that were seeded with human mesenchymal stem cells, and evaluated for their ability to induce differentiation into chondrocytes. In fact, the scaffold may mimic the native micro-environments of specific tissues and permit a guided regeneration of cartilage.

P-08-006
The various characteristics and antifungal effects of PMMA incorporating cerium nanoparticles

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Poly(methyl methacrylate) (PMMA) is commonly utilized in dentistry, however, it exhibits a limited ability to prevent the attachment of oral bacteria. To address these limitations, numerous research efforts have concentrated on altering the surface characteristics of PMMA or incorporating substances that possess antimicrobial capabilities. The objective of this study was to incorporate cerium nanoparticles (CNPs) into PMMA to enhance the antifungal efficacy while preserving the mechanical qualities. PMMA specimens with concentrations of 1%, 2%, and 4% of CNPs were produced. The specimens underwent analysis using SEM, EDS, and TGA. The assessment of surface characteristics included measurement of surface roughness, Vickers hardness (VH), and contact angle. The mechanical characteristics were assessed using a three-point flexural strength (FS), fracture toughness (FT), and dynstat impact strength (DI) test. Adhesion tests were performed with *C. albicans*, and the cell activity was evaluated using the Presto Blue reagent. The characteristics of CNPs-PMMA differed based on the amount of CNPs supplied. The presence of increasing levels of CNPs led to an increase in surface roughness and energy. It was found that the strength values of various mechanical properties decreased as the CNPs concentration approached 4%. The anti-adhesion effect was notably highlighted in the case of 4% CNPs-PMMA, with a stronger synergy when used with plasma. PMMA containing CNPs has potential as an antifungal denture material due to its mechanical strength meeting international standards and more effective anti-adhesion through plasma treatment. This work was supported by the Priority Research Center Program provided by the Ministry of Education (2019R1A6A1A11034536), the Ministry of Science and ICT (2020R1A2C1005867, 2022R1F1A1074892), and the Basic Science Research Program funded by the Ministry of Education (2022R1I1A1A01069606).

P-08-007
Use of *Callistemon citrinus* extract for the production of chitosan-based films for industrial applications

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This research addresses the global issue of plastic waste by exploring sustainable alternatives with a focus on bioplastic production[1]. Our study is dedicated to investigating the production of bioplastics using a casting method. The films are chitosan-based with an anthocyanin-enriched fraction of acidified ethanolic extract from *Callistemon citrinus* flowers (CE). *Callistemon citrinus* is an ornamental plant known for its biologically active compounds with potential health benefits[2]. The resulting hydrocolloid films exhibit promising mechanical properties, attributed to the plasticizing effect of CE, which enhances flexibility and manageability. Furthermore, analyses of anthocyanins reveal significant polyphenol content and inhibitory concentration values. Notably, films containing 30% GLY and 5% CE demonstrate improved hydrophobicity properties. Moreover, different concentrations of CE contribute notable antioxidant properties to the films, as demonstrated by various assays including TAC, FRAP, DPPH, and ABTS assays following the method described by Laganà et al.[2]. The FTIR spectra of the films with the addition of CE exhibit slight changes, particularly in the 1500–1700 cm^{-1} region, indicative of the presence of flavonoids. Additionally, the bio-composites obtained are characterized according to their barrier features towards H_2O, CO_2, and O_2. These findings suggest the potential of these bioplastics for various industrial applications, offering a sustainable solution to the environmental impact of conventional plastics and potentially mitigating secondary oxidative reactions in packaged materials. Further research will explore their effects on real food, evaluating their suitability for food packaging applications and potential benefits in food preservation. References: 1 Zhao X. et al., (2020) Environmental Science of Technology, 54 (8), 4712–4732. 2 Laganà G. et al, (2020) Plants, 9, 1045.

P-08-008
Renewable biopolymers SEC-TDA hydrodynamic characterization as a powerful tool towards the optimization of their biomedical applications

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Alginate (Alg), is among the most used biopolymers in the biomedical field, derived from brown algae and it is constituted by 1,4-linked D-mannuronic acid and a-L-glucuronic acid. Molecular-weight (MW) distribution, conformational-features, concentration of the Alg solution, exposed to crosslinking, are expected to have a great impact on final hydrogel performance. Despite this, Alg is not extremely characterized, in most cases, insufficient information is provided and, rarely, a SEC-MALS characterization is supplied. Thus, commercial Alg samples (low, medium, high viscosity LV; MV; HV), from the same supplier were analyzed by size exclusion chromatography-triple detector array (SEC-TDA) to accomplish a hydrodynamic characterization. Key hydrogel features (hydration, rheological properties and stability) were evaluated to correlate them with concentration and MW. SEC-TDA analyses revealed MW: 120, 250, 400 kDa for the LV, MV, HV with the LV and HV showing the largest and the narrowest MW/Mn (2.2–1.4) respectively. Solutions of the diverse Alg (1–6 wt%), exhibited a shear thinning behavior with the HV showing the more marked decrease in viscosity with the shear rate. Same aliquots from the solutions were frozen at −20°C, freeze-dried and then hydrated in presence of calcium ions. Oscillatory measurements confirmed hydrogel formation revealing G' (storage modulus) values exceeding G″ (loss modulus) ones with both the Moduli rather constant with frequency. We found, for each MW, G′ scaled as c^x with x in the range of 2.1–4.1 (R^2:0.96–0.99). The MW did not affect hydrogel stiffness

at low Alg concentration (G′ was around 10 kPa for 1 wt% Alg, regardless of the MW). When rising the concentration, G′ was more markedly dependent on the Alg MW (G′ in the range 66–200 kPa for Alg 3wt%). Regarding the swelling ratio for MV and HV, it scaled as c^x with x 0.4 and 1.9 (R^2: 0.81–0.93). Overall, these data may help in the conscious selection of specific alginates towards specific applications.

P-08-009
From basic principles of protein self-assembly to functional biomaterials

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Natural proteins display critical structural and bioactive properties that have evolved in nature for millions of years. However, depending on the specific protein, there may be useful functions, such as mechanical toughness, while other critical features may be more limiting, such as cell compatibility or a broader range of mechanical properties. Thus, for example, while silk fiber is known for its remarkable mechanical properties, there are still many mysteries surrounding mechanistic aspects of silk fiber formation and evolution of their physical properties, including unique mechanics, strength to weight ratio, self-healing abilities, biocompatibility and biodegradability and many more. Therefore, the mechanism of silk fiber formation is of great interest from both fundamental and applicative aspects. By combining state-of-the-art cryogenic sample preparation, fixation, imaging techniques and spectroscopy-based structural analysis, we were able to analyze silk feedstock processing *in-situ* at the nano- to micron-scales, imaging its macromolecular assemblies and phase transitions along the entire *Bombyx mori* silkworm silk gland. The results from our analysis gave rise to number of new findings, which indicate that the spinning process itself entails a series of structural transitions, from alignment of protein chains in liquid feedstock, through the formation of several fibrillated nano-structures, to nano-fibril bundles, and, in the final stage, a network of cross-linked nano-bundles, which is what determines the structure and properties of the final microfiber.

P-08-010
Gelatines: hydrodynamic parameters and their effect on gelatine-based hydrogels

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Gelatine, a well-known biocompatible polymer, is obtained by hydrolysing collagen extracted from bovine, porcine or fish waste materials. Depending on the specific collagen hydrolytic treatment, two main types of gelatine, A and B, are obtained and commercialized. Commercialized gelatine samples are defined basing on two physical parameters: bloom (a measure of gelatine gel strength) and viscosity. However, no proper characterization of molecular weight distribution is available for these polymeric materials. Gelatine is widely employed for biomedical uses, food industry, drug delivery, often after chemical crosslinking. Key aspects of gelatine-based materials are strongly dependent on the specific gelatine molar mass distribution, therefore a proper hydrodynamic characterization of commercialized gelatine samples as well as the investigation of the correlation between these parameters and the performance of the gelatine-based devices is of great interest. Here, the SEC-TDA system, representing the current most advanced technique for the hydrodynamic characterization of polydisperse biopolymers, was applied for characterizing several gelatines. Thanks to the TDA array comprising a refractive index detector, a four-bridge viscosimeter and two laser scattering detectors (Right Angle and Low Angle) the weight average molecular weight (MW), polydispersity index (MW/Mn), hydrodynamic radius (Rh) and intrinsic viscosity (η) were directly derived for each gelatine product. These samples were used for producing gelatine-based hydrogels by means of enzymatically catalysed crosslinking. The main features of these hydrogels (water-soluble fractions, hydration extent, rheological behaviour and stability under physiological conditions) were evaluated and a potential mathematical correlation with the initial gelatine hydrodynamic parameters was investigated. Data are expected to be helpful for the design and development of gelatine-based materials with specific performance.

P-08-011
Development of a bioinspired mat with chitosan functionality: engineered scaffold for tissue regeneration

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Tissue engineering and cell-based therapies are the two areas of regenerative medicine research that have received the greatest attention. It has been demonstrated that the choice of materials are critical success elements. Natural polymers are preferred by cells as binding scaffolds because they promote cellular adhesion, migration and proliferation. The goal of this work is to develop a biopolymer-functionalized cell scaffold that can mimic the extracellular matrix. Chitosan is a natural polymer known for its antimicrobial effect. Chitosan nanoparticles (CS-NPs) were manufactured by ionic gelation method. The morphologies of the NPs were evaluated by scanning transmission electron microscopy (STEM). CS-NP diameter, zeta potential and polydispersity index (PDI) were measured by Nanoplus 3 Nanoparticle Size/Zeta Potential Measurement Device. A biocompatible polymer, PCL, and a natural polymer, gelatin, were dissolved in hexafluoro isopropanol and the polymer suspension containing CS-NPs was electrospun. Electrospinning was performed using a high-voltage power supply set to 14 kV, a collector distance of 12.5 cm, and a flow rate of 0.5 ml/h. Fiber morphologies and diameters were determined by FESEM analysis. The attachment of keratinocyte cells onto the functionalized fiber mat was observed using SEM analysis and DAPI staining. The biocompatibility of the fiber mat was determined by XTT cytotoxicity tests after 72 h of incubation with keratinocyte cells. The results demonstrated that CS-NPs have spherical morphologies, with a mean diameter of 187 nm and a zeta potential of 35.75 mV. The electrospun bioinspired mat exhibited an average fiber diameter of 560 nm. Cell attachment and biocompatibility tests indicated that the CN-NP functionalized mat could be considered as a biocompatible scaffold to facilitate cell growth and regeneration. The authors acknowledge the support of the REGENEU project (no:101079123) funded by Horizon Europe.

Towards Sustainable Use of Natural and Renewable Resources

P-09-001
Enzymatic hydrolysis of agro-industrial solid leftovers to produce bioactive molecules

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The agri-food industrial sector generates yearly a significant waste stream that has an undesirable footprint on the environment and on the economic well-being of nations. For this reason, there is an urgent need to convert these residues into value-added products. In this context, enzyme-assisted extraction of compounds can represent a greener option compared to traditional non-enzymatic extraction, with benefits in terms of reduced solvent usage and potentiality of processes scale-up. In this study, different agro-industrial residues were subjected to enzymatic hydrolysis by means of endo-glycosyl hydrolase and endo-proteases to produce oligosaccharides and peptides that will be tested for their bioactivity. Each biomass was pretreated to remove lignin, and enzymatically hydrolysed using two commercially available endo-glycosyl hydrolases (endo-1,4-β-D-glucanase and endo-1,4-β-Xylanase) at different enzyme to substrate (E/S) ratio (IU/g). The obtained hydrolysates were analysed via HPAEC-PAD, showing the presence of both low molecular weight oligosaccharides of glucose and xylose (degree of polymerization 2-5) and unidentified peaks related to higher molecular weight oligosaccharides. On the other hand, proteins extracted from each biomass were subjected to hydrolysis by commercial endoproteases, alcalase, trypsin (serine endoproteases) and pepsin (aspartic endoprotease) at different E/S ratios. The results of the digestions were analysed by SDS-PAGE and mass spectrometry, confirming the hydrolysis of high molecular weight proteins after few hours of digestion. The phyto-stimulant and antimicrobial activities of the obtained hydrolysates is under investigation, and process parameters are going to be optimized to enhance the yield of bioactive molecules. This research was funded by Next Generation EU in the framework of National Biodiversity Future Center (NBFC).

P-09-002
Exploring the potential use of edible insect hydrolysates in cellular agriculture

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A major future challenge will be ensuring sustainable production and disposal of sufficient, nutritious food. Alternative protein sources (algae, insects, cell-based meat and seafood, plant-based dairy and meat supplements) offer opportunities to meet the growing global demand for extra protein. Edible insect farming provides greater biomass output and lower environmental impact than conventional livestock and fish farming. To commercialize cell-cultured meat successfully, addressing production cost and safety issues, especially using expensive and non-food-grade fetal bovine serum (FBS), is crucial. Current research seeks affordable alternatives to costly serum substitutes, focusing on sustainable options like insect hydrolysates. Thus, we subjected food-grade cricket flour (*Acheta domesticus*) to alcalase, trypsin and pancreatin digestion and studied the techno-functional properties and serum substitution potential of the obtained hydrolysates. Biochemical analysis of digestion mixtures included pH measurements, conductivity, protein/peptide concentration and profiling, calculation of degree of protein hydrolysis, and sugar and lipid composition analysis. We also tested the antioxidant capacity and other techno-functional properties such as emulsifying, oil holding and foaming capacity. Finally, the impact of different concentrations of protein hydrolysates on doubling time, cell biomass, and cell performance was monitored. In our hands, cricket hydrolysates significantly increased cell growth compared to complete (with FBS) or serum-free media without hydrolysates. Pancreatin hydrolysates showed the highest antioxidant activity and favourable composition, providing a suitable cell culture environment, replacing up to 90% of FBS in CHO-K1 cell culture while maintaining cell health, proliferation and morphology. This study is supported by the Ministry of Science, Technological Development and Innovation, RS. Contract No: 451-03-47/2023-01/200168 (200288).

P-09-003
Pyruvate production from protochatecuic acid, a degradation product of PET plastic

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Polyethylene terephthalate (PET) wastes are currently a heavily underused polymeric materials that represent an abundant source of bulk chemicals, useful for producing high value-added molecules. We focused on protocatechuic acid (PCA), an aromatic derivative originating from the depolymerization and biotransformation of raw materials. In details, PCA was used as the starting point for an enzymatic cascade bioconversion toward the production of pyruvate. The experimental approach is based on *E. coli* RARE, a strain with reduced catabolism toward aliphatic and aromatic aldehydes, transformed with up to 3 plasmids of the DUET series (pRSF-Duet 1, pET-Duet 1 and pCDF-Duet 1), to express a total of six proteins simultaneously: a dioxygenase (LigAB), a dehydrogenase (LigC), a hydrolase (LigI), a tautomerase (LigU), a hydratase (LigJ) and an aldolase (LigK). The main difficulties in establishing an efficient process arose from the hydration equilibria, involving the reactions catalyzed by LigI and LigJ enzymes and the tautomerization of 4-oxalmesaconate, which represents a kinetic bottleneck for the overall cascade [previously published in: Hogancamp TN et al. (2018) Biochemistry 19: 2837–2845]. We proved that the additional LigU enzyme is necessary to establish a fast process: at the moment, up to 10 mM of PCA has been successfully consumed. The employed process involves a whole-cell set-up (expressing the LigAB and LigC enzymes), coupled with a subsequent conversion performed by purified LigI, LigU, LigJ and LigK enzymes. This mixed approach was chosen to avoid the funnelling of pyruvate, the final product, into the central metabolism. This bioconversion cascade represents a valuable starting platform to produce a range of high value-added amino acids, highlighting the great potential of underused waste materials. This work is part of the ProPla project (Fondazione Cariplo 2022).

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P-09-004
Systems biocatalysis for renewable biomasses valorization: dream and/or reality?

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Developing a sustainable biobased process to convert low-value substrates of natural origin to high-value products is an increasingly attractive strategy due to the lower ecological footprint as compared to chemical synthesis. At the industrial level, lignin is a by-product of papermaking industry, currently under-utilized and routinely combusted: its effective valorization is essential for environmental sustainability and to enhance the economics of lignocellulose-based biorefineries. Moreover, wheat bran is an agricultural inexpensive by-product obtained in large amounts worldwide: most of it is used as a low value ingredient for livestock feed. Wheat bran can be processed to extract ferulic acid, a precursor of valuable fine chemicals such as vanillin and/or *cis, cis*-muconic acid (ccMA), a building block for the synthesis of plastic materials. Recently, we developed an efficient green process for producing such valuable compounds based on: (a) the optimization of the extraction procedures of vanillin from lignin and of ferulic acid from wheat bran; (b) the genetic engineering of an *E. coli* strain to modulate the expression of up to seven recombinant enzymes [previously published in: Molinari et al. (2023) ACS Sustain Chem Eng 11, 2476–2485]. In detail, when the optimized whole-cell biocatalyst expressing all seven enzymes was used, ccMA was produced in one-pot with a >95% conversion yield starting from ferulic acid in 10 h, corresponding to 0.73 g of ccMA/g of ferulic acid, and 2.2 mg of ccMA/g of wheat bran biomass. The proposed bioconversion system generating ccMA from different natural and renewable feedstocks (instead of petroleum) represents a starting tool to develop further innovative synthetic biocatalytic processes aimed at generating bioproducts towards a sustainable and biobased economy. This work is part of the project PRIN2022 which has received funding from the MUR (Grant 2022SYTYST).

P-09-005
Steps in development of a genetic engineering tool for *Paenarthrobacter nicotinovorans* ATCC 49919, a soil nicotine-degrading actinobacteria

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Paenarthrobacter nicotinovorans ATCC 49919 is a nicotine-degrading microorganism with biotechnological potential to convert this alkaloid into compounds of industrial and pharmaceutical importance like 6-hydroxy-L-nicotine (6-HLN), methylamine, succinic acid, or γ-amino-butyric-acid. A genetic engineering tool based on the CRISPR system 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 and we focus on inactivating or reducing the expression of 6-HLN oxidase (*6hlnO*), a key enzyme that catabolizes the conversion of 6-HLN to 6-hydroxy-methylmyosmine. Two approaches have been employed, one based on the CRISPR-Cpf1 system that allows gene knock-out and one based on CRISPR/dCasi9 system that allows partial and controlled inactivation of gene transcription. For the first approach, CRISPR-Cpf1 genes from pJYS3-ΔcrtYf plasmid were isolated by PCR using the following primer set: For: TCCGACGTCGTCGACTTTGCTGTTTACAATTAATC ATCGTGTGG; Rev:ACCACTAGTCCTAGGTTTTTGACAGC TAGCTCAGTCCT and cloned into the DraI linearized pART2 vector using Gibson Assembly. Positive recombinant plasmids were selected following digestions with SpeI and ApaLI. We are in the process of targeting the CRISPR-Cpf1 system for *6hlnO* by cloning a crRNA sequence and corresponding protospacers into the pART2-Cpf1. For the second approach, a 20 bp spacer targeting the gene of interest was obtained by annealing two 5′ phosphorylated synthetic oligonucleotides (For: GAAAAA GTTGCAGCATCCAAAGCG and Rev: AAACCGCTTTGGA TGCTGCAACTT) by incubating at 95°C for 3 min and then gradually cooling the mixture by 0.010°C every 10 seconds for 2 h. The spacer was cloned using Golden-Gate assembly into pCasiART. Positive colonies have been selected by blue-white screening. At this stage, pCasiART-Δ6hlnO and pART2-Cpf1 plasmids were obtained, and tests are underway for evaluating the efficiency of these genetic engineering tools.

P-09-006
Antioxidative, antimicrobial and anticancer evaluation of olive *Olea europaea* L. leaf extract

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The food industry, being a substantial source of biowaste, particularly in the form of biomaterials like leaves, has sparked considerable concern regarding effective waste management and its environmental impact. Given that these biomaterials are often rich in components possessing antioxidant and antimicrobial properties, the food industry is currently investigating potential applications for utilizing them as novel ingredients in the functional food. In that sense, we prepared olive (*Olea europaea* L.) leaf extract (OLE) with microwave-assisted extraction (MAE), to evaluate its possible health-protective properties. The obtained OLE contained a high content of total phenols and flavonoids (e.g. oleuropein, rutin, tyrosol) with the sum 35.23 ± 0.79 mg GAE g^{-1} and 3.56 ± 0.14 mg QE g^{-1}, respectively. The *in vitro* antioxidant power of OLE was evaluated using FRAP method and capacity was 38.67 ± 1.35 mg TEQ g^{-1}. Antimicrobial activity of OLE was tested using the disk diffusion, the microdilution, and the colony counting method. Out of 13 microorganisms used, only the growth of *S. aureus* was inhibited (MIC = 17.62 mg GAE g^{-1}). Further, since many antioxidant-rich plant extracts possess anticancer activity, we tested OLE effect on the breast (MDA-MB-231 and MCF-7) and prostate (PC-3) cancer-type cell models. After 24-h treatment the highest anticancer effect was observed on the most progressive cancer type, triple-negative MDA-MB-231 (IC_{50} = 89.67 ± 4.33 μg g^{-1}). Overall, antibacterial, anticancer and antioxidant potential make OLE a promising biomaterial to investigate its further effects in specific therapies. Acknowledgment: This study was supported by European Structural and Investment Funds (KK.01.1.1.04.0096), and by European Union – Next Generation EU (Class: 643-02/23-01/

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P-09-007
Value-added products from agri-food wastes through enzyme technology

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Agri-food wastes have gained increasing attention as a source of valuable bioactive compounds, such as polyphenols and fermentable sugars, with applications in the food, pharmaceutical, and cosmetic industries [1,2]. The use of sustainable methodologies to extract these compounds is an appealing way to replace harsh chemical treatments [3]. This work aimed to evaluate the total phenolic content (TPC), antioxidant capacity, reducing sugar content, antimicrobial compounds, and antiproliferative activities of agri-food extracts. In particular, spent coffee grounds (SCGs), sunflower, and citrus residues were subjected to an enzyme-assisted extraction (EAE) method by using different commercial cocktails such as Viscozyme L (V), Cellulase (C) or V + C (Novozyme). TPC and antioxidant capacity of SCGs, sunflower, and citrus extracts did not show significant differences between the diverse enzymatic treatments. Whereas a 57% increase in reducing sugar content was observed in the citrus residue extracted with V + C treatment than in the individual cocktails. The antimicrobial activity of waste extracts was also evaluated on several microorganisms. It is worth noting that the V + C treated citrus residue showed a significant inhibition of the growth of several microorganisms (MIC = 5 mg/ml) and the development of microbial biofilm at the sub-MIC concentration of 2.5 mg/ml. Interestingly only citrus extract resulted in no cytotoxic of on human dermal fibroblasts (HDF) whereas it exerted an antiproliferative effect on metastatic melanoma cells (WM266). Different combinations of EAE on food residues have highlighted that citrus extracts were the most promising showing low MIC value, high inhibition, and eradication of biofilm and these extracts did not affect the viability of normal cells but showed a cytotoxic effect on melanoma cells. References: 1. doi:10.3390/antiox9050438. 2. doi: 10.3390/antiox7050067. 3. doi:10.1093/fqs/fyx004. *The authors marked with an asterisk equally contributed to the work.

P-09-008
Phytochemical characterization of water and ethanol extract from *Micromeria friwaldszkyana*

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Medicinal plants contain different substances (i.e. flavonoids, alkaloids, tannins, and terpenoids) that reveal antioxidant and antimicrobial effects (Mladenova et al., 2021). The species *Micromeria frivaldszkyana* is a Bulgarian endemic, included in Appendix 3 of the Biological Diversity Act and the Red Data Book of the Republic of Bulgaria under the category endangered (Mladenova T et al., 2021). There are several studies on the antiinflammatory and antimicrobial effects of *Micromeria* against some pathogens (Mladenova T et al., 2021). Recently conducted UPLC-MS-MS assays on ethanol and water extract samples of *M. friwaldszkyana* showed that both extracts were equally rich in linarin. Except linarin, the extracts had approximately equal concentrations of eupatilin and quinic acid. Other secondary metabolites in the ethanol and water extract were flavonoids like quercetin-3-O-glucoside-7-O-glucoside; kaempferol-3-O-glucoside-7-O-glucoside and other kaempferol-3-O-glucosides. In ethanol extract diosmetin-7-O-glucoside and luteoloside were found, which were not presented in the water extract. In addition, threo-3-isopropylmalic acid, 5-feruloylquinic acid and 3-methylglutaric acid were detected in the water extract, but not in the ethanol extract. Moreover, rosmarinic acid concentration was significantly high in the ethanol extract, whereas in the water extract it was not even in the leading positions. In conclusion, the ethanol extract was abundant in rosmarinic acid, and the equal concentration of linarin for both solvents indicate that the ethanol extract had the potential for higher biological activity. Reference: Mladenova T. et al. Plants (Basel). 2021 Apr 7;10(4):710. doi: 10.3390/plants10040710. *The authors marked with an asterisk equally contributed to the work.

P-09-009
Recovery of bromelain from pineapple core: a waste valorization strategy

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Bromelain is a mixture of cysteine endopeptidases usually extracted from pineapple juice and widely used for the treatment of various human diseases, in the nutraceutical and cosmeceutical sectors, and in the food industry. Since bromelain demand has been quickly increasing in recent years, its recovery from pineapple wastes represents a sustainable waste management strategy. Pineapple core can account up to 15% of the total processing waste and is commonly richer in bromelain than other pineapple residues. In this project, we compared the enzymatic properties and composition of bromelain preparations extracted from either pineapple core or pulp to address the recovery of bioactive bromelain from pineapple core as a potential strategy to the valorization of this waste material. Despite the significantly different protein content of the two preparations, no differences were assessed in their proteolytic activity and in the effect of pH on their enzymatic activity. MS approaches identified the same peptidases in the fruit and in the core. This confirmed the possibility of using pineapple core to obtain relevant amounts of bromelain fully comparable to the enzyme obtained from the pulp, thus paving the way to more sustainable practices in this specific industrial sector.

P-09-010
Design of (bacterio)chlorophyll inspired antenna systems for artificial light-harvesting devices

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Renewable energy sources, particularly solar energy, are crucial to supply mankind's increasing demands. In this regard, artificial photosynthesis represents a promising research area where natural photosystems can be employed as models for designing and engineering synthetic light-harvesting devices. For instance, the light-harvesting system 2 (LH2) and its constituent bacteriochlorophyll (BChl) chromophores represent a great example of studying Nature's way of collecting and channeling solar energy. Understanding the mechanisms and the fine details regulating energy absorption and transfer in LH2 represents a major challenge. Within this research topic, in the presented project, we employ advanced computational chemistry tools to accurately describe the photochemical properties of novel, BChl-inspired, chromophore models, aiming to assess their spectroscopic characteristics. Moreover, the multichromophoric aggregates of natural and synthetic light-sensitive complexes absorb light at different wavelengths compared to their constituent molecules. Thus, the assessment of the underlying intermolecular interactions (i.e. couplings) is crucial when trying to understand and rationalize the behavior of such systems. Consequently, we evaluate the aggregate behavior for numerous dimeric derivatives of BChl-like systems thanks to a recently developed code, integrated into the OpenMolcas Program [1][2], that allows for the assessment of the inter-monomeric couplings, in the spirit of Frenkel Exciton Hamiltonian model. The implemented computational methods enable an accurate description (at the multireference, multiconfigurational level of theory) of the photochemical and photophysical properties of the pigment units and set the basis for the future development of novel artificial photosynthetic devices. References: 1. Kaiser et al. (2023) J Chem Theory Comput 19, 10, 2918–2928. 2. Li Manni et al. (2023) J Chem Theory Comput 19, 20, 6933–6991.

P-09-011
Polyphenol-functionalized selenium nano-formulations: utilizing waste-derived bioactive compounds for improved functionality

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Selenium nanoparticles (SeNPs) have been investigated intensively as the novel forms of selenium for nutritional supplementation. Their major advantages, in relation to inorganic or organic forms of Se are increased bioavailability and therapeutic potential due to high Se-density of the nano-formulation. Because of the large surface area, SeNPs show immense potential as carriers of bioactive compounds and therapeutics improving their absorption and enabling targeted delivery. In this work, polyphenol-functionalized selenium nano-formulations were synthetized using extracts obtained from agricultural wastes: olive and tomato pomace. Tomato pomace-derived pectin was used as stabilization agents for SeNP while olive waste-derived polyphenols were used for SeNP surface stabilization. Cytotoxic and antioxidative activities of functionalized SeNPs (fSeNPs) were compared to inorganic selenium forms and selenium nano-formulations obtained through standard synthesis using chemical stabilizers (sSeNPs) SeNPs were characterized in terms of shape, particle size distribution and zeta potential. Their cytotoxicity and antioxidative activity were investigated in human hepatocellular carcinoma (HepG2) and human colorectal adenocarcinoma (Caco2) cell lines. Green synthesis of SeNPs resulted in formation of stable nanoparticles with satisfactory physico- chemical properties. fSeNPs showed lower cytotoxicity and wider range of direct antioxidative mechanisms in comparison to sSeNPs and inorganic selenium forms. The significant impact of fSeNPs on intracellular antioxidative mechanisms has been observed and it was dependent on physico-chemical properties, applied concentration and type of the cell culture, indicating the overall complexity of involved mechanisms. *The authors marked with an asterisk equally contributed to the work.

P-09-012
BPLH a waste-based biomaterial from *Lupinus albus*: sustainable scaffolds in wound healing and bone tissue repair

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Reprocessing vegetal waste to produce new biomaterials with biomedical applications represents a new and impactful research field [Cancelliere R. et al. (2021) Talanta 121671; Buonvino S. et al. (2023) Biomaterials 293, 121984]. Recently we have obtained from *Lupinus albus* hulls (LH) a sustainable biomaterial named BPLH (Bioplastic-Lupin Hulls) with notable scaffolding properties of mesenchymal stem cells (MSCs) and normal human dermal fibroblasts (NHDFs) [Buonvino S. et al. (2023) Biomaterials 293, 121984]. Thanks to its high content of cellulose and beneficial phytochemical substances, LH represents a suitable, low-impact and low-cost source for fabricating biocompatible and bioactive scaffolds. Here the LH reprocessing protocol was modified to obtain a mesoporous BPLH biomaterial (BPLHp) with the aim to increase gas permeability and enhance the material's bioactive properties, also allowing for functionalization approaches. BPLHp was characterized by confocal-fluorescence microscopy and scanning electron microscopy. BPLHp is characterized by an autofluorescence property, which was here used for the direct monitoring of live cells growing in the scaffold without any staining procedure opening the way for biosensing and nanotechnological applications. Furthermore, an interesting property of BPLHp was the ability to induce the alignment of NHDFs cultured on plate suggesting potential application of the BPLHp in wound healing. The growth of MSCs on the BPLHp scaffold was also here tested and after three weeks of cell culture the stem cells showed an osteo-differentiation. Our work represents an effort toward the recycling and valorization of the vegetal waste, showing the remarkable properties of promising BPLH material as cell-scaffold useful for topical patches in wound healing and bedsores treatment and in bone regeneration.

P-09-013
Removal of synthetic dyes from water solutions using egg-white proteins amyloid fibrils

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Water pollution represents one of the global leading factors for illness and death. Synthetic dye compounds originating from the textile, paper, cosmetic, and leather industries are frequently discharged to environmental waters. It has been proven that synthetic dyes may have harmful and toxic effects on the environment, even in low concentrations. Therefore, it is of great significance to explore novel methods for their removal. In this research, we have prepared amyloid fibrils using sustainable protein source and quantified amyloid's dye-binding capacities for multiple synthetic dyes. Alongside this, we have examined the effect of acidic/alkali conditions on the amyloid dye-binding capacity. It is shown that the pH level prominently altered the dye-binding capacities implicating that the binding process is, at least partially, electrostatically driven. We have put the foundations of a novel method for the purification of water contaminated by synthetic dyes, emerging on its simplicity, low cost, and eco-friendliness.

P-09-014
Citrus sinensis wastes as renewable resource of active molecules

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The exploration of sustainable alternatives for crop protection suggests that biopesticides, bioactive compounds produced from living organisms, could be a notable substitute for synthetic pesticides, acting in a totally eco-sustainable manner. Numerous studies have reported that the use of essential oils (EOs) is effective against pests, and their potential antimicrobial and antioxidant properties will be a help for the development of novel biopesticides for eco-friendly agriculture. These could be extracted from waste products which account for a huge amount each year. This study is focused on the EOs isolation from *Citrus sinensis*, through comparing extracts from two different organs of waste orange plants, the peel and the albedo. An innovative solid-liquid extractor, the Extractor Naviglio, works at room temperature to preserve the bioactive compound content[1], with a processing time between 2 and 4 h, and ethanol as solved is used. The chemical compositions of volatile compounds, which were determined by using gas chromatography, appear to overlap in both samples, although the different compounds are present in different quantities. The most high-boiling components obtained by Naviglio extractor method are more representative than other methods conventionally obtained[2]. Furthermore, the antioxidant activity and total phenolic content were determined by using the DPPH method and the Folin-Ciocalteu method to understand their potential applications[3]. There was a linear correlation between the antioxidant activity and the total phenolic content of the samples. This represents the first step for processing waste material to obtain a valuable material such as essential oils that could have a high market value and useful for various applications. References: 1. Naviglio D et al. (2003) Anal Lett 36,8, 1647–1659. 2. Cholke PB et al. (2017) RJLBPCS 2,5, 41–51. 3. Torres-Alvarez C et al. (2017) Cyta – J Food 15,1, 129–13.

P-09-015
Genomic mining of *Geobacillus stearothermophilus* GF16 for xylose production from hemicellulose-rich biomasses using secreted enzymes

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The valorization of lignocellulosic biomass is a sustainable approach to enhance production chains while reducing environmental impact. Microbial enzymes, especially glycoside hydrolases (GHs) like xylanases, play a key role in cellulose

degradation and xylose production for bioethanol and other industries[1]. In this context, enzymes from thermophilic bacteria are stable, efficient and represent a convenient alternative to fungal secretomes[2]. This study reports the genomic characterization of the thermophilic bacterium *Geobacillus. stearothermophilus* GF16 to identify genes encoding putative enzymes involved in lignocellulose degradation. We assess the activity of thermo-stable GHs secreted by this bacterium, grown under different conditions, on various natural polysaccharides and synthetic substrates, revealing a palette of inducible GH activities. In particular, the concentrated secretome exhibits thermo-stable xylanase and β-glucosidase activities, highlighting a potential for biomass valorization. Therefore, the hemicellulose hydrolysis of different agri-food wastes by the concentrated secretome was evaluated and the monosaccharides released estimated. The findings reveal that xylose is the main sugar produced and the concentration is 300-fold higher than that produced by a commercial cocktail. The new strain is a promising candidate for low-cost enzyme production, decisive for converting biomass into high-value products like xylan. References: 1. Salzano, F. et al. (2024) Int J Biol Macromol 264, 130550. 2. Carbonaro, M. et al. (2022) Int J Mol Sci 24 (1), 243.

P-09-016
Chitosan- and chitosan-polaxamer 407 based nanoparticles: green nanotechnology tools for biomedical applications

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Biopolymers are a sustainable, available, and low-cost alternative suitable for the development of green technologies. For this purpose, chitosan nanoparticles (ChNPs) have been widely studied as promising tools in a wide variety of areas, including pharmaceutical and nutraceutical delivery [1]. Nevertheless, ChNPs' stability in aqueous solution has some limitations. In this work, we studied how to improve the stability of ChNPs using an FDA approved non-cationic surfactant – Polaxamer 407, a tri-block co-polymer with polyoxyethylene (POE) (A) and polyoxypropylene (PPO) (B) units in an A-B-A arrangement (POE-PPO-POE) [2]. Ionic gelation (IG) was the physical crosslinking method used for producing ChNPs due to its low level of toxicity (using a non-toxic crosslinker, STPP) and high production yield [3]. Nanoparticles were synthesized using a Ch/STPP mass ratio (5:1, 5:2, 5:4 and 5:6) and physical-chemical characterization through FTIR, TGA and DLS were performed. The 5:2 mass ratio provided better results in size (Z-Average, d.nm) (334.5 ± 29.39 d.nm), polydispersity index (PDI) (0.43 ± 0.03) and zeta potential (ZP) (ζ) (+32.2 ± 1.98 mV). On another set of experiments, Polaxamer 407 was used as a stabilizer for ChNPs preparation following 5:2 Ch/STPP mass ratio. The DLS results confirmed a significant improvement in size (Z-Average, d.nm) (265.6 ± 11.07 d. nm), (PDI) (0.307 ± 0.042) and ZP (ζ) (+44.2 ± 0.907 mV) parameters. In conclusion, Polaxamer 407 addition greatly enhanced ChNPs stability in water. Also, the non-toxic feature of these materials makes them suitable candidates for biological and medical applications. References: 1. Shirvan AR et al. (2018) Prosp Green Chem Textile Techn. 107–133. 2. Cortés H et al. (2021) Materials 12, 1–39. 3. Bavel NV et al. (2023) Molecules 28, 1–14 PhD Project funded by FCT (Fundação para a Ciência e Tecnologia), Fellowship Ref[a] 2022.09574.BDANA. *The authors marked with an asterisk equally contributed to the work.

P-09-017
Treatment of *Lactuca sativa* seeds with non-thermal plasma and plasma activated water stimulates germination, seedling growth and activates biochemical processes

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Rapidly increasing needs for agricultural products urge the development of the sustainable agricultural technologies directed towards enhancement of yields and quality of production, using environmentally friendly methods as an alternative to the traditional ones, based on the intensive use of chemicals for fertilization and plant protection. Among such technologies, the field of plasma agriculture has gained increasing attention. The aim of this study was to compare the effects of the combination of seed treatment with non-thermal plasma (NTP) and imbibition in plasma activated water (PAW) on germination, early sprout growth and the amounts of secondary metabolites in 10-days-old seedlings of two lettuce (*Lactuca sativa*) cultivars: cv. Perl germ and cv. Cervanek. Low-pressure NTP and atmospheric dielectric plasma discharge (DBD) plasma irradiation devices were used for seed treatment. Gliding arc discharge plasma device was used for PAW production from the deionized (DW) or tap water (TW). Both seed treatment with NTP and PAW(TW) increased germination rate (by 6%) and early growth of seedling roots (up to 15%) of cv. Perl germ when applied separately, however the combination of NTP pre-treatment and PAW did not enhance the stimulatory effect. Treatments were less effective for cv. Cervanek; only DBD plasma stimulated germination by 3%. Only PAW produced from TW affected seed germination due to higher concentration of hydrogen peroxide, as compared to PAW generated using DW. The amounts of photosynthetic pigments and total phenolic compounds (TPC) were 9–15% higher in leaves of *L. sativa* seedlings from positively affected treatment groups, but the size of effects on these biochemical parameters was strongly cultivar-dependent. This work was supported by the Research Council of Lithuania under Grant S-MIP-23-8. *The authors marked with an asterisk equally contributed to the work.

P-09-018
Heavy metal chelating capability of microbial levan: an opportunity for sustainable agriculture

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Pseudomonas strain 2ASCA, an EPS producer microorganism, was isolated from sediment sample of permafrost thaw pond (thermokarst lake) in subarctic Québec. Strain 2ASCA grows optimally at temperature of 15°C and can produce an exopolymer (yield 1.17g/l), located in the loosely bound cell membrane fraction, in the presence of sucrose 6% (w/v) as sole carbon source, after 96 h of incubation. Chemical and spectroscopy studies revealed the levan-type nature of exopolymer released by strain 2ASCA with a repeating unit consisting of β-(2,6)-linked fructose and a molecular mass higher than 2 000 000 Da. Scanning electron microscope-energy dispersive X-ray spectroscopy (SEM-EDS) showed the levan capability to sequester different heavy metals, such as Cu (II), Zn (II), Pb (II), Fe (III) and Cd (II). Furthermore, a strong affinity was revealed for Cr (III), which has never been previously reported for levan polymer, highlighting an interesting biosorption potential, for instance in agriculture field. Recently, the modifications of soil microbial communities drove by the controlled addition of bacteria as plant growth promoter (PGP), represent a new tool in sustainable agriculture. *Azospirillum*, *Pseudomonas*, *Enterobacter*, *Bacillus*, *Serratia*, *Burkholderia*, *Variovorax* and *Klebsiella* are the genera that have shown the most beneficial effects on plant proliferation. The genus *Pseudomonas* is the one that has caught the attention of researchers for its ability to colonize roots, production of enzymes and metabolites, nutrient solubilisation, synthesis of indole acetic acid and siderophores, acting as a biocontrol agent and inducing systemic resistance to plant disease. Ongoing studies are testing the effects of heavy metal chelating capability of levan from strain 2ASCA on barley (model organism plant) thank to projects PRIN 2022LPPFTY, -TREASURE, MICS (Made in Italy – Circular and Sustainable) from the Next-Generation EU PNRR n. PE00000004 and the "SUS MIRRI" n. IR0000005.

P-09-019
Extremophilic bacteria as smart tool for vegetable waste valorization

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Extremophilic bacteria are microorganisms able to survive and to grow optimally under extreme conditions and represent a biotechnological treasure by producing a large portfolio of biomolecules such as thermozymes, exopolysaccharides, biopolymers, organic compounds, that have the potential to be valuable resource for the development of a bio-based economy. The ICB-CNR owns an extremophilic microorganisms' collection (strain library) consisting of thermophiles, thermoacidophiles, hyperthermophiles, halophiles, haloalkaliphiles and psycrophiles, which have been isolated from samplings carried out in various extreme habitats (volcanoes, glaciers, salt and soda lakes, etc.) distributed all around the world. Processing and selection of fruits and vegetables in addition to residues of dedicated crops, generate high amounts of wastes which represent an economical and environmental issue for the agroindustrial sector. Nevertheless, according to the so called "biorefinery" approach, this waste biomass can be exploited as sole carbon source to produce microbial biomasses and their related molecules. The α-amylase production by the thermophilic *Anoxybacillus amylolyticus* was described, by growing the strain in submerged and solid-state fermentation on rhizomes from *Arundo donax* L., on steam and leaf from *Cynara cardunculus* and potato peel wastes. The xylanase produced by the thermophiles *Parageobacillus thermantarcticus* was increased by using a cheaper medium containing residues of *C. cardunculus* with the respect to standard growth media. The levan-type exopolysaccharide released by *Pseudomonas* strain 2ASCA was increased by setting up microbial fermentation conditions based on the re-use of the molasses obtained from sugar beet processing. The present work was partially financed by PNRA19_00073 (TENORE), PNRA18_00232 (AMICI), MICS (Made in Italy – Circular and Sustainable) from the Next-GenerationEU PNRR n. PE00000004, and by PRIN 2022LPPFTY -TREASURE.

P-09-020
Evaluation of an H_2 fuel cell electrochemical system powered by microbial cells

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Escherichia coli and *Cupriavidus necator* H16 are model organisms in the generation of O_2-tolerant [NiFe]-hydrogenases (Hyds), significant biocatalysts for biological fuel cells (BFCs). It is important to get active Hyds economically, which means using cheap waste materials. Glycerol is a main sidestream of biodiesel and other industries. The efficiency of applying the 3 µl (1.5 mg cell dry weight) of *E. coli* and *C. necator* H16 intact cells on the 0.5 cm^2 as anode catalyzers in the bio-electrochemical system was evaluated. The flow rates of gases into the samples were maintained at 0.5 L per min. The reaction continued as long as Hyd substrate H_2 was supplied and stopped immediately if the supply was interrupted. Whole immobilized cells of *E. coli* and *C. necator* H16 (grown on glycerol) produced an electrical potential of 0.65 and 0.75 ± 0.03V when pure H_2 was supplemented into the system. The system operated at a temperature of 37°C. The system exhibited no or negligible change in signal when pure N_2 was supplied. However, supplementation of pure CO_2 initiated ~0.15 ± 0.05V electrical potential generation during the operation of *E. coli* cells. The sensitivity of the system operating under various concentrations of H_2 (ranging from 20% to 80% H_2 in a gas mixture of 80% air, N_2, and CO_2, respectively) with immobilized *E. coli* was investigated. In all samples, as the concentration of H_2 in the gas mixture increased, there was a corresponding rise in electrical potential. Compared to NO_2 and CO_2, the electrical potential generation is reduced when air is supplemented with varying amounts of H_2, indicating the negative impact of O_2 in the air. On the contrary, the highest potential is attained when H_2 is mixed with 20 and 80% CO_2 reaching up to 0.6 ± 0.01 V mixed with CO_2. The findings will foster the development of microbial-based fuel cell (BFC) sensors, offering significant promise for renewable green energy and diverse applications.

P-09-021
Boosting antioxidant activity and phenolic content in carrot roots: harnessing the power of natural biofungicides

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This study examines the effects of natural plant-based extracts as biofungicides on carrot roots' antioxidant activity and total phenolic content. Natural alternatives are being explored in response to concerns over the environmental and health impacts of chemical fungicides. Field trials were conducted at the experimental fields of the Institute of Horticulture, Lithuanian Research Centre for Agriculture and Forestry, in 2022 and 2023. Carrots were sprayed five times during the vegetation period with investigated *Thymus vulgaris* and *Syzygium aromaticum* plant extracts. A control without extract treatment was used to compare the results. After harvesting, the total phenolic content (TPC) and antioxidant activity of fresh roots were analyzed. The antioxidant activity of the samples was tested using the DPPH (2,2-diphenyl-1-picryl-hydrazyl-hydrate) and ABTS (2,2′-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid)) assays. Our research demonstrates that applying natural biofungicides leads to notable increases in TPC and antioxidant activity in carrot roots compared with the control. The highest content of phenolic compounds (34 mg GAE 100 g^{-1} fw) and strongest antiradical activity (DPPH, 1.20 μmol TE g^{-1} fw and ABTS, 4.14 μmol TE g^{-}1 fw) had carrot roots after *S. aromaticum* extract treatment in the field. Meanwhile, *T. vulgaris* essential oil weakly influenced TPC and antioxidant activity in carrot roots. These findings suggest that the phytochemicals present in plant extracts induce stress-response mechanisms in carrots, enhancing their resilience to fungal pathogens while simultaneously promoting their nutritional quality. Such results not only highlight the efficacy of natural biofungicides but also underscore their potential to contribute to sustainable agricultural practices. This research provides valuable insights into optimizing crop protection strategies while promoting food safety and nutritional benefits. *The authors marked with an asterisk equally contributed to the work.

P-09-022
The influence of different carbohydrate sources in defined medium on the exopolysaccharides and carotenoids production by *Rhodothermus marinus* DSM 16675

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Rhodothermus marinus, is an extremophile, well-known for its diverse carbohydrate-degrading enzymes, resulting in potential to utilize different feedstocks as carbon sources. However, there is limited data on growth kinetics and secondary metabolite productivity in defined medium, necessitating further investigations of its potential to grow on various carbon sources. This study evaluated the growth kinetics, and production of native carotenoids and exopolysaccharides (EPSs) by *R. marinus* DSM 16675 in an optimized defined medium supplemented with 1–5 g/l of various mono-, di-, tri-, and polysaccharides. The monosaccharides, arabinose (2 g/L), xylose (2 g/l), galactose (5 g/l), and glucose (5 g/l) supported substantial growth and cell dry weight (CDW), while fructose (1–5 g/l) and mannose (1–5 g/l) did not result in visible growth. The disaccharides, lactose (5 g/l), maltose (5 g/l), and sucrose (5 g/l) were fully utilized, whereas use of cellobiose (1–5 g/l) as carbon source, did not result in visible growth. The trisaccharide raffinose and the polysaccharides starch, and xylan, all supported growth but were not completely utilized. Notably, growth also occurred in alginate containing medium (1–3 g/l) when glucose was used as a co-substrate, indicating the potential to use marine alginate as a carbon source for *R. marinus* DSM 16675. Among the tested carbohydrates, galactose (5 g/l) yielded highest biomass (CDW: 2.5 ± 0.00014 mg/l) and EPSs production (3.915 ± 0.1008 mg/l), while sucrose (5 g/l) resulted in the highest total carotenoids production (1.63 mg/l). The monosaccharide content of the produced EPSs varied with the carbon source, indicating an effect on EPSs composition. Both the carotenoids, and EPSs production were shown to be correlated with the carbon consumption. This study sheds light on the potential of the strain *R. marinus* DSM 16675 as a biorefinery organism, expanding the utilization of 2nd and 3rd generation feedstocks as carbon sources.

P-09-023
Riboflavin production by a mutant *Limosilactobacillus* fermentum in vegetable beverages

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Some lactic acid bacteria (LAB) possess the ability to synthesize riboflavin, a trait linked to the rib operon. Riboflavin (vitamin B2) is a key vitamin involved in essential biological pathways and nutrient metabolism, and the capability of riboflavin synthesis is a strain dependent property. Several strains of *Limosilactobacillus fermentum* have shown probiotic properties, applications in the biomedical and food field. In fact, different strains showed immunomodulatory as well as anti-infectious properties, and it has recently been used as food preservative and to obtain functional foods with improved health properties [previously published in: Naghmouchi et al. (2020)]. The aim of the present work was to select a riboflavin over producing food grade LAB for the vitamin biofortification of fermented foods. The presence of riboflavin biosynthesis genes, namely ribG, ribB, ribA and ribH was investigated and verified in an *L. fermentum* strain isolated from buffalo milk [previously published in: D'ambrosio et al. (2022)]. Successively spontaneous roseoflavin-resistant riboflavin overproducing mutants were selected to improve riboflavin titers. The mutant strains, overproducing riboflavin were used to ferment oat beverage in small scale bottle experiments and the results successfully demonstrated the enrichment of oat beverage with riboflavin up to 0.79 mg/L. The scale up of the process in controlled bioreactor conditions to further optimize riboflavin production and characterize strain physiology is ongoing.

P-09-024
Cupriavidus necator H16 growth and H2-oxidizing activity using mixture of glycerol and roasted coffee waste

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Coffee as one of the most consumed beverages globally, generates substantial volumes of spent coffee grounds (SCG) daily. In this study the potential utilization of spent coffee grounds (SCG) through the application of the chemolithoautotrophic β-proteobacterium *Cupriavidus necator* H16. *C. necator* H16 demonstrates a diverse metabolic spectrum. Bacterium is known for its capacity to synthesize different O_2-tolerant [NiFe]-hydrogenases (Hyds) and serves as potential anodic biocatalysts in microbial fuel cells. SCG were collected from a local cafeteria: they were oven dried at 70°C until constant weight. 4% SCG were treated by physicochemical method at 121°C. Total H_2-oxidizing activity of bacterial cell was measured by methylene blue (MB) reduction, 570 nm, 30°C, using a spectrophotometer (Cary 60 UV–vis, Agilent Technologies, USA). Growth parameters, oxidation-reduction potential (ORP) and pH kinetics and H_2-oxidizing activity was investigated upon utilization of SCG hydrolysate supplemented with 4 g L^{-1} glycerol. Bacterial growth was followed for 72 hours. With the increase in biomass, the decrease in medium ORP and pH was stated. However, glycerol supplementation enhanced the growth ~ 1.5-fold. H_2-oxidizing activity of bacterial cell identified during bacterial late stationary phase reached up to 0.5 U mg^{-1} cell dry weight (CDW). Nevertheless, upon glycerol supplementation the H_2-oxidizing activity was ~ 4.6 and 12 U mg^{-1} CDW at 48 and 72 hours, respectively. The results reveal opportunities for developing novel methodologies to enhance oxygen-tolerant Hyds, consequently opening avenues for diverse applications.

P-09-025
Antibacterial, hemolytic and anticancer activities of silver nanoparticles biosynthesized by phycocyanin extracted from *Spirulina*

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The use of cyanobacteria and their metabolites as natural and renewable resources in the biosynthesis of nanoparticles (NPs) provides a cost-effective and environmentally friendly option compared to physicochemical methods. In this work, phycocyanin (PC) pigment extracted from cyanobacteria *Spirulina platensis* Pc-005 was used as a stabilizing agent in the biosynthesis of silver NPs (PC-AgNPs). UV-Vis absorption peak at 410 nm confirmed the synthesis of PC-AgNPs. Dynamic light scattering measurements demonstrated that these NPs have a hydrodynamic radius of 38.4 ± 6.0 nm with a polydispersity index of 26.5%. FTIR spectroscopy analysis of NPs revealed the participation of certain functional groups of cyanobacteria biomolecules in the PC-AgNPs formation. The antibacterial potential of PC-AgNPs was evaluated against conditionally pathogenic bacteria *Staphylococcus aureus* MDC5233, *Pseudomonas aeruginosa* Gar-3, and *Salmonella typhimurium* MDC1759. PC-AgNPs demonstrated concentration-dependent bactericidal activity, with Gram-negative bacteria having higher susceptibility to NPs than Gram-positive strains. The hemolytic potential of PC-AgNPs was determined to assess their biocompatibility with human blood cells and erythrocytes. At low concentrations, these NPs showed no hemolytic activity against erythrocytes. To elucidate the anticancer activity of PC-AgNPs, their cytotoxicity was investigated in human lung adenocarcinoma A549 cell culture. A decrease in cell viability of about 40–70% was established, depending on the concentration of NPs. In addition, a 4-fold increase in the quantity of TNF-α (Tumor necrosis factor alpha) and a 2.5-fold decrease in VEGF-A (Vascular endothelial growth factor A) indicated the apoptosis-induced properties of NPs. Additional research will be conducted to clinically validate the anticancer properties of PC-AgNPs. The work was supported by the Higher Education and Science Committee of RA, in the frames of the research project 21T-1F179.

Marine Biochemistry

P-11-001
An *in silico* workflow for mining glycoside hydrolase enzymes from nature

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The identification of enzymes highly specialized for a substrate of interest is relevant for new biotechnological applications but may require expanding the known enzymatic toolbox in new regions of the sequence space. This will require specificity and kinetics studies on purified enzymes, which is not affordable on the several thousands of new enzyme sequences added in public databases each year. High-throughput specificity assays may be a possible solution, but the required technical equipment is possessed by few labs and only for specific enzyme classes. Therefore, in the last decades several *in silico* sequence-based methods were proposed to help in shortlisting new efficient enzymes with the specificity of interest. However, most of the proposed methods rely on sequence signatures or similarity thresholds with respect to characterized enzymes; therefore, they do not allow predictions that traverse the sequence space of enzyme families. Glycoside hydrolases (GHs), enzymes active in the hydrolysis of poly- and oligosaccharides, form one of the greatest classes of enzymes, with the possibility to find, within the same GH family, many different substrate specificities and promiscuity, tunable by few key amino acids substitutions or the addition of substrate-binding loops. In this work available databases and end-to-end deep learning (DL) predictors were tested for predicting GH substrate specificity, starting from primary sequence and minimal reaction mechanism information. The results indicate that sequence-based DL methods are prone to systematically fail on hard case sequences that are far from the characterized sequence space. A DL workflow which explicitly models the enzyme-substrate complex was shown to be able to predict, at least in part, also those hard cases. The workflow was applied to predict the specificity of new bacterial glycoside hydrolases belonging to different families, subsequently experimentally characterized in the lab.

P-11-002
Phaeodactylum tricornutum extracts: protective effects against *in vivo* induced chemical toxicity and inhibition potential of human acetylcholinesterase and pancreatic alpha-amylase

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It is well established that microalgae are a sustainable source of biologically active molecules. This research focused on evaluating the potential of extracts derived from *Phaeodactylum tricornutum* to inhibit human acetylcholinesterase, pancreatic alpha-amylase, and demonstrating *in vivo* protective effects against induced hepatic and nephrotic toxicity. Ultrasound assisted methanol extraction (10% w/v) was carried out on different lyophilized *P. tricornutum* biomasses, obtained under different culture conditions by varying media composition based on phosphorus and nitrogen contents (conway, ASW-algal and f/2 media). Our results showed that the f/2 extract has interesting inhibition capabilities of both acetylcholinesterase and amylase (IC50 of 0.71 mg/ml and 0.14 mg/ml respectively). This extract was further characterized by measuring its antioxidant activity by the DPPH scavenging assay (IC50 = 0.43 mg/ml), the total polyphenols (10.58 mg EAG/g dry extract) and the carotenoids contents (7.2 mg/g dry extract). Investigation of the protective effect against *in vivo* induced hepatotoxicity and nephrotoxicity by CCl4 and cisplatine respectively was also studied for the same extract. This was assessed compared to negative and positive controls of Wistar mouse groups by analyzing several blood parameters (urea, uric acid, creatinine, transaminases, lactate deshydrogenase), by measuring in both organs antioxidant enzymes and yield of lipid peroxidation, and by examining the obtained histological sections. Overall, the results showed a dose dependent protective effects up to 65% compared to the response toxicity induced par the chemical reagent. These interesting biochemical and protective properties could be considered as a result of intensive secondary metabolites production provoked by nutritive stress brought by the f/2 medium. They showed the potential of *Phaeodactylum tricornutum* or its extracts to manage neurodegenerative diseases and diabetes.

P-11-003
Decrypting the function of recurrent ancillary enzymes in bacterial phosphonate catabolism

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Phosphonates (organic molecules containing a direct C–P bond) occur in the environment both as natural products and as anthropogenic pollutants. Despite the remarkable stability of the C-P bond, numerous environmental microorganisms are able to catabolize phosphonates, contributing to the marine phosphorus redox cycle. Notably, many bacteria possess so-called "hydrolytic" pathways for the degradation of 2-aminoethylphosphonate (AEP), the most prevalent natural phosphonate. These pathways are highly specialized for AEP as substrate and typically begin with the conversion of AEP to phosphonoacetaldehyde (PAA), operated by the transaminase PhnW. Through a combination of bioinformatics, organic chemistry and enzymology, we have been addressing the role of functionally uncharacterized enzymes whose genes are recurrently found in the bacterial gene clusters for these hydrolytic pathways. For example, we have recently focused on a rather heterogeneous group of FAD-dependent amine oxidoreductases, which are frequently encoded in these clusters. We characterized three of these oxidoreductases in detail

and showed that – in spite of significant mechanistic differences they all efficiently oxidize N-methyl 2-aminoethylphosphonate (M_1AEP), another common natural phosphonate, converting it to PAA. In the absence of these FAD enzymes, M_1AEP could not be processed by the hydrolytic pathways. Furthermore, some of these enzymes can also efficiently oxidize plain AEP to PAA, hence surrogating the role of PhnW in organisms that do not possess the transaminase. [Previously published in: Zangelmi E et al. (2023) *iScience* 26, 108108]. We are currently analyzing the function of other oxidoreductases (NAD(P)-dependent) also associated with AEP catabolism. Overall, such ancillary enzymes appear to offer a selective advantage by allowing a bacterium to consume multiple AEP-related compounds, which otherwise would not be efficiently degraded, through a single catabolic pathway. *The authors marked with an asterisk equally contributed to the work.

P-11-004
Extreme marine and coastal environments as a source of glycoside hydrolases involved in the degradation of oligosaccharides and polysaccharides

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Extreme marine and coastal environments, as polar regions and hypersaline habitats, force bacteria to counteract a variety of stressful conditions, such as low temperatures, salt stress and scarce nutrient availability. In these harsh situation, hydrolytic enzymes as glycoside hydrolases (GHs) are pivotal in the breakdown of oligosaccharides and polysaccharides, which serve as carbon and energy sources for microorganisms. Here we report our studies on GHs from bacteria living in different extreme environments: *Marinomonas* sp. ef1, an Antarctic bacterium able to grow at low temperatures, and *Bacillus altitudinis* strain CML04, an endophytic halotolerant bacterium isolated in Crete. The Antarctic bacterium *Marinomonas* sp. ef1 possess three different GHs belonging to family 3, namely M-GH3_A, M-GH3_B and M-GH3_C, which have different architectures and low sequence identity. While M-GH3_C was produced as an insoluble, M-GH3_A and M-GH3_B show different thermal and structural properties: M-GH3_A is a bona fide cold-active enzyme, while M-GH3_B shows mesophilic-like properties. Moreover, M-GH3_A is a promiscuous β-glucosidase, mainly active on cellobiose and cellotetraose, whereas M-GH3_B is a xylanase active on xylan and arabinoxylan. The mediterranean bacterium *Bacillus altitudinis* strain CML04 can degrade xylan-based polysaccharides and, moreover, enhances degradation activity in presence of salinity stress. Genome mining identifies different GHs putatively involved in the degradation of xylan, and here we report the discovery of two extreme halotolerant xylanases belonging to GH family 11 and 30. These two enzymes are both active on xylan-based polysaccharides, have different biochemical and structural properties and can to tolerate salinity stress up to 2,5 M. Future analyses will help us to understand how the expression of these enzymes is affected by salinity stress and how these enzymes act in polysaccharides degradation.

P-11-005
Nutraceuticals from the cold: impact of global warming on the polyunsaturated fatty acid biosynthesis in the Antarctic diatom *Fragilariopsis cylindrus*

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Diatoms, a key component of polar marine ecosystems, represent one of the major groups of photosynthetic algae and account for more than half of the total primary production. They are also primary producers of omega-3 polyunsaturated fatty acids (PUFAs), including eicosapentaenoic and docosahexaenoic acids. Since diatoms' PUFA composition can be affected by temperature, light, and nutrient conditions, understanding the influence of environmental changes on the PUFA biosynthetic pathway can provide insights not only into their ecological future in the global warming scenario but can also offer opportunities for their biotechnological exploitation. Among cold-adapted species, *Fragilariopsis cylindrus* is the first polar diatom whose genome has been sequenced and annotated. In this work, we have grown *F. cylindrus* at temperatures mimicking a normal and a warm Antarctic summer using Xanthella LTD© photobioreactors. Upon (1) *in silico* identification of the genes encoding for the desaturases and elongases involved in PUFA production from linoleic acid, we have (2) cloned and sequenced their allelic variants, (3) analyzed by RT-qPCR their transcription levels in *F. cylindrus* grown at different temperatures, (4) explored by liquid chromatography-high resolution mass spectrometry the resulting differential lipidomic profile and (5) recombinantly expressed the enzymes to evaluate substrate specificity. Exploring the pathway responsible for PUFA production in *F. cylindrus* will offer promising avenues for advancing our understanding of lipid metabolism in polar microorganisms and obtaining a more sustainable production of marine PUFAs for nutraceutical applications. *The authors marked with an asterisk equally contributed to the work.

P-11-006
Diving deep: how marine bacteria's transport proteins shed light on global nutrient patterns

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SAR11 bacteria dominate the global ocean, shaping ecosystems with their efficient nutrient uptake. By exploring the molecular feature of their transport proteins, we've uncovered unprecedented affinity and specificity, shedding light on their adaptation to nutrient-poor environments. Our findings not only unveil new carbon sources for SAR11 but also offer insights into global nutrient cycles, shaping our understanding of marine biogeochemistry.

P-11-007
Bioinformatics and molecular docking studies for the structural and functional characterization of lipoxygenases from diatoms

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Diatoms are a major class of unicellular algae in the phytoplankton, are at the base of the trophic pyramid in the oceans, lakes and rivers and play a crucial role as bioindicators for the health of aquatic ecosystems. Under environmental pressures, diatoms, as many plants, animals, cyanobacteria, and some fungi, synthesize oxylipins, part of a wide variety of secondary metabolites derived from oxygenated poly-unsaturated fatty acids (PUFA) by lipoxygenase enzymes (LOX). These compounds impact not only on the growth of phytoplankton, such as diatoms themselves, but also on the growth of numerous organisms constituting zooplankton. Due to the possible biotechnological applications, ranging from ecology to medicine, researchers' interest in diatoms' lipoxygenases and oxylipins have increased. Using bioinformatics and molecular docking tools, we investigated the lipoxygenases of diatoms and their possible interaction with substrates. By analyzing large-scale sequence resources, we retrieved 45 sequences of lipoxygenases from diatoms. Through comparison and analysis of the sequences by multiple alignments and phylogenetic trees, we investigated the possible clustering in phylogenetic groups. Then, we modelled the 3D structure of representative enzymes of the different groups and investigated in detail the structural and functional properties by docking simulations with possible substrates. The results enabled us to suggest a classification of diatom lipoxygenases according to their sequence features, potentially manifesting in specific structural differences, and possible substrate specificity. Previously published in: Giordano D et al. (2024) Biomolecules 14(3):276.

P-11-008
Characterization of the structure and antibacterial activity of mutants peptides derived from the Trematocine isolated from an Antarctic fish

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Antimicrobial resistance (AMR) is a severe problem for public healthcare system. In fact, already in 2019, more than one million deaths were directly attributable to drug resistance [1] and this number should increase to 10 million in 2050 [2]. A way to contrast the emergence of bacterial resistance to classical antibiotics is the development of new therapeutic agents, like antimicrobial peptides (AMP), that can replace or support existing antibiotics. AMP usually display a short amino acid chain, a net positive charge and high hydrophobicity. They constitute a fundamental part of the immune responses of all organisms and act principally by altering permeability and stability of bacterial cell membranes. This work aimed to characterize the AMP Trematocine isolated from an Antarctic fish and two mutant peptides (KH-Trem and KHS-Trem) obtained from its scaffold. We studied, by spectroscopic methods, their interaction with two membrane systems and their capability to permeabilize the membrane of Gram-negative and Gram-positive bacteria models. Subsequently we analysed their antimicrobial activity against human pathogens bacteria. Finally, we performed *in vitro* toxicity studies against a human primary cell line and mammalian erythrocytes and *in vivo* toxicity studies against *Galleria mellonella* larvae. The results showed that KH-Trem and KHS-Trem peptides have an increased antimicrobial activity against some of the tested antibiotic-resistant bacterial strains compared to the Trematocine wild type. Moreover, these peptides revealed a low haemolytic and cytotoxic activity at the concentrations needed to kill bacteria and no toxicity during the *in vivo* experiments. These results highlight the potential pharmacological applications of these AMP in the battle to fight AMR. References: 1. Antimicrobial Resistance Collaborators. (2022) Lancet. 399(10325):629–655. 2. O'Neill J. (2016) The Review on Antimicrobial Resistance. 3. Della Pelle G. at al. (2020) Antibiotics (Basel). 9(2):66.

Clinical Trials, Preclinical Studies and Basic Research Related to Physical Activity

P-12-001
Effects of atorvastatin and simvastatin on oxidative metabolism in astrocyte cells

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Statins, the most prescribed drugs in the world, prevent serious cardiovascular disorders in patients with hypercholesterolemia. The mechanism of action of these drugs is to block a key enzyme of the mevalonate pathway, which leads to cholesterol biosynthesis. A product of the same pathway is also coenzyme Q (Q), an important cellular antioxidant and a key electron carrier in the mitochondrial respiratory chain. The aim of this study was to investigate the effects of chronic 6-day exposure to two lipophilic statins, atorvastatin and simvastatin, on cell function and oxidative metabolism of cultured rat astrocytes (CTX TNA2). The tested statins at a concentration of 200 nM did not reduce cell viability. However, they reduced the levels of Q9 and Q10, as well as cellular and mitochondrial ROS production. These results indicate that statins did not cause an increase in oxidative stress, as evidenced by unchanged levels of oxidative stress markers (lipid peroxidation marker and protein peroxidation marker). In statin-treated astrocytes, mitochondrial respiration with the substrates of carbohydrate catabolism (glucose and pyruvate) was reduced, while the oxidation of the most potent respiratory

substrate (glutamine) was unchanged. For all substrates tested, we observed a significant increase in proton leak, indicating greater mitochondrial uncoupling in statin-treated cells. We also measured the activity of respiratory chain complexes in digitonin-permabealized control cells and statin-treated cells with various respiratory substrates under phosphorylating and non-phosphorylating conditions. We further confirmed lower ATP levels in statin-treated cells by observing reduced respiration associated with ATP synthesis. Thus, statins modulate astrocyte energy metabolism, leading to changes in the cell's energy state, coenzyme Q redox balance, and mitochondrial respiratory function. This research was funded by National Science Centre, Poland, OPUS2020/37/B/NZ1/01188

P-12-002
Cynaropicrin suppresses cell proliferation by inducing mitophagy through p38-mediated mitochondrial ROS in Hep3B hepatocellular carcinoma cells

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Cynaropicrin, a sesquiterpene lactone, has diverse pharmacological activities, including anticancer activity, but its mechanism of action in hepatocellular carcinoma (HCC) is not fully defined. Therefore, current study investigated the cytotoxic effect of cynaropicrin and examined the action mechanism on human HCC Hep3B cells. The results demonstrated that cynaropicrin significantly induced cytotoxicity and autophagy, which was related to the induction of mitochondrial reactive oxygen species (ROS) production and mitochondrial membrane potential loss. Under cynaropicrin-treated condition, the expression of microtubule-associated protein 1 light chain 3, which is involved in elongation of the phagophore membrane, was upregulated, whereas the expression of Beclin-1 and p62, which are essential for the formation of autophagosomes, was downregulated. In addition, the expression of mitophagy regulators, PTEN-induced kinase 1 (PINK1) and Parkin, in mitochondria was increased, suggesting induction of autophagic flux for mitochondria. However, N-acetyl-l-cysteine, a ROS scavenger, counteracted the cynaropicrin-induced effects. Moreover, cynaropicrin increased phosphorylation level of p38 mitogen-activated protein kinase (MAPK) and SB203580, p38 MAPK inhibitor, notably reversed cytotoxic effects and mitochondrial ROS production. Importantly, SB203580 reversed cynaropicrin-promoted expression levels of PINK1 and Parkin in mitochondria. Collectively, our findings demonstrate that cynaropicrin can exert cytotoxic effect against HCC Hep3B cells by inducing mitochondrial autophagy through p38 MAPK-ROS pathway activation, indicating that cynaropicrin could be a potential therapeutic compound for liver cancer treatment.

P-12-003
Exposure to polystyrene nanoplastics induce premature cellular senescence through mitochondrial ROS generation in murine myoblast C2C12 cells

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Nanoplastic is emerged as a novel environmental disrupter. Recently, increasing evidence indicates that nanoplastics accumulate in various tissues and exert toxicological effects on physiological systems. Polystyrene, the most predominant type present in the atmosphere, is one of the types of plastic widely used in consumer goods and has been found to have harmful effects on the human body. However, the link between polystyrene nanoplastics (PS-NPs) and cellular senescence in muscle cells is not well understood. Therefore, we evaluated the impact of PS-NPs (100 nm) accumulation on cellular senescence and the potential regulatory role of mitochondrial reactive oxygen species (mtROS) in murine myoblast C2C12 cells. According to our results, senescence marker proteins (p16 and p21) as well as β-galactosidase activity were markedly increased in C2C12 cells exposed to PS-NPs. We also investigated the regulatory role of mtROS in PS-NPs-induced senescence in C2C12 cells and found that mitochondrial superoxide levels were noticeably upregulated. In addition, mitochondrial membrane potential, mitochondrial contents and ATP levels were significantly decreased and mitochondrial fragmentation was largely increased in PS-NPs-treated C2C12 cells, demonstrating that mitochondrial dysfunction was induced. In contrast, in the presence of the mtROS scavenger MitoTEMPO, cellular senescence induced by PS-NPs was clearly attenuated, as observed by decreased β-galactosidase activity. Collectively, these results indicate that exposure to PS-NPs promotes premature cellular senescence through mtROS-dependent mitochondrial dysfunction in murine myoblast C2C12 cells.

P-12-004
Association of autophagy-related proteins in serum and synovial fluid with the severity of knee osteoarthritis

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Osteoarthritis (OA) is a highly prevalent painful joint disease that significantly reduces the quality of life and causes physical disability around the world. OA is pathologically characterized by articular cartilage degeneration, synovial inflammation, and abnormal subchondral bone changes. Even though OA causes an enormous socioeconomic burden, there is no currently effective treatment option to impede the progression of the disease and ameliorate clinical outcomes. Autophagy is a dynamic process of

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recycling that is essential for maintaining cellular homeostasis. Autophagy dysfunction contributes to the onset and development of OA. However, there is no study examining the role of autophagy-related proteins in the serum and synovial fluid of patients with OA. Here, we aim to investigate the relationship of autophagy-related proteins with the radiographic findings and symptomatic severity of knee OA. We enrolled 43 OA patients and 37 patients with other knee joint disorders (non-OA control) in the study. Autophagy-related proteins (Beclin-1, Autophagy-related 3 [ATG3] and LC3A) were measured with an enzyme-linked immunosorbent assay. The radiographic grading and symptomatic severity of OA were evaluated using the Western Ontario McMaster University Osteoarthritis Index (WOMAC) scores and the Kellgren-Lawrence classification, respectively. Serum Beclin-1 expressions in the OA group were higher than in the non-OA group ($p < 0.05$). The levels of autophagy-related proteins (Beclin-1, LC3A, and ATG3) in the synovial fluid of patients with OA were markedly elevated compared to the non-OA group ($p < 0.05$). Moreover, ATG3 expressions in the synovial fluid of patients with OA were significantly correlated with the severity of OA symptoms. SF LC3A and ATG3 expressions in grade 4 OA patients were higher than in grade 3 OA patients ($p < 0.05$). Our data suggests that autophagy-related proteins might have diagnostic and prognostic value for the prevention and treatment of OA.

P-12-005
The impact of hyperoxia and hypoxic interval training on the plasma metabolome in skiing athletes

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Metabolomics is a promising tool for investigating molecular changes induced by physical exercise, as metabolic adaptations to external stimuli can occur in a short period of time. Moreover, these studies enable to monitor both the physiological response and the recovery after an injury or a competition, thus providing the possibility to develop a personalised training. This study aimed to evaluate the influence of hypoxic interval training with and without hyperoxic recovery on the blood metabolome in eleven male trained alpine skiing athletes. Each athlete performed two different test trials, involving hypoxic exercise sessions at a simulated altitude of 3500 m interspersed by 15 min of passive recovery. During one trial, 100% oxygen was administered during recovery. In the second trial, recovery was conducted simulating 3500 m oxygen level. Blood was collected before, immediately after, and seven days after the trials. Untargeted high-resolution mass spectrometry approaches were used to perform both the metabolome and the lipidome analyses of plasma extracts. Systematic alterations of relative blood metabolite and lipid concentrations in trained athletes were observed in response to a single session of high intensity eccentric/concentric exercise performed during simulated altitude. Specifically, short-term metabolome differences involved the fatty acid β-oxidation and the tricarboxylic acid cycle, while the long-term variations were associated with malate-aspartate shuttling, urea cycle alterations, lysine degradation, and branched chain amino acids degradation as a consequence of varying oxygen availability. Similarly, lipidome analysis showed specific changes according to the type of oxygen exposure and short term versus long term recovery. Overall, we observed systematic alterations of plasma metabolite and lipid concentrations in response to high intensity exercise performed at different simulated altitudes.

P-12-006
Blood microsampling for untargeted metabolomics: a preliminary study on patients affected by myocardial infarction

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Blood microsampling is a promising tool for blood collection as it is simple and minimally invasive. It allows multiple sampling and is optimal for longitudinal studies. For these reasons, it has gained attention and has been applied to metabolomics studies. Physical activity induces metabolic changes and a correlation between metabolism and cardiovascular risk has been demonstrated. For example, physical exercise has been shown to improve the catabolism of branched-chain amino acids (BCAA). The involvement of BCAA in ischemic cardiomyopathy is well known: their plasma levels correlate with the presence and severity of the disease. In this preliminary study, blood samples were collected with dried blood spots (DBS) from patients affected by myocardial infarction participating in a physical rehabilitation program. Blood was collected at 7 time points: before the start of the rehabilitation protocol, before and after the first training, before and after training halfway through the rehabilitation protocol, and before and after the last training. Untargeted metabolomic analysis was performed to assess exercise-induced changes, correlation with clinical outcome and cardiovascular risk factors in these patients. Polar metabolite analysis was performed on DBS with UHPLC-MS. The results showed the most altered metabolic pathways were associated with purine metabolism and histidine metabolism. In particular, xanthine and histidine levels were high at the beginning of rehabilitation and gradually decreased with the training time. Comparing the metabolic phenotype at the beginning and at the end of rehabilitation highlighted a reduction of xanthine, urocanic acid and C5-carnitine. Overall, the use of DBS simplified longitudinal blood sampling and allowed the investigation of metabolic changes during the physical rehabilitation program after myocardial infarction.

P-12-007
The disturbances of redox homeostasis in normal skin cells triggered by the phototoxic action of meloxicam – *in vitro* study on melanocytes and fibroblasts

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Meloxicam (MLX) belongs to the non-steroidal anti-inflammatory drug group and is a selective COX2 inhibitor widely used in the pharmacotherapy of musculoskeletal pain, mainly osteoarthritis and rheumatoid arthritis. The simultaneous skin exposition to the MLX and UVA irradiation (UVAR) result in the phototoxic reaction occurrence that contributes to disturbances of redox homeostasis. The main aim of this study was to assess the content of reactive oxygen species (ROS) in melanocytes and fibroblasts exposed to MLX and UVAR and to determine changes in the activity and expression of antioxidant enzymes – superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GPx) in normal skin cells. Confocal microscopy using CellROX Green Reagentâ was used to assess ROS levels in skin cells. SOD, CAT and GPx activities were analyzed using assay kits, and the expression of antioxidant enzymes was assessed using the western blot technique. The obtained results indicated that UVAR enhances meloxicam-induced oxidative stress in melanocytes and fibroblasts. It was found that MLX differently affects the expression and activity of SOD, CAT and GPx depending on the analyzed cell line. In the case of fibroblasts, a decrease in the expression and activity of SOD, CAT and GPx was observed. Conversely, an increase in the activity and expression of antioxidant enzymes was found in melanocytes. Due to the fact that MLX has the ability to bind to melanin biopolymers, creating its long-term reservoir, the concentration of free drug in melanocytes is lower than in fibroblasts. The demonstrated differences indicate that melanin may have protective properties against the phototoxic effects of MLX. Funding: this research was funded by Medical University of Silesia in Katowice (grant numbers: BNW-2-018/K/3/F; BNW-1-012/K/3/F).

P-12-008
Blockade of identity loss during transplantation through regulation of UPR proteins in hepatocyte spheroids

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Hepatocyte transplantation presents a promising alternative to orthotopic liver transplantation, mitigating challenges such as donor shortages and surgical risks. However, hepatocyte transplantation encounters hurdles, including susceptibility to dedifferentiation due to adverse conditions during the transplantation process. This dedifferentiation, often accompanied by cell apoptosis, leads to the loss of hepatocyte identity. While spheroidal structures offer high biological similarity to native organs, it remains unclear whether hepatocyte spheroids can withstand identity loss during transplantation and the underlying mechanisms. Here, we utilized spheroid formation as a strategy to preserve intercellular connections, thereby maintaining hepatocyte identity and bolstering resistance to inflammatory cytokines, a major contributor to the post-transplantation harsh environment. We examined differences in the expression of hepatic function genes between single cells and hepatocyte spheroids, focusing on genes involved in IRE and PERK signaling pathways. Notably, we identified the spliced Xbp1 protein, a component of the unfolded protein response (UPR) signaling pathway, as a primary factor contributing to enhanced hepatic function observed in spheroids. This was corroborated through siRNA knockdown or ligand treatment experiments. Furthermore, our findings suggest that activated STAT3 in spheroids may serve as an upstream regulator in this axis. In summary, we elucidated the mechanism underlying the inhibition of hepatocyte dedifferentiation during transplantation in hepatocyte spheroids. Our discoveries highlight the potential of utilizing hepatocyte spheroids to enhance transplantation efficiency, offering promise in overcoming limitations associated with traditional hepatocyte transplantation. *The authors marked with an asterisk equally contributed to the work.

P-12-009
A one-two punch strategy for vestibular schwannoma treatment

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Vestibular schwannomas (VS) are benign tumors that arise from the Schwann cells of the cochleovestibular nerve. They can be classified into two groups: sporadic VS and those associated to the rare syndrome neurofibromatosis type 2 (NF2). VS grow slowly and can cause hearing loss both due to compression of the auditory nerve and the release of ototoxic substances. They can also cause dizziness, facial paralysis and death due to compression of the brainstem. Currently, patients undergo tumor surgery or radiotherapy as treatment but there are no pharmacological FDA-approved therapies to treat these tumors. Cellular senescence is a crucial response against cancer development and a defining feature of some benign tumors. Here, we analyze the senescence nature of VS in patient derived primary cells. Our data show that VS primary cell cultures show high levels of senescence markers: the senescence-associated β-galactosidase (SA-β-GAL) activity, the cyclin-dependent kinase inhibitor p21 and the expression of senescence assciated secretory phenotype (SASP) components. The treatment with DNA damage drugs, such us bleomycin, increases the levels of these senescence features. We also explore a two-hit combination therapy for VS based on the induction of cellular senescence by DNA damage agents and the subsequent activation of apoptosis by senolytic drugs treatment in patient derived primary cells and the immortalized VS cell line HEI-193 derived from a NF2 patient. Our results also show that treatment with navitoclax, a senolytic agent, decreases the viability of the bleomycin- induced senescent cells by activating the extrinsic and intrinsic apoptosis pathways. These results suggest that a one-two punch strategy based in the combination of senogenic and senolytic agents could constitute a potential alternative for the treatment of VS.

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P-12-010
The response of macrophages to infection is dependent on mycobacterial growth phase

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Mycobacterial infections are known as hard to eradicate, requiring long multi-drug treatments associated with poor patient compliance and low success rate. This is mostly due to ineffective drugs, urging the need for new and more effective treatments. However, traditional methods for evaluating *in vitro* activity do not adequately mimic the complexity of the infection, thus failing at predicting clinical effectiveness. Therefore, our main objective is to develop complex *in vitro* infection models to test new molecules in a high throughput setting, taking advantage of genetically engineered bacteria. While optimizing the infection of macrophages by *Mycobacterium avium* and *Mycobacterium abscessus*, we found out that the ability of macrophages to control the intracellular bacterial load and its response to antibiotics is species-specific and dependent on the bacteria's fitness upon internalization. After infecting murine bone marrow-derived macrophages with mycobacteria in different growth phases, we performed live cell imaging using high-content fluorescence microscopy to monitor the autophagic flux, quantify lysosomal accumulation and evaluate ROS/RNS production and correlated it with bacterial load. We observed that the activity of clinically approved antibiotics against intramacrophagic bacteria depends on the growth phase of the bacteria upon infection. Therefore, when testing new compounds against mycobacteria, the growth phase of the bacteria should be taken in consideration. Overall, we are contributing to accelerate the drug discovery pipeline by establishing practical and reliable in vitro tools to select clinically promising drug candidates. This work was financed by Portuguese national funds through FCT – Fundação para a Ciência e a Tecnologia, within the project PTDC/BIA-MIC/3458/2020 and PhD fellowships UI/BD/150830/2021 to CMB and 2021.07335.BD to GSO.

P-12-011
Effect of Empagliflozin compared to placebo on the plasma lipidome in patients with type 2 diabetes mellitus – results from the EmDia trial

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Sodium-glucose cotransporter 2 (SGLT2) inhibitors, such as Empagliflozin, are antidiabetic drugs that reduce glucose levels and have emerged as a promising therapy for patients with heart failure (HF), although the exact molecular mechanism underlying its cardioprotective effects remain unknown. The EmDia study, a randomized, double-blind trial conducted at the University Medical Center of Mainz has confirmed the beneficial effects of Empagliflozin in HF patients after both one and twelve weeks of treatment. In this work, we aimed to analyze changes in lipid profiles using lipidomics data from the EmDia study to elucidate the role of Empagliflozin in HF and gain insights into its cardioprotective mechanisms. Lipid signatures after treatment for one week, obtained with sparse group LASSO regularized regression models, consisted of 37 lipids of lipid groups lysophosphatidylcholine (LPC), phosphatidylcholine (PC), phosphatidylethanolamine (PE), sphingomyelin (SM), and triacylglycerol (TG). Following twelve weeks of treatment, the signature comprised 24 lipids from the same five lipid groups as well as Ceramides (Cer). Five lipids overlapped between the two timepoints. Using linear regression models could highlight associations between significantly changed clinical traits and lipids, and clear differences of lipid abundance on selected clinical subgroups (left ventricular ejection fraction LVEF, left ventricular hypertrophy LVH, obesity, HbA1c, eGFR and uric acid). Establishing direct associations between individual lipids and specific clinical events proved challenging, however utilizing lipid classes instead of individual lipids showed promise in elucidating patterns driven by Empagliflozin treatment. Future research will involve integrating lipidomics data with other omics datasets to provide a more comprehensive understanding of the identified lipid signatures and their potential roles in health and disease.

P-12-012
The bloodbrain barrier in the context of glioma therapy using the oncolytic myxoma virus studies on an *in vitro* model

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Current glioma therapy is based on tumor resection and local chemotherapy aimed at eradicating residual glioma cells. The bloodbrain barrier (BBB) poses significant glioma treatment limitations and influences the choice of alternative therapeutic approaches, e.g. systemic therapy. Intraarterial application of oncolytic viruses (OV) in glioma therapy requires methods of checking the efficiency of virus crossing this barrier. *In vitro* artificial BBB model imitating the real one makes it possible to check this premise. We assessed the efficacy of penetrating such barrier by myxoma viral genetic construct (vMyx-M011L-KO-EGFP) designed to eliminate brain tumor initiating cells (BTIC), responsible for CNS tumor recurrence. In the study we investigated the viral construct delivery via non-immunogenic mesenchymal adipose-derived stem cells (ADSC). ADSC allow avoiding unfavorable host antiviral response before OV reaching the tumor bed. The in vitro BBB model tested is based on murine endothelial cells (Bend.3) cultured on Matrigel-covered inserts placed inside a multi-well plate and measuring the so-called transendothelial resistance parameter (TEER) of the system. When adequate resistance values were obtained, corresponding to the presence of a suitable layer of Bend.3 cells we applied unshielded myxoma viral construct as well as myxoma viral

construct-loaded ADSCs and tested their effect on infection progression in murine (GL261) and human glioma cells (T98G, LN18) cultured below the inserts. Efficiency of OV penetration through endothelial cells layer was assessed by microscopy and cytotoxic effect of the OV in the BBB system measured by MTS assay. The results demonstrate myxoma viral construct ability to cross the tested BBB, infect target glioma cells and induce apoptosis. No adverse effects of the viral construct on endothelial cells forming the BBB were noted. This points to feasibility of OV-infected ADSC systemic delivery *in vivo* and application in glioma therapy.

P-12-013
Exploring frailty and aging biomarkers: preliminary insights from the Novara Cohort Study

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This study is part of a broader research initiative focused on identifying biomarkers, whether individually or in combination, to enable early detection of accelerated aging and serves as a pilot study within the Novara Cohort Study (NCS), a longitudinal cohort study focusing on aging and involving the population of the Novara province. The primary goal is to establish a proof of concept for identifying blood indicators that can serve as markers of subclinical deficits. These deficits are representative of physiological decline, increased vulnerability, and reduced resilience to stressors, ultimately contributing to the development of frailty. By pinpointing these indicators, the aim is to enhance frailty assessment by detecting early signs of decline in physiological function and resilience before it becomes clinically apparent. The preliminary results from the analysis of selected blood biochemical markers in the plasma of 123 participants from the Novara Cohort Study (NCS) representing the general population are presented. A Frailty Index based on laboratory tests (FI-Lab) was calculated to quantify the frailty status of the participants. Additional analyses were carried out to investigate the relationships between these biomarkers, age, and health status. An explorative metabolomics analysis was also conducted in acute renal disease patients (AKI), representing a model of accelerating aging, revealing metabolites that hold potential as biomarkers of aging. Overall, these findings provide a preliminary investigation into the feasibility and potential outcomes of identifying biomarkers for early detection of accelerated aging.

P-12-014
Evaluation of osseointegration in alveolar bone defects: a preclinical modelling

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Osseointegration is a dynamic process influenced by cells and mediators in bone tissue as a direct structural and functional connection between living bone and implant surface. This study explored the possibility of regenerating alveolar bone tissue by inhibiting local activators of bone destruction. In order to reduce bone osseointegration time, experiments were done using various doses of anti-sclerostin and anti-dickkopf-1 antibodies and their mixtures by protein based coated method on the implant surfaces. *Sus scrofa domesticus*, male pigs (50–70 kg) were divided into 5 groups (control, graft, implant + Scl, implant + DKK1, implant Scl + DKK1). Volumetric tomography slice imaging, histomorphologic analysis, and radiologic measurements were performed. The bone trabeculae showed various stages of formation. Especially compared to the Scl-ab group, vascularization, and regular bone formation were detected at lower levels in both graft 3-week and graft 6-week groups. In the DKK1-ab 3-week group, new bone formation was more advanced than in the graft 3- and 6-week groups. However, bone maturation and the amount of newly formed bone were detected more limited compared to the Scl group. The increase in the number of inflammatory cells observed was more advanced in the mixed 3-week group compared to the mixed 6-week group. Vascularization was detected, osteoconductive growth was observed, and osteoblasts were found at the periphery of bone tissue fragments. Around the implant material throughout the extraction area, mature bone tissue formations in lamellas and partly osteoid tissue areas were observed. The present study reports that using a therapeutic composition (Scl + DKK1 antibody) on dental implants increased bone volume and filled the insufficient bone gaps. These findings point towards the potential of Scl + DKK1 as an effective agent for enhancing bone regeneration and integration in dental implant procedures.

P-12-015
Correlation between myokines and cardiometabolic parameters during a maximal exercise cardiopulmonary test

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Exercise is known to promote upregulation of proteins and enzymes involved in fatty acid oxidation, while stimulating a comprehensive anti-inflammatory state. Myokines such as irisin, apelin, musclin and BDNF have been consistently shown to be released by skeletal muscles in response to exercise, exerting

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positive physiological and metabolic effects, influencing the redistribution of metabolic fuel from lipids to catabolic pathways, modulating the metabolism of WAT (white adipose tissue) and SkM (skeletal muscle). The present study aimed to investigate the association between myokines and the oxidation of energy substrates during cardiopulmonary exercise test (CPET) in marathon runners. Seventy-six healthy marathon runners (mean age ± 41.4 years) participated in this study. Blood samples were collected for plasma levels of irisin, apelin, musclin and brain-derived neurotrophic factor (BDNF) by enzyme-linked immunosorbent assay. CPET used a conveyor belt protocol (TEB Apex 200, TEB, São Paulo, Brazil). The expirated gases analysis was performed in a respiratory-to-breath system (CPET Quark, Cosmed, Rome, Italy) at anaerobic threshold (AT), respiratory compensation (RC) and peak. Correlations between molecular parameters and CPET were performed using Spearman's test. Statistical significance was set at $p < 0.05$ in all analyses. The analyses revealed a positive correlation of irisin, apelin and musclin myokines ($r = 0.3325$, $p = 0.0033$, $r = 0.3235$, $p = 0.0044$, $r = 0.3882$, $p = 0.0005$, respectively) with lipid oxidation and BDNF ($r = -0.4954$, $p < 0.0001$) was correlated with carbohydrate oxidation, all at the anaerobic threshold (AT). Our results suggests that the myokines irisin, apelin and musclin are essentials to improve lipid metabolism and to promote better resistance in marathon runners. *The authors marked with an asterisk equally contributed to the work.

P-12-016
Disrupted circadian rhythm results in diabetes in the offspring of rats

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The circadian rhythm is a significant biological pathway in humans and other mammals, and it is affected by light, sleep, and human activity. The natural sleep–wake of 24 h is controlled via complicated cellular pathways with feedback loops affected by genetic and epigenetic factors. We aimed to show that diabetes mellitus might be the result of environmental factors to which the mother is exposed and the endocrine effect of maternal hormonal disorder on the fetus. The role of exposure to 480 nm blue light during the sleep period was investigated. Experimental groups comprised 14 pregnant Wistar rats of 7 control and 7 experimental subjects and were followed for 23 days for the expected pregnancy period with 12 h of dark and light. The experimental group was exposed to blue light instead of dark. Blood samples were taken from pregnant rats on the last day of the study. C-peptide, total antioxidant, and oxidant status were measured. It can be concluded that the group exposed to light exhibited a significant decrease in offspring count. Moreover, the non-exposed group did not display any glycaemic response, while the blue light-exposed group had elevated blood glucose levels compared to the control group. Notably, there was a significant distinction in the C-peptide levels of the experimental and control pup groups ($p = 0.04$) that could lead to the onset of diabetes mellitus. Therefore, it is essential to consider the possible long-term effects of light exposure on the development of diabetes. The study's findings also revealed a substantial difference in the total oxidant levels between the experimental and control pup groups ($p = 0.040$).This implies that the experimental group may have been subjected to higher levels of oxidative stress, which could adversely affect their health and growth. Changes in the circadian rhythm can alter the metabolic pathways and could affect the fertility and the frequency of diabetes mellitus.

P-12-017
New strategies for performance acute stress monitoring in volleyball players: the role of ROS, amylase and cortisol levels

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It is now well-known how performance anxiety can affect performance in sports. To date, a way to highlight its levels and study possible restraining actions has not yet been found. We enrolled in our study 20 female volleyball players aged 13 ± 1 years old who played in two different teams during a regional championship final. Saliva was collected before and after the match. To evaluate the neuroendocrine effectors involved in stress and performance anxiety, we analyzed cortisol, amylase as well as ROS levels through an Elisa assay and a standard kit. The results showed a significant statistical decrease in salivary cortisol levels between pre- and post-match (7.7 ng/ml vs 4.5 ng/ml) only in the winning group ($p < 0.039$). An opposite trend was detected for salivary alpha-amylase (sAA) concentrations, wherein the winning group the change was statistically significant (pre match: 166.01 ± 250 U/ml vs post-match: 291.59 ± 241 U/ml; $p = 0.01$). As regards ROS levels, there were no significant differences between T0 and T1 in the winner group nor the loser group ($p > 0.05$) within and between group factors. However, it is interesting to note that while its levels decreased in the winning group between before and after, in the losing group the trend was the opposite. This trend emphasizes a correlation with performance anxiety based on the results of the match. In conclusion, the parameters analyzed are proposed as markers of sports performance-induced stress although future studies will be needed to confirm these preliminary findings. Previously published in: Rossi C et al. (2024). PeerJ 12, 1–17.

P-12-018
The impact of a nutritional intervention program on eating habits, body composition and performance in Italian kickboxing and boxing athletes

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A balanced diet is a fundamental component of athletes' health, training, and performance. The majority of athletes choses adequate quantities of macronutrients but, at the same time, they do not respect World Health Organization dietary guidelines, eating a lot of discretionary food and not drinking enough water. In relation to this, athletes need more nutritional education in order

to improve the quality of their food choice. However, in sports where weight categories are contemplated, maintaining some healthy and correct eating habits turns out to be very difficult for some athletes, especially in fighting disciplines. The aim of our study was to evaluate the effect of nutritional intervention program on eating habits, performance and body composition in a group of Northen Italy athletes practicing kickboxing and boxing. We organized meetings, detected eating habits (before and after the meetings) using a food frequencies questionnaire, body composition (BMI and body fat mass) and physical performance (Cooper test, speed endurance test, squat test jump and the maximum bench press test). We found that nutritional intervention positively affected participants consumption of vegetables ($p < 0.05$) and nuts ($p < 0.05$), while the consumption of sweets ($p < 0.05$) and snacking ($p < 0.05$) decreased. Although there were some variations in some anthropometric measurements during the study, the average fat mass percentage did not decrease. All four performance tests showed significant improvements. Our finding suggested that nutritional intervention could promote healthy eating habits among athletes. If sport nutrition expert, coaches, personal trainer, sport medicine expert and athletes collaborate, they could guarantee athletes' health status.

P-12-019
Identification of potential non-invasive biomarkers in diastrophic dysplasia

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Diastrophic dysplasia (DTD) is a recessive chondrodysplasia caused by pathogenic variants in the *SLC26A2* gene encoding for a sulfate/chloride antiporter of the cell membrane. Its functional impairment causes reduced cytosolic sulfate level resulting in proteoglycan undersulfation. Research on an animal model of DTD has suggested possible pharmacological treatment approaches. In view of future therapeutical approaches to DTD, the identification of non-invasive biomarkers is crucial to assess the efficacy of the treatment. In this work, we have considered urinary glycosaminoglycan (GAG) sulfation and N-terminal fragment of collagen X (CXM) in blood as biomarkers in children with DTD. Urinary GAG composition has been related to various metabolic disorders such as mucopolysaccharidoses. CXM is considered a real-time marker of endochondral ossification and height velocity; it has been studied in patients with achondroplasia and Osteogenesis Imperfecta. In this study 15 patients were enrolled and for each patient the clinical history was recorded. For GAG sulfation analysis, GAGs were isolated from urine and chondroitin sulfate disaccharide analysis was performed by HPLC after GAG digestion with chondroitinase ABC and ACII. CXM was assessed in blood samples or in dried blood spots. Results from DTD patients were compared with an age-matched control population. Undersulfation of urinary GAGs was observed in DTD patients with some relationship to the clinical severity and underlying *SLC26A2* variants. Lower than normal CXM levels were observed in most patients, even if the marker did not show a clear pattern in our small patient cohort because CXM values are highly dependent on age, gender and growth velocity. In summary, both non-invasive biomarkers are promising assays targeting various aspects of the disorder including overall metabolism of sulfated GAGs and endochondral ossification. Work supported by MIUR "Dipartimenti di Eccellenza 2023-2027" (to AF and AR).

P-12-020
Phototoxicity induced by chloroquine – an *in vitro* study using various experimental models

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Chloroquine (CQ) is a medication used to treat and prevent malaria. It is also prescribed for certain autoimmune diseases, such as rheumatoid arthritis and systemic lupus erythematosus. Long-term use of CQ may produce phototoxic side effects both in the skin and in the eye. This may be due to the drug's high affinity for melanin and its accumulation in pigmented tissues. Melanin biopolymers may therefore be of great importance in the development of phototoxicity in patients using chloroquine. The purpose of this study was to investigate the phototoxic potential of CQ using various experimental models on skin cells with different pigmentation (human dermal fibroblasts, lightly-pigmented melanocytes, darkly-pigmented melanocytes) and incubation time with the drug prior the exposure to sunlight (1 h or 24 h). Cells were incubated with CQ (0–100 μg/ml) and then irradiated with the SXL-3000V4 sunlight simulator. The WST-1 assay was used to determine cell viability, and EC50 (concentration causing a 50% reduction in cell survival) and PIF (photo-irritation-factor) values were calculated. It was demonstrated that the extension of incubation time with CQ augments the drug phototoxic potential reflected by an increase in PIF values. A dramatic difference was observed between the cell types tested – fibroblasts were found to be significantly more sensitive to the phototoxic effect of CQ compared to melanocytes. The results indicate that the phototoxicity of CQ depends on cell type and drug exposure time. Therefore, optimal in vitro models used to evaluate the phototoxic potential of melanin-binding compounds should include cultures of cells with different pigmentation. Moreover, the preincubation time of 1 h, which is currently considered the standard, should be extended. Funding: This research was funded by the National Science Centre, Poland (SONATA 16, project 2020/39/D/NZ7/01206).

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P-12-021
A single stiletto dance class can induce tissue damage and inflammation

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The stiletto dance style, which has experienced a remarkable surge, is a powerful tool for enhancing self-esteem and addressing psychosocial factors. However, that the prolonged use of high heels can lead to chronic injury and inflammation, playing a significant role in the genesis of synovitis and arthritis. A recent study from our group demonstrated that the stiletto class caused joint and muscle damage and did not alter the plasma concentration of pro-inflammatory cytokines (TNF, IL-1, IL-6, and IL-8) (Silva, J. et al 2023). However, it is important to define markers related to lesions and inflammation to define training protocols and the impact of inflammation on dancers. Herein, we amplify the inflammatory mediators' measurement of injury and inflammation in dancers after a single bout of stiletto dance class. The plasma biomarkers of joint damage (MMP-3), muscle damage (Mb, CK, LDH, and Lac), inflammation (PCR and SAA), and adaptation/repair (VEGF) were quantified before and immediately after a single stiletto class (60 min) of moderate intensity. Sixteen volunteers (23.4 ± 3.8 years; 61.7±8.1 kg; 23.4±2.3 kg/m^2; and 27.2±3.8% body fat) participated in the study. After class, our data showed that there was an increase in biomarkers for tissue injury (MMP-3 [56%]; Mb [113%]; CK [21%], and Lac [21%). We also observed an increase in markers of systemic inflammation (CRP [29%]; SAA [24%]), as well as a marker for angiogenesis/tissue damage repair (VEGF [26%]). We concluded that a single bout of stiletto dance class caused joint impairment and muscle damage followed by systemic inflammation. These findings, while highlighting the potential risks, also pave the way for developing safer training methods and preventing chronic injuries. Acknowledgments FAPESP (20/12267-5; 14/21185-1; 11/21441-0), CAPES (Grant number 88887.908560/2023-00, finance code 1), and CNPq (309644/2021-6).

Understanding of well-being homeostasis: the role of physical activity

P-13-001
Disruptions in the homeostasis of normal skin fibroblasts and melanocytes induced by doxycycline phototoxicity – model studies using a sunlight simulator

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Drug-induced phototoxicity constitutes a significant medical issue, taking into account epidemiological aspects, therapy safety, and the consequences of skin cell damage. Typically, the initial risk assessment for the occurrence of phototoxic reactions is carried out using the NRU test and mouse fibroblasts 3T3. The aim of this study was to assess changes in the homeostasis of human skin fibroblasts (HDF) and melanocytes with light (HEMn-LP) and dark (HEMn-DP) pigmentation caused by phototoxic action. Doxycycline (DOX) was selected as a model drug due to exhibiting phototoxic potential and forming complexes with melanin biopolymers. Cells were exposed to simulated sunlight from the SXL-3000V2 simulator (SERIC) at a dose of 5 J/cm^2 for UVA spectrum. Considering the possibility of doxycycline accumulation in melanocytes, cells were incubated with the drug for either 1 or 24 h before exposure. The experimental panel included a screening analysis of cyto- and phototoxicity using the NRU assay, glutathione level measurements, cell cycle analysis, and DNA fragmentation studies. It was demonstrated that DOX had cyto- and phototoxic potential towards all examined cells, proportionally to the drug concentration. As a result, intracellular reduced glutathione levels decreased, and cell cycle disturbances occurred. The observed disturbances were more pronounced in the case of longer cell incubation with DOX. Analysis of DNA fragmentation showed greater damage to genetic material in melanocytes compared to fibroblasts. The noted effect may be associated with the formation of drug-melanin complexes and the accumulation of DOX in pigmented cells. The conducted studies indicate the need to assess the phototoxicity of drugs using cells with varying degrees of pigmentation. Funding: This research was funded by the National Science Centre, Poland (SONATA 16, project 2020/39/D/NZ7/01206).

P-13-002
Investigating circulating extracellular vesicles in response to physical exercise

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Physical exercise represents a highly complex perturbation of homeostasis in a large number of tissues, largely consequential of the increasing metabolic demands of contracting skeletal muscle. When regularly performed, it produces physiological adaptations associated with improved health and longevity [Whitham M

et al. (2018) Cell Metab 27, 237–251]. Although the benefits of exercise are well-established, the molecular mechanisms underlying exercise adaptations remain ill-defined and are actively being investigated. Extracellular vesicles are a major source of regulatory signals that play a key role in cell-to-cell communication and have recently emerged as a potential tool through which the muscle communicates with other tissues and organs [Maggio S et al. (2023) Int J Mol Sci 24, 3039]. For this reason, the present project aims to investigate and characterize circulating EVs released in response to high intensity and duration exercise. To isolate EVs, serum and plasma samples were collected from ultra-marathoners pre- and post-race and then processed with size exclusion chromatography (SEC). The obtained SEC fractions were characterized by nanoparticle tracking analysis to check particle counting and size distribution, which resulted comparable with those reported in literature, as previously published in Maggio S et al. (2023) Int J Mol Sci 24, 3039. Dot blot assay, employed for EV marker detection, showed that EV surface protein CD9, muscle-related marker CAV3 and stress-related marker HSP60 expression increased in post-race in most marathoners. These findings suggest that EVs could be involved in the systemic responses to high intensity and duration exercise. Circulating EVs could therefore represent an innovative source of exercise biomarkers which could give a helpful insight on the physiological activation state in response to physical exercise.

Molecular mechanisms of functional foods and their bioactive compounds

P-14-001
Bioaccessible fraction of manna inhibits inflammatory response in a co-culture model of intestinal epithelial Caco-2 cells and RAW264.7 macrophages

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Inflammatory bowel diseases (IBD) involve intestinal barrier dysfunction, with chronic inflammation being a key factor in their occurrence. Manna is a product obtained from the spontaneous solidification of the sap of certain *Fraxinus* species and is used in medical therapy at 30 g/day as a laxative, due to its high mannitol content. Manna is also a rich source of (poly)phenols with reducing, antioxidant, and anti-inflammatory properties[1]. In this study, manna (5 g) was subjected to *in vitro* gastrointestinal digestion (INFOGEST 2.0) to obtain the bioaccessible fraction (BFM) and we assess its anti-inflammatory effect in a co-culture model of Caco-2 and RAW264.7 cells. Caco-2 cells were differentiated on the apical side of bi-cameral Transwell inserts while RAW264.7 cells were grown on the basolateral side. Inflammation in RAW264.7 was induced using LPS (1 μg/ml), therefore BFM was added to the apical side. Compared to unstimulated cells, treatment of macrophages with LPS for 24 h resulted in increased release of proinflammatory IL-8 and NO by Caco-2 cells, higher iNOS protein levels, and triggered redox imbalance as evidenced by intracellular ROS generation. Dysfunction of the absorptive cell monolayer was also evident by the decrement of the transepithelial electrical resistance (TEER). A 90-min pre-treatment of the apical Caco-2 monolayer with non-toxic BFM (6 mg/ml) significantly inhibited the increase of inflammatory markers and restored the TEER value, comparable to 1 μM Budesonide used as a positive control. Additionally, BFM protected from LPS-induced inflammation by inhibiting the induction of COX-2 level, the release of proinflammatory molecular mediators such as IL-6, TNF-α, PGE_2 and preventing the nuclear translocation of the p65 NF-κB subunit. Our study suggests that manna, used at a lower non-laxative dose, can be considered a natural product capable of preventing IBD. Reference: [1]Attanzio A et al. (2019) Antioxidants 18;8(10):494.

P-14-002

Abstract withdrawn.

P-14-003
From metabolomics to medicine: exploiting the functional potential of plant extracts in human health, with emphasis on *Ribes nigrum, Ficus carica* and *Vitis vinifera*

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The challenge of drug resistance in anticancer chemotherapeutics poses a significant hurdle to effective treatments. Modern cancer therapies, rooted in a nuanced understanding of tumor biology and molecular genetics, have shifted towards targeted strategies. The study presents a thorough metabolomic characterization of plant extracts derived from *Ribes nigrum*, *Ficus carica* and *Vitis vinifera*, using LC-Q-Orbitrap HRMS analysis along with quantification of total phenolic and flavonoid content. Noteworthy is *V. vinifera*, exhibiting the highest levels of flavonoids and phenolics, identified through 144 major peaks, predominantly comprising flavonoids and phenolic acids. Comparative analysis reveals a spectrum of shared and unique compounds across the plant samples. Given their recognized high redox potential, flavonoids and phenolic compounds emerge as potential contributors to anticancer therapies. The evaluation extends to the cytotoxicity and chemotherapeutic modulatory properties of these plant extracts on human colon adenocarcinoma HT-29 cells, encompassing both doxorubicin-resistant and sensitive phenotypes. The extracts exhibit significant growth inhibitory effects, with *F. carica* emerging as particularly potent. Hydroethanolic extracts of grape and blackcurrant showcase notable cytotoxicity towards HT-29 human colon cancer cells. The growth inhibition observed with *F. carica*, *R. nigrum* and *V. vinifera* extracts suggests the presence of diverse postulated anticancer constituents. Furthermore, resistance-modifying experiments on doxorubicin-resistant and susceptible HT-29 cells demonstrate that all three extracts not

only exhibit intrinsic cytotoxicity but also enhance the cytotoxicity of doxorubicin. These compelling findings underscore the potential of these plant extracts as promising components in functional foods, emphasizing their prospective role in promoting human health, particularly within the realm of functional foods designed for applications in cancer therapy. *The authors marked with an asterisk equally contributed to the work.

P-14-004
Protective action of anti-aging formulations in counteracting DNA damage in human keratinocytes

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Dietary intervention is a safe preventive strategy for slow aging due to several factors like genomic instability. This study aimed to evaluate the protective effect of different compounds against DNA damage: calcium alpha-ketoglutarate, di-magnesium malate, pterostilbene, lithium aspartate, glycine, glucosamine sulfate, fisetin, spermidine, and trehalose, widely used as ingredients in food supplements, to test their possible anti-aging activity once combined in different formulations. The compounds safety was assessed through spectrophotometric assays for cytotoxicity and cell viability in human keratinocytes (HaCaT), pretreated for 24 h at different concentrations, to find the best for each ingredient to add to the final formulation. Six different formulations were used to evaluate DNA damage by immunofluorescence using the Anti-53BP1 antibody. After 24 h of pretreatment with the supplements (1:10 dilution), cells were stimulated for 2 h with the genotoxic agent neocarzinostatin (NCS 0.13 μM). Foci (F) were counted, indicating the double-strand breaks as follows: mild damage $F < 5$; intermediate damage $5 \leq F \leq 20$; acute damage $F \geq 20$. 24-h treatment with the diluted 1:10 formulations showed no cytotoxic and cell viability-modulating effects on human keratinocytes. Analysis of DNA damage through 53BP1 foci count showed that the formulations do not induce DNA damage and significantly prevented DNA damage in pretreated cultures challenged with NCS ($p < 0.0001$). Next, the antioxidant activity of the four most promising formulations was tested using a chemiluminescent assay to detect intracellular H_2O_2 in 2D and 3D HaCaT cell models. Results showed that 24-h treatment with the formulations reduced acute DNA damage and some formulations also showed antioxidant activity, thus suggesting their possible anti-aging effect. This work was supported by MIUR-PRIN 2022 (Prot. 2022LW54KC) to SH, CC, and AP.

P-14-005
MAPKs and NF-κB inhibition are involved in the neuroprotective effects of a cinnamon/curcumin/turmeric spice blend in an *in vitro* model of Alzheimer's disease

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Alzheimer's disease (AD) is a neurodegenerative disorder whose exact aetiology remains elusive, challenging the entire scientific community. However, it is acknowledged that AD is characterized by an increased deposition in the brain of β-amyloid (Aβ) protein, which triggers pro-inflammatory events in the surrounding nervous tissue. The aim of this study was to investigate the neuroprotective effects of a spice blend composed of an extract of cinnamon bark and two different turmeric root extracts (CCSB) in Aβ-exposed THP-1 cells, employed as a model of neuroinflammation. In abiotic ORAC assays, CCSB exhibited up to 3-fold greater reactive oxygen species (ROS) quenching ability than the standard Trolox. In THP-1 cells, CCSB also demonstrated antioxidant potential by reducing ROS, induced by the amyloid fragment by up to 39.7%. Furthermore, CCSB diminished the Aβ-stimulated secretion of the pro-inflammatory cytokines IL-1β and IL-6 by up to 24.9% and 43.4%, respectively, and their gene expression by up to 25.2% and 43.1%, respectively. The mechanism underlying this effect seems to involve the mitogen activated protein kinases (MAPKs) ERK, JNK and p38, whose phosphorylation was reduced by up to 51.5%, 73.7%, and 58.2%, respectively. In addition, phosphorylation of p65, one of the five components forming NF-κB, was reduced by up to 86.1%. Our data indicate that CCSB can counteract the neuroinflammation induced by Aβ-stimulation of THP-1 cells, thus targeting the events characterizing the early stages of AD. We thank NeoLife International LLC for the financial support of this research project.

P-14-006
Innovative method for the study of glucose release in foods

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In recent years, research has highlighted the importance of studying the bioaccessibility of nutrients, defined as the fraction of nutrient released from the food matrix and available for intestinal absorption. In fact, the mere evaluation of the food proximate composition is not sufficient for the reliable nutritional assessment as the food matrix and the processing strongly modulate nutrients bioaccessibility. The study of glucose release is of particular importance as it provides a reliable insight on the impact of food intake on glycemia. In our study, a new protocol for the evaluation of glucose release was developed, in which the INFOGEST consensus method [1] was added of an additional step including mucosal enzymes for carbohydrates digestion. The novel protocol was tested on two commercial products (cracker) having similar starch content but different dietary fibre content.

Briefly, the oral, gastric and the first part of duodenal phase of the digestion were performed according to Brodkorb et al., 2019. After 1 h of duodenal digestion, the enzyme amylo-glucosidase was added and glucose release from foods was assessed spectrophotometrically after 0, 30, 60, and 120 min. At T0 and T30, the glucose concentration in the digested fraction was similar in the two products and increasing with time. Starting from T60, the release of glucose was higher in the crackers not containing dietary fibre. Our results confirm that the presence of dietary fibre can modulate starch hydrolysis and glucose bioaccessibility. The novel protocol will be further applied to other commercial and innovative, which will be compared with their conventional counterparts. Reference: Brodkorb, A., et al. (2019) Nat Protoc, 14 (4): p. 991–1014.

P-14-007
Dietary protein and host homeostasis via modulating gut microbiota

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Gut microbiota has been proposed to be involved in the host homeostasis. Diet is the primary factor in host nutrition and metabolism; however, the consumption of various diets results in alterations to gut microbial composition, microbial diversity, and specific bacterial taxa. Although wheat is a common cereal in the Western diet and an important source of protein and fiber, the excess intake of gliadin, which is included in wheat protein, is considered the greatest risk factor due to the intestinal inflammation. Recently, some researchers suggested that the intake of excess gliadin may have a pivotal role in gut homeostasis via modulating gut microbiota. However, the detailed molecular mechanisms underlying implications among the gliadin intake, gut microbiota, and gut homeostasis have not been clarified. Here, we investigated the effects of gliadin intake on the gut microbiota and host energy homeostasis. The metabolic parameters, such as body and tissue weight, and blood glucose level, in high-fat diet-induced obesity were similar between HFD-fed control and gliadin-fed mice. On the other hand, the excess intake of gliadin induced changes in the gut microbial composition and diversity, and aggravated the intestinal inflammation. Moreover, we further clarified that the intake of excessive gliadin decreased insulin signaling in the gut and liver, which inhibited lipid metabolism. Additionally, the mRNA expression of insulin signaling-related genes in the gut and liver was changed in gliadin-fed mice. These results suggested that the intake of different protein consumption in diets may promote changes in the gut microbial compositions and may play a role in host homeostasis. Our findings shed light on the regulation of host homeostasis and the development of novel functional foods by targeting the gut microbiota.

P-14-008
Prebiotic effects of exopolysaccharides produced by lactic acid bacterium via SCFA receptors

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Indigestible polysaccharides, including dietary fiber and oligosaccharides, improve host insulin sensitivity and reduce the risk of obesity through production of short-chain fatty acids (SCFAs; acetate, propionate, and butyrate), because they are metabolized to SCFAs by gut microbial fermentation in the colon, after escaping digestion and absorption in the small intestine. Indigestible polysaccharides-derived gut microbial SCFAs are not only host energy sources but also important signaling molecules for exerting physiological functions related to host energy homeostasis via the G-protein coupled receptors GPR41 and GPR43. Exopolysaccharide (EPS) as polysaccharides produced by some of lactic acid bacteria is also expected to have indigestible polysaccharides-associated host beneficial effects. Here, we demonstrated that EPS produced by lactic acid bacteria *Leuconostoc mesenteroides* have significant metabolic benefits for the host. *L. mesenteroides* converts sucrose to glucose and fructose via dextransucrase, and utilizes fructose as the energy source while producing dextran as EPS from glucose. *L. mesenteroides* producing EPS (LmEPS) was an indigestible α-glucan, and LmEPS intake improved glucose metabolism and energy homeostasis through SCFAs production and changing gut microbial composition. Thus, *L. mesenteroides* producing EPS exerts prebiotic effects by regulating host energy homeostasis. These findings may contribute to the development of functional foods for the prevention of lifestyle diseases such as obesity and diabetes.

P-14-009
Gut microbial metabolites improve host hepatic metabolism via polyphenol receptor

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The gut microbiota has become a significant factor with the potential to impact various physiological functions and pathophysiological processes, including obesity and type 2 diabetes mellitus. Increasing evidence indicates that gut microbial metabolites play a crucial role as essential molecules in interactions between the host and microbes. Phenolic compounds, which are abundant in plants, fruits, and beverages, have been extensively studied in animal models and clinical trials for potential health benefits. Conversely, a significant proportion of dietary polyphenols remain unabsorbed in the small intestine and accumulate in the colon. The gut microbiota plays a critical role in the extensive metabolism of these polyphenols into smaller, more readily absorbable metabolites, contributing to a variety of beneficial effects on the host. Comprehensive studies of the bioaccessibility and bioavailability dynamics of polyphenols and their metabolites are essential for understanding their physiological functions. However, the mechanisms underlying the interplay among dietary polyphenols, gut microbiota, and host health are still poorly

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understood. Therefore, the aim of this study was to investigate the beneficial metabolic effects of polyphenolic microbial metabolites on a high-fat diet-induced obese mouse model and to elucidate the molecular mechanisms involved. In addition, a global analysis of hepatic gene expression changes after polyphenolic microbial metabolites administration was performed to understand the detailed mechanisms of improved hepatic lipid metabolism. Furthermore, an exploratory investigation was also conducted to identify specific receptors for these metabolites in the body. Our findings provide valuable insights for the development of functional foods containing polyphenol microbial metabolites and preventive drugs targeting polyphenol receptors to treat metabolic disorders. Reference: Ohue-Kitano R et al. (2023) Sci Rep 13, 21246.

P-14-010
Hispidulin inhibits the vascular inflammation triggered by *Porphyromonas gingivalis* lipopolysaccharide

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Hispidulin is a natural bioactive flavonoid that has been studied for its potential therapeutic properties, including its anti-inflammatory, antioxidant, and neuroprotective effects. The aim of this study was to explore whether hispidulin could inhibit the endothelial inflammation triggered by *Porphyromonas gingivalis* (*P. gingivalis*) lipopolysaccharide (LPS). The adhesion of monocytes to the vascular endothelium was evaluated through *in vitro* and *ex vivo* monocyte adhesion assays. We analyzed the migration of monocytes across the endothelial layer using a transmigration assay. The results showed that treatment with hispidulin decreased the *P. gingivalis* LPS-induced adhesion of monocytes to endothelial cells and their migration by suppressing the *P. gingivalis* LPS-triggered expression of intercellular adhesion molecule-1 (ICAM-1) through downregulating nuclear factor-κB (NF-κB). In addition, hispidulin inhibited *P. gingivalis* LPS-induced mitogen-activated protein kinases (MAPKs) and AKT in endothelial cells. Altogether, the results indicate that hispidulin suppresses the vascular inflammation induced by *P. gingivalis* LPS. Mechanistically, it prevents the adhesion of monocytes to the vascular endothelium and migration and inhibits NF-κB, MAPKs, and AKT signaling in endothelial cells. Previously published in: Yeon Kim et al. (2023) Molecules 28, 6717.

P-14-011
MCT oil suppresses non-alcoholic steatohepatitis via MCFA receptor

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Medium-chain triglycerides (MCTs), composed of medium-chain fatty acids (MCFAs), are unique dietary fats with various health benefits. Among MCFAs, particularly C10:0 and C12:0 are recognized as ligands for G protein–coupled receptor 84 (GPR84). However, GPR84 is still considered an orphan G protein–coupled receptor (GPCR) because of the low plasma levels of endogenous MCFAs, and the nutritional signaling of dietary MCFAs via GPR84 remains unclear. In this study, we demonstrated that MCFA-mediated GPR84 signaling protected hepatic functions from diet-induced lipotoxicity. Under high-fat diet (HFD) conditions, GPR84-deficient mice exhibited nonalcoholic steatohepatitis (NASH) and the progression of hepatic fibrosis, but not steatosis. With significantly increased hepatic MCFA levels under HFD, GPR84 suppressed lipotoxicity-induced macrophage overactivation. Thus, GPR84 is an immunomodulating receptor that suppresses excessive dietary fat intake–induced toxicity by sensing increases in MCFAs. Additionally, administering MCTs, MCFAs (C10:0 or C12:0, but not C8:0), or GPR84 agonists effectively ameliorated NASH in mouse models. Therefore, intake of MCT oil may be a potential strategy for treating NASH.

P-14-012
Bioactive peptides from fermented sourdough: potential role in modulation of gut inflammation and intestinal barrier function

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Dietary bioactive peptides (BPs) produced during microbial fermentation represent valuable ingredients to reduce the risk of chronic disorders, such as inflammatory bowel disease [Previously published in: Cicchi et al. (2023), Biology, 12, 1162]. In this context, an antioxidant and anti-inflammatory activity of BPs from sourdough and bread obtained with wheat flour was demonstrated. [Previously published in: Luti et al., (2020), Food Chem 322, 126710]. In this study, sourdough from spelt flour (*Triticum spelta*) was obtained through fermentation with selected bacterial starters. Water extracts were fractionated by RP-HPLC. The amounts of recovered peptides showed that fermentation with bacterial starters results in a higher yield of peptides when compared to fermentation with only *Saccharomyces cerevisiae* (used as control), confirming the high protease activity of some bacterial starters. [Previously published in: Galli et al. (2018), Int J of Food Mic 286, 55–65]. Experiments on jejunum organoids, to test the effects of these products on the intestinal epithelium, are ongoing. Organoids were stimulated with LPS. Preliminary results show that farro flour digested with bacterial starter decreases the expression of Tlr4, Cxcl1 and Tjp1 genes. Induction of antibacterial peptides Reg3 and Defa1 was observed, together with that of Cldn2 and 4. In turn, farro flour digested only with *S. cerevisiae* showed a different profile, only inhibiting Cxcl1 expression. To study the impact of BPs on intestinal functionality, experiments with Caco2 monolayers are in progress. Their effect on intestinal permeability can be assessed by measuring the passage of Lucifer Yellow via the paracellular space and analysis of proteins involved in barrier integrity, such as Zo-1 and Occludin. In conclusion, the use of bacterial starter for the fermentation of sourdough generates peptides with a wide range of size and sequences that has a higher impact on gene regulation in the intestinal epithelium.

P-14-013
In vitro and *in silico* approaches to assess modulation of digestive proteases by food-derived bioactives

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The interactions between various food components and digestive tract enzymes can alter how food nutrients are absorbed and have an impact on an individual's health. Some food components – especially polyphenols – reportedly inhibit some digestive enzymes and are commonly referred to be anti-nutritional factors. Reports on this subject frequently contradict one another, emphasizing the necessity of using consistent methodologies to assess the potential impact of bioactive compounds. In this study, the activity of pepsin, trypsin, and chymotrypsin was evaluated *in vitro* using ovalbumin, gluten, and hemoglobin as substrates in the presence/absence of twenty-five bioactive compounds at physiological concentrations. Results show that bioactives may affect proteolytic activity in opposite ways, depending on the substrate and the enzyme. A computational approach based on molecular docking and dynamics simulations was then applied to investigate the interactions that selected bioactives may have with the enzymes and substrates. Piceid and phloridzin dihydrate were studied, along with their respective aglycones (resveratrol and phloretin). Resveratrol, piceid, and phloridzin dihydrate enhanced the activity of chymotrypsin on ovalbumin, whereas phloridzin dihydrate aglycone (phloretin) inhibited it. *In silico* results show that all four polyphenols can interact with chymotrypsin but only those polyphenols enhancing the *in vitro* proteolytic activity (resveratrol/piceid; phloridzin dihydrate) could alter the structure of ovalbumin determining a partial unfolding. Thus, a substrate-dependent relationship can be brought forward to explain why polyphenols may affect protein digestion in opposite ways. The evidence gathered here suggests caution in generalizing polyphenols as a single category of 'anti-nutritional factors' and highlights the potential of individual molecules within this class as 'digestive modulating agents'.

P-14-014
Production and receptor mediated recognition of taste-related peptides

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Humans perceive five tastes, sweet, bitter, sour, salty, and umami, through the taste buds of the tongue. Sweet taste is primarily mediated through sweet taste receptors, which are membrane-bound heterodimeric structures composed of T1R2-T1R3 belonging to the G protein-coupled receptor class C. In this study, we expressed the N-terminal domain of hT1R3 (hT1R3-TMD) in *Escherichia coli*, purified it through immobilized metal affinity chromatography, and then refolded it using the dilution refolding method. Circular dichroism spectroscopy was used to confirm successful refolding of hT1R3-TMD, and tryptophan fluorescence quenching assay was used to investigate the interaction of hT1R3-TMD homodimers with the sweet protein brazzein. Meanwhile, with regard to the human salty taste perception mechanism, it is known that a mechanism through TRPV1, which is different from the previously known ENaC pathway, is involved in salty taste perception, but the specific mechanism of action has not been revealed. We established a yeast expression system for recombinant peptides and optimized expression conditions for efficient production of salty peptides derived from sweet tasting protein brazzein. We also performed molecular modeling and docking simulations between TRPV1 and salty peptides to derive a binding model, and based on this, we attempted to reveal the salty taste recognition mechanism related to TRPV1. As a result, S1-S2 linker of TRPV1 was shown to be the critical site for the interaction between TRPV1 and salty peptides.

P-14-015
Antiferroptotic potential of oleuropein and *Olea europaea* cultivars

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Olea europaea L. (Oleaceae) is a species that is widely distributed in Mediterranean countries. Especially products such as olive and olive oil have a critical importance in dietary and protection against various diseases. In addition, it has been suggested that olive leaves have rich phenolic contents and therefore may have antioxidant roles. Ferroptosis is an iron-dependent form of cell death that was identified for the first time in 2012. This pathway is characterized by increased intracellular iron ion triggering lipid peroxidation. Increasing lipid hydroperoxide levels causes cell death. In our study, the aqueous ethanolic extracts of olive leaves were prepared from five different cultivars (Ayvalık, Memecik, Gemlik, Domat and Manzanilla) and evaluated for their antiferroptotic activities. The extracts (10 and 100 μg/ml) and oleuropein (1, 5 and 10 μM) were applied to SH-SY5Y cells for 2 h. After incubation, cells were exposed to erastin (50 μM), a known ferroptosis agent, for 24 h. The changes in cell viability were evaluated by the MTS method. According to our results, 100 μg/ml concentrations of all extracts, independent of cultivars, significantly increased cell viability compared to erastin-treated cells ($p < 0.05$). A similar observation was found when cells were treated with 10 μM oleuropein ($p < 0.05$). Ferroptosis is a newly identified cell death pathway whose mechanism has not yet been elucidated. It has been demonstrated that extracts rich in phenolic compounds prepared from olive leaves contribute to the reversal of elastin-mediated ferroptosis. However, the data need to be supported by further *in vitro* and *in vivo* studies. This study was financially supported by the Scientific and Technological Research Council of Türkiye (TUBITAK) under 1005-National New Ideas and Products Research Funding Program (Project number: 222O592). *The authors marked with an asterisk equally contributed to the work.

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P-14-016
Bioactive natural products for atopic dermatitis

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Atopic dermatitis (AD) is a chronic, relapsing skin disease accompanied by itching and dry skin caused by a complex interaction of innate and adaptive immune responses. AD treatments include glucocorticoids and immunosuppressants. However, long-term treatment can have serious side effects. Thus, an effective AD treatment with fewer side effects is required. Natural materials, including herbal medicines, have potential applications. In DNCB-induced mice, BS012 (a mixture of *Asarum sieboldii*, *Platycodon grandiflorum*, and *Cinnamomum cassia* extracts) showed potent anti-atopic activity, such as reducing AD-like skin lesions and inhibiting the expression of Th2 cytokines and TSLP. In TNF-α/IFN-γ-stimulated keratinocytes, BS012 inhibited the expression of pro-inflammatory cytokines and chemokines via blockade of NF-κB and STAT signaling pathways in a dose-dependent manner. Serum metabolic profiles of mice showed significantly changed in lipid metabolism, which is related to the inflammation of AD. In the intracellular metabolome, BS012 treatment affected the metabolism associated with not only inflammation but also the skin barrier function and lipid organization of the stratum corneum. This study proved that BS012 has an anti-atopic activity by reducing the Th2-specific inflammatory response and improve the skin barrier function *in vivo* and *in vitro* models of AD. The metabolic mechanism of the therapeutic effects was investigated using metabolomics and discovered that the effects were closely related to the inflammation and lipid organization. It suggested that the BS012 could be a potential therapeutic alternative for AD and this study could provide valuable insights into the development of natural products for AD treatment.

P-14-017
Anti-aging effects of plant extracts on eukaryotic models of Parkinson's disease

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Aging and age-related neurodegeneration are among the main challenges in modern medicine [Tripodi F. et al. 2020, Aging 12 (19), 19785–19808] and Parkinson's disease (PD), characterized by the misfolding of α- synuclein protein, affects about 10 million people worldwide [Oliveira L. et al. 2021, NPJ Parkinsons Dis 7 (1), 65]. Plants are historically recognized as a source of bioactive molecules useful in many applications in the fields of medicine, cosmetics, and food industry. In recent years, the protective effects of several bioactive compounds have been highlighted on a wide variety of diseases among which diabetes, cardiovascular and neurodegenerative diseases [Pohl F. et al, 2018, Molecules 23 (12), 3283]. In response to the substantial waste generated by the conventional linear food industry, there is a growing emphasis on exploring alternative and environmentally friendly strategies to mitigate food waste. One such avenue involves the utilization of cocoa shells, a by-product typically discarded during the roasting process of cocoa beans (Pagliari S. et al, 2022, Food, science and technology). This study is dedicated to unlocking cocoa shell components by investigating their potential inhibition effects on α-synuclein aggregation. Moreover, to valorise the huge Italian flora biodiversity, we made a screen of 63 extracts of endemic Italian plants, performed in the context of the National Biodiversity Future Centre (NBFC, PNRR). The aim of this study is the identification of new plant-based bioactive molecules, considering their potential antioxidant, anti-aging and neuroprotective properties. Using eukaryotic cell models overexpressing human α- synuclein, we have assessed the effects on cellular longevity, oxidative stress as well as protein aggregation, all phenotypes associated with α-synuclein-dependent toxicity. The most promising extracts are now under investigation for their effects on cellular metabolism and on the induction of catabolic processes.

P-14-018
Delivery of DNA and mRNA vaccines by human milk exosomes

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Delivering DNA and mRNA vaccines into the body is still challenging, and there is currently no universal solution. Artificially synthesized liposomes have a number of well-known disadvantages. This work aimed to create a delivery system for mRNA and DNA vaccines (pVAX1) containing the RBD gene in laboratory mice bodies using milk exosomes and auxiliary reagents. To isolate exosomes from milk preparations, we used a previously developed method that included the stages of centrifugation, ultrafiltration, and ultracentrifugation. We also purified exosomes from co-isolating impurities using gel filtration. The resulting preparations were loaded with DNA and mRNA vaccines; laboratory mice of the CD1 line and other strains were immunized with the loaded exosome preparations. The vaccine was administered twice, with an interval of two weeks; two weeks after the second immunization, the animals were slaughtered, and the titer of antibodies against RBD was analyzed. It has been shown that DNA and mRNA vaccine preparations are effectively loaded into milk exosomes. A statistically significant increase in antibody titers to RBD was observed in laboratory animals' blood plasma, comparable to that after the immunization with the RBD with Freund's adjuvant. The results obtained indicate that milk exosomes can be used as delivery vehicles for DNA and mRNA vaccines.

P-14-019
Chemical composition, antioxidant and antibiotic-modulating potential of *Ribes nigrum* extract

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Plants have the ability to synthesize diverse compounds possessing biological activity. The aim of our study was to determine the phytochemical composition of the ethanol extract of *Ribes nigrum* L. leaves and to investigate some peculiarities of the

mechanisms of its antimicrobial and antioxidant activity in different test-systems. The total phenolic and flavonoid contents in *R. nigrum* leaf extract were 167.15 ± 7.29 μg of gallic acid equivalent (GAE) mg^{-1} and 49.99 ± 0.86 μg quercetin equivalent (QE) mg^{-1}, respectively. The HPLC profiling showed that the main components of extracts were quercetin rutinoside, quercetin hexoside, quercetin glucuronide, quercetin malonylglucoside and quercitrin which all possessed expressed antioxidant capacity. The DPPH assay exhibited high antioxidant activity of investigated extract (IC50 value was 66.01 ± 1.65 μg ml^{-1}). *R. nigrum* extract antibacterial and antibiotic modulatory properties were elucidated on different Gram-positive, Gram-negative bacteria and yeasts. The plant extract at non-toxic concentrations (0.125 and 0.25 mg/ml) for the test bacteria combined with kanamycin and ampicillin decreased the MIC value of the antibiotics ~2 fold against tested bacteria. The antioxidant activity of *R. nigrum* extract was expressed also by the alteration of the activity of different key enzymes, included in cell antioxidant defense system (SOD, catalase, acetyl-CoA oxidase). These was evaluated using BV-2 wild type (WT) cells and acyl-CoA oxidase 1 (ACOX1) deficient (Acox1-/-) microglial cell lines. Taken together, it can summarized that *R. nigrum* has high potential as a source of biologically active substances which can have antiobacterial and antioxidant affects. *The authors marked with an asterisk equally contributed to the work.

P-14-020
Polyphenols from Mediterranean herbs as bioactive molecules for improving human health

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Currently, the scientific community is focused on studying the beneficial properties of Mediterranean diet, specifically natural compounds such as aromatic herbs and molecules present in some foods (e.g. lycopene), on human health. This study explored the protective and/or anti-inflammatory abilities of carvacrol, thymol, eugenol and lycopene, alone or mixed in two pathological *in vitro* models; nonalcoholic hepatic steatosis (NAFLD) and osteoarthritis (OA). NAFLD *in vitro* model was obtained by insulting human liver cancer cells (HepG2) with a fatty acid mix [Stellavato, A., et al.(2018) Lipids Health Dis. 17:24]. After the addition of natural compounds to the cells, Oil Red O-staining displayed a reduction in fat accumulation. Peroxisome proliferator-activated receptors (PPARs) were modulated, towards improved lipid metabolism, by the presence of these extractive natural compounds and, above all, by the mixture of them. The second pathological *in vitro* model (OA) was established by using human primary pathological chondrocytes [Vassallo, V., et al.(2021) J Cell Biochem. 1021–36]. In this case, carvacrol, eugenol and lycopene were tested combined with pharma-grade chondroitin sulfate (CS) that is usually employed as component of medical devices or food supplements for OA management. Here, the combination of natural compounds with CS enhanced its already well-known effects in modulating specific markers OA related. In fact, qRT-PCR results showed the formulations ability in preserving cell phenotype by collagen type 2A1 and aggrecan up-regulation. Moreover, western blotting and ELISA assays showed that the formulations also reduced the main biomarkers involved in inflammation and cartilage degradation (e.g. NF-kB, metalloprotease-13). Overall, these results increased our knowledge about natural molecules involvement into specific biochemical pathways and their potential application in enriched/functional foods for diseases prevention or as adjuvant of pharmaceutical treatments.

P-14-021
Biological activity of oat globulins hydrolysates after hydrothermal treatment

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Oat proteins are considered as a reservoir of peptides with biological activities, including inhibitors of dipeptidyl peptidase-IV, angiotensin converting enzyme and antioxidative peptides. The aim of this study was to analyze oat globulins after hydrothermal treatment as the source of peptides with antidiabetic, antihypertensive and antioxidant activity. The study was carried out using computation tools available in UniProt (https://www.uniprot.org/), BIOPEP-UWM (http://www.uwm.edu.pl/biochemia/), and METLIN (https://metlin.scripps.edu/) databases as well as Fragment Ion Calculator (http://https://proteomicsresource.washington.edu/protocols06/). According to the INFOGEST method, the *in vitro* digestion procedure included the following steps: oral – 2 min, gastric – 2 h, pH = 3.0, intestinal – 2 h, pH = 7.0. Then, hydrolysates were analysed for their DPP-IV and ACE inhibition and antioxidant activities. Based on the *in silico* systematic screening for DPP-IV and ACE-inhibitory, as well as antioxidative peptides, the amino acid sequences of bioactive peptides were identified in hydrolysates using LC-Q-TOF-MS/MS method. It was found that all oat globulins are potential source of sequences of antioxidative fragments and moreover ACE- and DPP-IV-inhibiting ones. The intestine hydrolysate demonstrated the highest degree of DPP-IV inhibition (98.85%; IC50 = 0.26 mg/ml) and ACE inhibition (92.75%; IC50 = 0.64 mg/ml) and moreover antioxidative activity (ABTS = 97.23%; DPPH = 88.05%; FRAP = 72.08 μM Trolox/mg of sample). The DPP-IV inhibitory (i.e. dipeptides GL, HF) and ACE inhibitory fragments (i.e. dipeptides GF, PR) as well antioxidative peptides (i.e. PW, VY) were identified. It can be concluded that the hydrolysates obtained were characterized by the highest ability to inhibit the tested enzymes and the highest antioxidative activity after the intestinal phase of digestion. Founded by the Minister of Science under "the Regional Initiative of Excellence Program". *The authors marked with an asterisk equally contributed to the work.

P-14-022

Abstract moved to speed talks.

P-14-023

The hidden potential of biological properties of mushroom polysaccharide extracts

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Mushroom polysaccharides (MP) have demonstrated the ability to modulate several immune responses. Identifying their chemical composition is crucial for understanding the cellular mechanisms underlying immunomodulation. However, only a few mushrooms have been studied for polysaccharide profiles and the diversity of extraction systems has been limited. In this work, three water-soluble (WSP), one water-insoluble (WIP), and one alkaline-soluble polysaccharide (ASP) fractions from commercial biomasses of three mushroom species (*Coriolus versicolor*, *Pleurotus ostreatus*, and *Hericium erinaceus*) were obtained. Chemical characterization, polysaccharide and protein molecular weight distribution (MWD), and dynamic light scattering analysis of the extracts were conducted. The total phenolics content (TPC) and antioxidant activity were assessed, as well as the antimicrobial activity against Gram-positive and -negative bacterial strains. The results indicated that α-glucan content was higher than 40% in the three species. In opposite to wild mushrooms, these higher levels of α-glucans when compared to β-glucans, should be analyzed as an opportunity to evaluate their role in biological processes. Variations in protein content (0.5-4.8%), MWD, and particle sizes (112–204 nm) were also detected in the different extractions. TPC was higher in WSP fractions (100–650 mg GAE/100g of extract), and lower in ASP fractions (100–150 mg GAE/100 g of extract, and WIP fractions (20–50 mg GAE/100 g of extract), in agreement with the related antioxidant activity. Growth inhibition values of 20–90% for *S. epidermidis, S. aureus, and E. coli* strains were obtained, evidencing the role of both phenolic compounds and polysaccharides in the antimicrobial activity. Further, the characterization of the immunomodulatory response of these extracts will also be assessed aiming at their use to tackle new therapeutic strategies.

P-14-024

Olive oil hydroxytyrosol prevents mercury-induced phosphatidylserine exposure in human erythrocyte membrane: focus on the effect on scramblase and flippase activities

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Erythrocytes (RBC) are anucleate cells with an average lifespan of 120 days. The exposure of phosphatidylserine (PS) on the outer side of the plasma membrane is the key mechanism for removing aged/stressed RBC by splenic macrophages, acting like an "eat me" signal. However, this membrane alteration represents a critical event in the increased prothrombotic activity of RBC, as PS exposure promotes the adhesion of RBC to endothelial cells, thereby contributing to thrombus formation and subsequent microvascular occlusion. PS translocation is mainly mediated by two enzymes, ATP11C flippase and PLSCR1 scramblase. Several toxic substances can increase PS exposure, including mercury (Hg). In this respect, phenolic compounds in olive oil have attracted considerable interest as protective agents, particularly hydroxytyrosol (HT). We show that HT is able to reduce Hg-induced PS exposure in RBC membrane, therefore counteracting heavy metal poisoning. The aim of our study was to identify the molecular mechanisms underlying HT-protective effect. We showed that treatment with Hg induced alterations in ATP11C and PLSCR1 enzyme activity, but pre-treatment with HT restored the physiological situation. By western blotting, we also determined changes in the levels of these enzymes on the RBC membrane. Again, treatment with HT led to a decrease in the Hg-induced effect. Furthermore, as ATP11C is an ATP-dependent and PLSCR1 Ca^{2+}-dependent enzyme, we determined intracellular Ca^{2+} and ATP levels after Hg treatment and demonstrated how HT restores physiological levels. Finally, we performed a 'molecular docking' analysis and demonstrated how HT is able to interact directly with enzymes, in particular ATP11C, modulating their activity. Our work demonstrated how the beneficial properties of bioactive components of olive oil provide biochemical bases for nutritional strategies in the prevention of Hg-related diseases particularly related to Hg-induced endothelial dysfunction.

P-14-025

Artemisia gmelinii facilitates the expulsion of particulate matter by enhancing mucociliary clearance mechanisms

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Particulate matter (PM) is one of the most harmful inhaled pollutants and known to cause severe health problems to humans. Mucociliary clearance serves as an innate defense mechanism in the respiratory system against inhaled PM by the ciliated and goblet cells. In this study, we established an air-liquid interface (ALI) model system using human airway epithelial cells (hAECs) and screened the mRNA expression of MUC5AC across 15 medicinal plants. Among them, *Artemisia gmelinii* Weber ex Stechm extract (AGE) demonstrated superior effects on MUC5AC expression in ALI model of hAECs. Therefore, we evaluated the effects of oral administration of AGE on the emission of 89Zr-incorporated PM in a mouse model. In particular, AGE group showed rapid emission effects of 89Zr -incorporated PM at 144 h in lung tissues. Moreover, the expression of MUC5AC enhanced in lung tissues of the AGE group. Thus, we investigated the effect and mechanism of AGE in ALI model of hAECs. The AGE increased the ciliary beating frequency, adenosine triphosphate activity by enhancing the mRNA expression of dynein axonemal light chain 1, dynein axonemal heavy chain (DNAH) 10, DNAH 11, and sperm associated antigen 6, MUC5AC, and ZO-1 in ALI model. These data suggest that the administration of AGE is an effective approach for enhancing mucociliary clearance of inhaled PM and promoting respiratory health.

P-14-026
A proteomic study of the effects of an olive oil extract on endothelial cells reveals its antiangiogenic potential

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Extra virgin olive oil (EVOO), a staple of the Mediterranean diet, is rich in phenolic compounds recognized for their potent bioactive effects, including anti-cancer and anti-inflammatory properties. However, its effects on vascular health remain relatively unexplored. In this study, we examined the impact of a "Picual" EVOO extract from Jaén, Spain, on endothelial cells. Proteomic analysis revealed modulation of angiogenesis-related processes. In subsequent *in vitro* experiments, the EVOO extract inhibited endothelial cell migration, adhesion, invasion, ECM degradation, and tube formation while inducing apoptosis. These results provide robust evidence of the extract's anti-angiogenic potential. Our findings highlight the potential of EVOO extracts in mitigating angiogenesis-related pathologies, such as cancer, macular degeneration, and diabetic retinopathy. Grants: PID2023-148504OB-I00 (Spanish Government).Funds from BIO 267 (Andalusian Government, University of Málaga, EU FEDER).

P-14-027
Kumquat fruit intake ameliorates dysmetabolism-related neurodegeneration in the high-fat diet-fed mice

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Metabolic dysfunctions are strictly interconnected with neurological disorders. Accordingly, pathogenetic mechanisms underlying insulin resistance (IR) and Alzheimer's disease (AD) are deeply intertwined [Sędzikowska A et al. (2021) Int J Mol Sci 22, 9987]. Kumquats fruit contains flavonoids and polyphenols [Barreca S et al. (2017) Biofactors 43, 495] and is edible as a whole with its phytochemical-rich peel. We here investigate whether and how Kumquat fruit intake (8 mg lyophilised powder/kg/day for 24 weeks) exerts beneficial effects in a model of dysmetabolism-related neurodegeneration, i.e. the High-Fat Diet (HFD)-fed mice (Terzo S et al., 2023, Int J Mol Sci 24, 3467). Neurodegeneration was evaluated in terms of neuroapoptosis by TUNEL assay and by assessing levels of p27, Fas-L, BCL-2, BDNF by PCR. Alterations in the expression of selected genes involved in AD, i.e. insulin receptor (InsR), Ins degrading enzyme (IDE), beta-secretase-2 (BACE-2) and clusterin (Clu) were determined by RT-qPCR. Expression levels of proteins involved in brain insulin signalling, i.e. InsR, p-GSK-3, p-AKT and p-Tau were evaluated by western blot. Our results show that Kumquat intake significantly reduced the HFD-induced IR, evaluated by the HOMA-IR. Moreover, the fruit counteracted the dysmetabolism-related neuroapoptosis by decreasing the number of apoptotic nuclei, reducing the expression of p27 and Fas-L and increasing the levels of BCL-2 and BDNF. Remarkably, Kumquat hampered the HFD-induced downregulation of InsR and IDE gene expression and the upregulation of the BACE-2 and Clu one. Coherently, the fruit significantly improved brain insulin signalling, increasing InsR, p-AKT and p-GSK-3 protein levels and reducing p-Tau ones. As a whole, our data demonstrate that Kumquat intake counteracts dysmetabolism-related neurodegeneration, reducing neuroapoptosis and modulating the expression of crucial IR-related effectors of neurodegeneration at protein and gene level.

P-14-028
Computational evaluation of the human health effects of the main compounds found in *Artemisia dracunculus*

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Artemisia dracunculus is a perennial plant that grows wild and is also cultivated for its use in food, pharmacology and traditional medicine. This plant has been shown to have antibacterial, antifungal, antihemintic, antiseptic, anti-inflammatory, antipyretic, carminative, digestive and stimulant activities. Currently, this herb is the subject of professional phytochemical and pharmacological research. The main components of *A. dracunculus* have undergone safety checks using tests on laboratory animals, but it is already known that discoveries from animal experiments are frequently problematic to transfer to humans. Consequently, computational assessment of the human health hazard of chemical compounds found in this herb become important. In this study, the main organic compounds (with a content of the least 100mg 100g) in *A. dracunculus* were identified using the FOODB database as: caffeic acid (764 mg/100 g), 17alpha- ethynyl estradiol (550 mg/100 g), anethole (505 mg/100 g), 1 methoxy- 4 -(2 propenyl)benzene (376 mg/100 g), nerol (369 mg/100 g), maltose (275 mg/100 g), thujene (204 mg/100 g) and methyleugenol (160 mg/100 g). The possible toxicological effects on humans of these compounds were evaluated using admetSAR2.0 and SwissADME computational tools. The results obtained reveal that these compounds can inhibit organic anion and cation transporters, P glycoprotein, and cytochromes involved in endo- and xenobiotic metabolism, and can produce skin sensitization and hepatotoxicity. In addition, 17-alpha-ethynyl estradiol may lead to cardiotoxicity, respiratory toxicity, endocrine disruption, and reproductive toxicity. Anethole, caffeic acid and methyleugenol are the compounds that are also likely to produce reproductive toxicity. This information is important especially for those that are professionally exposed to higher quantities of these compounds.

P-14-029
Intestinal permeability in a mouse model of autism spectrum disorder: evaluation of the effects of food-borne *Lactiplantibacillus plantarum*

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In the frame of defining the role of the microbiota-gut-brain axis in neuropsychiatric health, it has been highlighted a strong correlation between autism spectrum disorder (ASD) and variations in gut barrier function, suggesting a prominent modulatory role of the intestinal microbiota. Intestinal dysbiosis associated with ASD results in increased intestinal permeability due to decreased expression of key junctional proteins belonging mainly to the claudin and zonulin families. Interestingly, the alteration of these proteins appears to be influenced by the action of probiotic strains. In this study, the administration of a mixture of two strains of food-borne *Lactiplantibacillus (Lpb) plantarum*, selected as probiotic candidates, was evaluated for 5 weeks in a monogenic murine model of autism, characterized by knock-in mice expressing the R451C mutation in Neuroligin3. After treatment, RT-qPCR analyses were performed to determine the mRNA levels of claudin 1, claudin 3 and zonulin 1 in colon and ileum regions. Our results showed that the gene expression of these proteins, involved in gut mucosal structure maintenance and permeability, were restored at the wild type levels in ASD mice nourished with *Lpb. plantarum*. These encouraging data suggest the potential impact of selected microbes' administration in alleviating ASD symptoms from the gastrointestinal perspective and deserve attention to move toward human clinical trials.

P-14-030
Encapsulation of bioactive nutraceutical compounds in donkey milk β-casein-based carriers

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β-casein (β-CN) can self-assemble into a micellar structure under appropriate conditions thanks to its amphiphilic structure, leading to the formation of intermolecular hydrophobic interactions as shown by Zhang J et al. (2024) Food Chem. 433 137285. Donkey milk β-CN showed similar self-assembling properties, but a low immunogenic potential if compared to the bovine counterpart, therefore could be used as a hypoallergenic nanocarrier. In this work, the encapsulation properties of donkey β-CN with vitamin D_2 or with the antioxidant molecule resveratrol have been investigated and the results were compared to those obtained with the bovine β-CN. The binding mechanisms and surface hydrophobicity of purified donkey β-CN or commercial bovine β-CN with resveratrol or vitamin D_2 were investigated by fluorescence spectroscopy, whereas complex particle size was measured by using dynamic light scattering. Finally, the encapsulation efficiency of resveratrol into β-CN micelles was evaluated by RP-HPLC. Fluorescence spectroscopy results indicated that vitamin D_2 significantly quenched the intrinsic fluorescence intensity of both proteins, and that resveratrol spontaneously binds to one binding site in both β-CNs. By encapsulation efficiency experiments it was observed that resveratrol entrapment into β-CN micelles is enhanced when the protein concentration is higher than 40 μM. The interaction process of both β-CNs with vitamin D_2 and resveratrol is driven mainly by hydrophobic forces; in the case of vitamin D_2, the enhanced surface hydrophobicity revealed that the binding leads to conformational changes in the structure of both β-CNs. In conclusion, these findings support the potential use of hypoallergenic donkey β-CN as a novel nanocarrier system for bioactive molecules in food applications. *The authors marked with an asterisk equally contributed to the work.

P-14-031
Chrysanthemum zawadskii flower extract rescues inflammation-related neuronal dysfunction by inhibiting acetylcholinesterase in microglia

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Although *Chrysanthemum zawadskii* (CZ) has been widely used in traditional medicine to treat various inflammatory diseases, its effect on inflammation-related neuronal dysfunction remains unclear. Therefore, we investigated whether an ethanol extract of CZ had anti-inflammatory effects on microglia and whether these effects could rescue inflammation-related neuronal dysfunction. CZ extract showed strong free radical-scavenging activity in vitro, with IC50 values of 186.04 μg/ml in a DPPH assay and 94.56 μg/ml in an ABTS assay. In lipopolysaccharide (LPS)-stimulated BV2 cells (a murine microglial cell line), CZ extract inhibited the productions of reactive oxygen species and nitric oxide production and the expression levels of iNOS and pro-inflammatory cytokines (e.g., IL-1β). CZ extract reduced the LPS-induced phosphorylation of IκBα and nuclear localization of NF-κB, suggesting that the observed anti-inflammatory effects may be mediated via inhibition of NF-κB signaling. Interestingly, CZ extract reduced inflammation-induced acetylcholinesterase expression in BV2 cells and directly inhibited its activity *in vitro*. Among the main flavonoids of CZ extract, quercetin and luteolin showed the best anti-inflammatory and antioxidant effects, and linarin showed the strongest inhibitory effect on acetylcholinesterase activity. *In vivo*, the CZ extract mitigated the learning and memory impairments induced by sleep deprivation in zebrafish. Together, these data suggest that CZ extract could reverse inflammatory neuronal dysfunction by alleviating inflammation-associated acetylcholinesterase activity in microglial cells, resulting in improved cognitive performance.

P-14-032
Anticancer properties of *Gratiola officinalis* extract on colorectal cancer cell lines

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Colorectal carcinoma (CRC) is one of the most common types of cancer worldwide. At the basis of its pathogenesis are mutations that impairs several molecular pathways that regulate cell growth, survival, proliferation, and migration. Furthermore, cancer cells also exhibit metabolic alterations that increase the amount of energy produced through glycolysis even in aerobic conditions, the so-called "Warburg effect". To treat such a heterogeneous disease, many drugs have been developed over the years. However, their efficacy is variable, and their administration may be related to the insurgence of strong side effects, urging the need to find new, complementary approaches. Amongst these, natural compounds present in plants have emerged as promising candidates, having shown antiproliferative and antioxidant activities on cancer cells *in vitro*. Here, we report that the ethanol extract derived from *Gratiola officinalis* has a stronger impact on CRC cell lines compared to healthy colon cells, altering their viability, proliferation rate and survival capability. These effects are particularly evident on the E705 CRC cell line, where the extract also causes the downregulation of glycolysis, thus reverting the Warburg effect. Taken together, these observations suggest that *Gratiola officinalis* could represent a novel player in the prevention and treatment of colorectal carcinoma.

P-14-033
What's brewing? Mapping the distribution of bioactive compounds and aroma/flavour precursors in green coffee beans through MS-driven spatial metabolomics

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Coffee is one of the most enjoyed beverages worldwide and whilst a wide array of secondary metabolites has been qualitatively and quantitatively characterised within the coffee bean, little is known about their distribution in different bean tissues. In this work, complementary mass spectrometry (MS) based approaches were utilised to determine the spatial metabolomic content of coffee beans, focusing on highlighting those which presented a regiospecific distribution and distinguished the different endosperm regions and the embryo. Preliminary exploration of the spatial metabolome of *Coffea arabica* coffee beans highlighted tissue-specific molecular features through an unsupervised statistical analysis. Then, the complementary information derived from higher resolution MS approaches resulted in the annotation of 13 metabolites which showed a specific enrichment in at least one tissue, and most of these were noted to be bioactive compounds or aroma/flavour precursors. Knowledge of the tissue distribution of these compounds could shed light on their biological role in plant physiology and bean development, but could also have relevant implications for their positive impact on human health due to coffee consumption as well as their possible effect on aroma and flavour upon roasting of green coffee beans. *The authors marked with an asterisk equally contributed to the work.

P-14-034
Apoptosis induction of cinnamon polyphenolic components on E705 and SW480 colorectal cancer cell lines

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Colorectal cancer is the third-most prevalent malignant tumor worldwide, with a constantly increasing incidence rate. The ideal treatment would achieve complete removal of the tumor and metastasis; however, for patients with unresectable lesions chemotherapy represents the only strategy. Given that chemotherapy has several drawbacks, new targeted approaches are being constantly developed, like the monoclonal antibodies against epidermal growth factor receptor, cetuximab and panitumumab. Since these targeted therapies are effective in no more than 30% of patients, there is an urgent need to identify new approaches such as the application of nutraceuticals. In fact, natural products, like cinnamon, have long been regarded as one of the potential materials for developing anticancer agents. In this work, the effects of hydroalcoholic extracts from bark of *Cinnamomum cassia* (CCHE) or *Cinnamomum zeylanicum* (CZHE), buds of *Cinnamomum cassia* (BCHE) and of the corresponding fractions enriched in polyphenols (B) have been evaluated on the healthy cell line CCD841 and on the cancer cell lines E705 and SW480. A 48-h treatment with fractions B is cytotoxic only on both cancer cell lines, suggesting a role in their antiproliferative activity either for B-type procyanidins or for flavonoid glycosides. Moreover, co-administration of fractions B with cetuximab on E705 cancer cells showed an additional cytotoxic effect. All cinnamon fractions B induced in all colorectal cancer cell lines a significant increase in early and late apoptosis and at the molecular level they up-regulated the expression levels of cleaved caspase 3 while down-regulating those of Bcl-2, mainly through increased ERK phosphorylation. These results allowed the elucidation of the anticancer activity and the potential molecular mechanism of cinnamon fractions enriched in polyphenols on human colorectal cancer cell lines.

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P-14-035
Comparative metabolomic analysis of *Moringa oleifera* leaves from different geographical origins and their antioxidant effects on C2C12 cells

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Moringa oleifera is widely grown throughout the tropics and increasingly used in therapeutic and nutraceutical properties. These properties are attributed to potent antioxidant and metabolism regulators, including glucosionlates/isothiocyanates as well as flavonoids, polyphenols, and phenolic acids. Research to date largely consists of geographically limited studies that only examine material available locally. These practices make it unclear whether moringa samples from one area are superior to another, which would require identifying superior variants and distributing them globally. Alternatively, finding that globally cultivated moringa material is essentially functionally equivalent means that users can simply sample material available locally. We brought together 20 accessions of *Moringa oleifera* from 4 continents and 9 countries and grew them together in a common garden. We performed a metabolomic analysis of leaf extracts (MOLE) using a LC-MSMS zenoTOF instrument. The extracts proved to be qualitatively and quantitatively different in their bioactive molecules, with some extracts richer in glucosinolates and others richer in polyphenols. Antioxidant capacity of MOLE evaluated by the total antioxidant capacity assay did not show any significant difference between the different extracts. MOLE were then tested for their antioxidant activity on C2C12 myotubes after an oxidative insult. Hydrogen peroxide (H_2O_2) was added to myotubes alone or after pretreatment with different leaf extracts. H_2O_2 exposure caused an increase of cell death that was diminished in all samples pretreated with moringa. Our results provide the very encouraging result that MOLE is effective in reducing the damaging effect of H_2O_2 in C2C12 myotubes irrespective of geographical provenance. These results are encouraging because they suggest that use of moringa for its therapeutic benefits can proceed without the need for lengthy and complex global exchange of material between regions.

P-14-036
Bioactive molecules from *Ganoderma adspersum* mycelium extracts exhibit selective cytotoxicity towards renal cancer cell lines

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Despite the progress in cancer treatment over the last decades, anticancer therapy poses a serious challenge for medical sciences. Although enormous applications, chemotherapeutics shows excessively adverse effects and often poor outcomes, demanding the exploration of new effective treatments. Within this context, natural products exhibit the potential to offer alternatives to chemotherapy, overcoming associated side effects and complications. Indeed, they have been used for the treatment of various diseases, becoming crucial in the field of drug discovery. Fungal organisms, such as *Ganoderma* spp., are extensively researched for their anti-cancer activities, and valued for their safety, low toxicity, widespread availability, and cost-effectiveness as alternative for new cancer treatments development. To explore the potential bioactivity of *Ganoderma adspersum* (G.ad), in this work, we assessed the cytotoxic effects of ethanolic extracts from the medium after fungal fermentation (extra) and from the mycelium content (intra) on human renal tumor (Caki-1 and RCC-Shaw) and non-tumor (HK-2) cell lines. We observed that the extracted compounds encapsulated within the mycelium (intra), but not the ones excreted in the medium (extra), significantly affected tumor renal cells viability, with up to 65% of viability decline in RCC after 48 h of treatment, while no significant impact on HK-2 proliferation was reported. The NMR-based metabolomics analysis of the two tested extracts demonstrated remarkable differences in their composition, highlighting the presence of succinic acid, alanine, xanthine and phenylalanine derivatives in the bioactive extract, that might be able to target mitochondrial function in the investigated cells. Further studies are needed to explore the therapeutic potential of the prioritized compounds within the context of cancer treatment and chemotherapy, focusing on their impact on tumor renal cell differential metabolism.

P-14-037
Proof of principle study on the effect of microRNA bta-miR-154c on *Escherichia coli* proliferation rates

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MicroRNAs (miRNAs) are small, non-coding RNA molecules, ranging from 18 to 23 nucleotides in length, that are synthesized in the nucleus and function in the cytoplasm. Emerging evidence has shown that miRNAs can also be found extracellularly and circulate in bodily fluids, influencing the gut microbiota. Furthermore, dietary miRNAs, known as XenomiRs, have been observed to resist digestive processes and affect the gut environment. Previous studies from our laboratory demonstrated that bovine miRNA bta-miR154c withstands *in vitro* digestion, suggesting a potential regulatory role in the gut microbiota[1]. This study aims to elucidate the effect of bta-miR-154c on *Escherichia coli* (*E. coli*), a facultative anaerobic bacterium and a component of the human microbiome. We identified potential binding sites for bta-miR-154c on *E. coli's* DNA and mRNA, indicating a possible mechanism for gene expression modulation. We then exposed *E. coli* cultures to synthesized bta-miR-154c mimics and observed a notable impact on bacterial proliferation compared to controls, confirming the miRNA's regulatory effect. Additionally, we explored the uptake of bta-miR-154c by *E. coli* using a GFP-expressing strain (*E. coli*-GFP) and Cy3-conjugated miRNA mimics. Through confocal microscopy and flow cytometry, we investigated the internalization of miRNAs by *E. coli*. Preliminary findings indicate miRNA uptake, although further research is needed to clarify the specifics of intracellular localization and its functional consequences. These findings provide valuable insights into the role of ingested miRNAs in regulating the gut microbiota, enhancing our understanding of their potential impact on human health and disease. [1] Previously published in: Pieri M et al. (2022) FEBS Open Bio, 12(5), pp. 925–936.

P-14-038
Carnosine mitigates dysregulation in epigenetic modifications in pancreatic β-cells exposed to glucolipotoxic metabolic stress

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Recent evidence has shown the existence of a tightly regulated crosstalk between epigenetic modifications and metabolic control in cells. However, metabolic stress-mediated alterations in the regulation of this important cellular crosstalk is one of the emerging hallmarks of type 2 diabetes. Carnosine is an endogenous dipeptide consisting of β-alanine and L-histidine with promising antidiabetic potential. We therefore investigated whether carnosine could mitigate dysregulation of epigenetic modifications caused by glucolipotoxic metabolic stress in pancreatic β-cells. Rat insulinoma islet β-cells of the pancreas (INS-1) were exposed to RPMI media supplemented to glucolipotoxic (GLT) concentrations of glucose (28 mM) and 200 μM free fatty acids (palmitate and sodium oleate) respectively, with or without 10 mM carnosine for 5 days treatment. Our data shows that exposure of INS-1 cells to GLT media resulted in a significant increase in the mRNA and protein expression of major histone acetyltransferases (HAT1, GCN5, KAT7 and KAT5) with concomitant decrease in the mRNA and protein expression of class III histone deacetylases (sirtuin 1, 3 and 6). This was associated with a significant increase in the levels of acetyl histones (H4, H3 and H2A), acetyl-isocitrate dehydrogenase and acetyl-superoxide dismutase. Co-immunoprecipitation of the histones and subsequent posttranslational modification studies with mass spectrometry revealed several residues that were abnormally acetylated due to incubation in GLT media. Conversely, supplementation with carnosine significantly decreased the mRNA and protein levels of the HATs, increased sirtuins and further decreased levels of acetyl histones and mitochondrial proteins. Carnosine evidently reversed deleterious GLT effects and collectively, this provides new insight on the antidiabetic actions of carnosine through mitigation of epigenetic dysregulation of pancreatic β-cells gene expression associated with diabetic metabolic stress.

P-14-039
Biochemical and molecular mechanism after acute and chronic TBI: focus on high monomeric polyphenol berry extract (HMPBE)

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Traumatic brain injury (TBI) has a significant economic and social impact due to the costs associated with medical care, rehabilitation, and lasting consequences on both quality of life and work productivity. Monomeric polyphenol berry extract (HMPBE) is a polyphenol-rich formulation with proven clinical efficacy in ameliorating learning and memory, assisting cognitive functions such as accuracy, working memory and concentration. The aim of this work was to investigate the potential benefits of HMPBE in a mouse model of acute and chronic TBI induced through a controlled cortical impact (CCI) method. In the acute TBI model, trauma was induced and HMPBE was administered 1 h after the trauma, while in the chronic model, after induction of trauma HMPBE was administered daily for 30 days at a dose of 15 mg/kg dissolved in water. Our studies show that HMPBE effectively reduced histological alterations as well as biochemical impairments like oxidative stress and lipid peroxidation. HMPBE has demonstrated positive effects limiting head trauma by increasing the Nrf-2 pathway, facilitating the synthesis of antioxidant enzymes, and decreasing the nuclear translocation of Nf-kb. As a conquences also proinflammatory agents and apoptosis were reduced. In the chronic TBI model, HMPBE improving behavioral aspects such as anxiety and depression, reversed the decline of tyrosine hydroxylase (TH) and dopamine transporter (DAT), preventing the accumulation of α-synuclein in the midbrain region. This involves a connection between the beneficial

effects of HMPBE administration and the onset of parkinsonism related to traumatic brain damage.

P-14-040
Exploring the potential of *Weizmannia coagulans* MA13 as a probiotic delivery of prebiotic molecules

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Weizmannia coagulans (formerly known as *Bacillus coagulans*) MA-13[1] is a thermophilic lactic acid (LA) producer exhibiting the optimal growth temperature at 55°C. Nowadays, there is a growing interest in spore-forming probiotics because of their ability to withstand harsh conditions of the gastrointestinal tract as well as of the food processing. Bacillus spores have long been used as a surface display system with applications in a variety of biotechnological fields[2]. In addition, MA-13 was proven to over produce glycosyl hydrolases enzymes such as α- and β-galactosidases that improve the digestibility of some nutrients. A full biochemical characterization of the MA-13 β-galactosidase belonging to the GH42 family[3] showed its ability to synthesize prebiotics such as galactooligosaccharides (GOS). The purpose of this study is to test the feasibility of MA-13 as probiotic and as a delivery system for diverse molecules/enzymes. Preliminary results showed that MA-13 meets the safety requirements for its use in foods and supplements established by EFSA. Indeed, it is sensitive to diverse antibiotics and it does not show haemolytic activity. Future studies will be addressed to test MA-13 spores as scaffold for the immobilization β-galactosidase enzyme through adsorption methods in order to develop a delivery system of biotherapeutic molecules. References: 1. Aulitto, M. et al. (2017) *Biotechnol Biofuels* 10, 210 2. Sirec, T. et al. (2012) *Microb Cell Fact* 11:100 3. Aulitto, M. et al. (2021) *Microb Cell Fact*. 18;20 (1):71

P-14-041
Leveraging multi-omics approaches to identify how the microbiome influences brain gene regulation

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A central question in behavioral biology is how environmental and genetic factors shape behavioral traits, allowing for substantial individuality. Homeostatic behaviors like foraging and food preferences are prime examples of genetic predispositions interacting with the food environment to produce diverse feeding habits. However, these programs are further modified by host-associated microbiomes, suggesting the overall systems controlling behavioral phenotype is far more complex than first anticipated. My lab studies the of role gut microbiome in host behavioral modulation using a gnotobiotic approach that permits precise configuration of the fly microbiome. In this presentation, I will share our recent findings that suggest host behavioral responses of food microbial and nutrient cues are shaped by the microbiome; and our ongoing work integrating transcriptomic, metabolomics, and proteomic approaches to elucidate the mechanisms underlying behavioral modulation by the microbiome.

P-14-042
Biochemical and functional properties of enzyme-assisted extracts from leaves and roots of *Aralia cordata* Thunb.

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Enzyme-assisted extraction (EAE) is a sustainable and effective method for obtaining bioactive extracts of various plant materials. Hence, the leaves and roots of the medical plant *Aralia cordata* Thunb. (*A. cordata*) can possess promising functional properties. This plant belongs to the same family as ginseng—Araliaceae. This study aimed to investigate the biochemical and antioxidant properties of *A. cordata* leaves and root extracts using EAE. The extracts were obtained under optimal conditions (3:15 h of extraction, temperature 45°C, pH: 4.9, and 1% Viscozyme L enzyme (v/w of dry leaves); the extract under optimal conditions without enzyme were made as a control. Successful hydrolysis of hydrolytic enzymes was observed by implementing the cell wall's scanning electron microscopy (SEM) imaging technique. The antioxidant potential of *A. cordata* leaves and roots extracts were tested using 2,2'-azino-bis(3-ethybenzothiazoline-6-sulfonic acid) ($ABTS^{\bullet+}$) radical cation assay and ferric reducing antioxidant power (FRAP) assay. The total phenolic content was determined using the Folin-Ciocalteu method. Sugar and organic acid profiles were evaluated using high-performance liquid chromatography (HPLC). The total protein content was obtained using the Lowry assay. The results indicated that the yields after EAE extraction for leaves and roots were 33.6 g/100 g DW and 31.9 g/100 g DW, while the controls yielded 23.8 g/100 g DW and 22.3 g/100 g DW, respectively. Additionally, the $ABTS^{\bullet+}$ and FRAP antioxidant activity increased only in *A. cordata* leaves EAE extract by 21% and 50%, respectively. Furthermore, the protein content in leaves EAE extract was two times higher than in root EAE extract. In general, the findings of this study present valuable insights into the use of different *A. cordata* morphological parts and their significant potential for functional food or supplement development.

P-14-043
Investigating the influence of amarogentin on mast cell activation through bitter taste receptor (TAS2R) agonism

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Mast cells play an essential role in mediating allergic and immune reactions through the release of histamine, leukotrienes, prostaglandins and inflammatory cytokines upon activation. The present study investigates the immunomodulatory effect of amarogentin, a bitter-tasting compound, by targeting TAS2R. Initial screening of amarogentin affinity to one or more of the TAS2R subtypes was performed *in silico* using the BitterX web tool. Among 13 TAS2R subtypes, the highest probability of amarogentin binding was evaluated for the TAS2R14 subtype. Presence of TAS2R14 was then demonstrated on human mast cell line LUVA using immunofluorescence analysis. *In vitro* experiments revealed that amarogentin significantly inhibited histamine secretion, assessed through β-hexosaminidase release assays. To validate the results of *in silico* analysis, TAS2R14 antagonist sulfamoyl-benzoic acid was used in the β-hexosaminidase release assay, which blocked the inhibitory effect of amarogentin on histamine release. Due to the significance of intracellular Ca^{2+} elevation on mast cell activation, we monitored the impact of amarogentin on thapsigargin-induced Ca^{2+} signalling using Fura 2 AM fluorescence analysis. Our investigations reveal that amarogentin attenuated the elevation of intracellular Ca^{2+}, suggesting a potential regulatory role in mast cell activation pathways. Our findings underscore the therapeutic potential of amarogentin, TAS2R agonist, as a modulator of mast cell activation, potentially offering avenues for developing novel treatments for allergic and inflammatory diseases. However, further *in vivo* and *in vitro* studies are needed to fully elucidate the mechanism of action and therapeutic potential of amarogentin and other TAS2R agonists. The study was financially supported by grants: APVV-19-0033, VEGA 1/0314/21, VEGA 1/0253/19, VEGA 1/0042/24, GUK /188/2023.

P-14-044
Isolation of a substance from Begger's rosehip plants and study of its chemical composition

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Growth of pharmaceutical production is one of the main objectives of the state program for innovative industrial development in Kazakhstan. Due to the limited scholarly research on the phytochemical makeup of *Rosa beggeriana* (specific for Asia and scarcely studied in chemical profile plant), our GC-MS findings were compared with the data on other *Rosa* species. It is important to highlight that this investigation marks the first-ever exploration of the fatty acids' profiles associated with this plant species. Diverse range of compounds of the leaves incorporates terpenoids, such as (-)-aristolene and phytosterols, like stigmastan-3,5-diene. Moreover, saturated and unsaturated fatty acids, along with their corresponding esters, were identified. Fatty acids include myristic acid, palmitic acid, palmitoleic acid, ethyl linoleate, (e)-9-octadecenoic acid ethyl ester, ethyl oleate, alpha-linolenic acid, 11-octadecenoic acid (z)-, 11-octadecenoic acid (e)-, oleic acid (z)-, stearic acid, 9,12-octadecadienoic acid (z, z)-, and 2-hydroxy-1-(hydroxymethyl)ethyl ester. A comprehensive analysis of the collective findings underscores the substantial abundance of saturated and unsaturated acids, terpenoids, and other substances in both leaves and fruits of the species. Among these, β-sitosterol, betulin, (+)-catechin, lupeol, and ethyl linoleate were recognized, having been previously found in the *Rosa* genus, but not specifically in *Rosa beggeriana*. Two compounds, namely 3β,23-dihydroxyurs-12-ene and ethyl linolenoate, were discovered for the first time, offering novel insights for both the genus and *Rosa beggeriana*.

P-14-045
Effects of hydrogen peroxide and radiation on the resilience of *Lactobacillus rhamnosus* MDC 9661 bacteria

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Lactobacillus rhamnosus MDC 9661 isolated from Armenian dairy products is considered as efficient starter for production of dairy products, functional food and preserving strain in food production. In an era marked by rising global radiation levels, understanding the intricate radioresistance of an organism like *L. rhamnosus* MDC 9661 to environmental stressors such as hydrogen peroxide (H_2O_2) and radiation becomes increasingly imperative. We aimed to assess how these stressors affect the growth parameters, oxidation reduction potential (ORP) and pH levels, and survival of *L. rhamnosus* MDC 9661. ORP was measured using platinum (Pt) and titanium-silicate (Ti-Si) electrodes. The bacteria were exposed to radiation using AREAL (Advanced Research Electron Accelerator Laboratory), a laser-driven 5 MeV electron linear accelerator. Bacterial growth was inhibited ~ 1.8 fold at 0.9 kilogray dose of electron beam radiation, whereas 1 kilogray proved lethal for the bacteria. In control experiments, ORP readings drop form positive (+70 ±10 mV and +55 ±10 mV, Pt and Ti-Si electrodes, accordingly) to negative values were observed during bacterial logarithmic phase growth, while after 24 hours, it increased to +180 ±10 mV and +150 ±10 mV, respectively, and pH changed from 7.5 to 3.8. Exposure to 0.1% H_2O_2 and 1.4 kilograys of X-ray radiation (MultiRAD 350) led to a significant decrease in ORP, with values dropping to +70±10 mV and +50 ±10 mV for the Pt and Ti-Si electrodes, respectively, over 24 hours, accompanied by a decrease in pH from 7.5 to 5. After 24 hours, cell counts halved compared to the control, showing a significant decline in the viability of *L. rhamnosus* MDC 9661 due to H_2O_2 and radiation. These findings shed a light on the possible role of ORP in *L. rhamnosus* MDC 9661's radioresistance mechanisms and their implications for diverse biotechnological applications.

Nutraceuticals Effects on Cell Metabolism and Chronic Diseases

P-15-001

Erectogenic, anti-oxidative and anti-inflammatory properties of enriched cookies of sandpaper leaves in hypertensive rats

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There is an existing crosslink between hypertension and erectile dysfunction owing to pressure exerted on the vascular walls in these pathologies. Therefore, this study aims to investigate the role of four variants of sandpaper leaves [*Ficus exasperata* (FE), *Ficus asperifolia* (FA), *Ficus mucuso* (FM), and *Ficus capensis* (FC)] formulated into functional cookies fed to hypertensive-erectile dysfunctional (HYP-ED) rats. Experimental rats (n = 8) were grouped and administered with 40 mg/kg/day L-NAME while treatment groups were given atenolol (10 mg/kg/day), 2.5 g, and 5.0 g FE, FA, FM, and FM-enriched cookies respectively for 14 consecutive days. Hemodynamic parameters and sexual behavioral experiments were assessed in normotensive and hypertensive rats with erectile dysfunction. Additionally, the activities of Angiotensin-converting enzyme (ACE-1), arginase, phosphodiesterase-5 enzyme (PDE-5), nitric oxide (NO) levels, sexual hormones (follicle stimulating hormone, testosterone, and luteinizing hormone), histological staining, and genetic expression of ACE-1, superoxide dismutase (SOD), tumor necrosis factor (TNF-α), and endothelial nitric oxide synthase (eNOS) using quantitative real-time polymerase chain reaction (RT-qPCR) were evaluated in this study. There was a significant ($p < 0.05$) reduction in elevated blood pressure, arginase, ACE-1, and PDE-5 enzyme activity and an equally significant elevation in NO level and antioxidant enzyme activities in the heart and penile of HYP-ED rats. Interestingly, FC and FM-enriched cookies showed a promising role in modulating the expression of TNF-α, SOD, and eNOS genes in HYP-ED rats comparatively. Findings from this study revealed the anti-hypertensive, anti-inflammatory and aphrodisiac properties of enriched cookies of varieties of sandpaper leaves owing to their ability to modulate related enzymes' action and expression in HYP-ED rats. *The authors marked with an asterisk equally contributed to the work.

P-15-002

Unveiling the antitumor potential: impact of natural oligoribonucleotides in complex with D-mannitol on cell viability, cycle, and molecular pathways

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A complex of oligoribonucleotides with D-mannitol (ORN-D-M), consisting of purified fragmented yeast RNA in complex with D-mannitol exhibits antiviral, antioxidant, and immunomodulatory effects. Previously, we demonstrated that the simultaneous administration of ORN-D-M and B16 mouse melanoma cells did not result in tumor formation in animals (Kraievska I. et al., 2019, 5th International Electronic Conference on Medicinal Chemistry). Our study aimed to investigate the impact of ORN-D-M on cell cultures. Firstly, we check the sensitivity of tumor (B16, U251) and non-tumor (MDCK, MEF) cell lines to ORN-D-M treatment by MTT assay and the trypan blue differential staining. We have established that tumor cell lines are more sensitive than non-tumor, which was reflected in lower IC_{50} doses. Further, we performed flow cytometry analysis with propidium iodide staining to investigate the influence of ORN-D-M treatment on the cell cycle of the B16 cell line. It was observed the disturbance in cell cycle distribution: arrest in G_0/G_1 phase with the concomitant decrease in the proportion of cells in G_2/M phase, and the appearance of an apoptotic Sub G_0 phase. As well, we investigated the relative mRNA expression of genes of some cellular ribonucleic acid receptors, their regulated pathways, and some apoptosis regulators. We have noted a rise in the expression of *Tlr3, Tlr7*, Tlr8, and *Eif2ak* receptors, *Nfkb1*, and *Ifna2*, and *Ifnb1*, while the decline of *Tnfa* and *Il1b* expression. Among the apoptotic regulators, a slight increase in the expression of *Bax* and *Casp3* with a decrease in *Bcl2* expression was shown. In conclusion, ORN-D-M reduces the viability of tumor cells, causes cell cycle arrest in the G_0/G_1 phase, and promotes apoptosis. This effect may be related to the involvement of TLR- and EIF2AK-dependent pathways, as evidenced by the increased expression of genes of these receptors and some components of their regulated pathways, as well as apoptosis-regulating genes.

P-15-003

From agro-bio-waste to application: the power of bilberry aerial part extract as anti-cancer and antibiotic-modulating agent

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Bilberry (*Vaccinium myrtillus* L.) leaves and stems are considered as bio-waste. However, they contain much higher contents of phenolic compounds than fruits. The study aimed to investigate the antimicrobial and anticancer potential of aerial part extracts from *V. myrtillus* L. plants harvested at high altitudes in Armenian landscape and characterize the bioactive phytochemicals. For evaluation of antioxidant properties, chemical-based (ABTS and DPPH tests) and cellular antioxidant activity (CAA) assays were applied. Genotoxicity and cytotoxic properties of the extract alone and in combination with fluorouracil were explored in human cancer and normal cell lines. Antibacterial properties of

V. myrtillus extract alone and in combination with antibiotics, as well as their effect on proton-flux rate trough cell membrane were explored on bacterial strains. The characterization of active phytochemicals was done using liquid chromatography-quadrupole-orbitrap high-resolution mass spectrometry (LC-Q-Orbitrap HRMS). The *V. myrtillus* aerial part extract demonstrated promising antioxidant properties in all tests. The selective cytotoxic activity was documented against various cancer cell lines (HT29, MCF-7 and HeLa), while it did not inhibit the growth of tested human normal primary renal mixed epithelial (HREC) cells even at 10-fold higher concentrations. The extract did not have genotoxic properties in comet assay. The investigated extract did not directly inhibit the growth of *Escherichia coli* and *Salmonella typhimurium* strains at up to 1 mg/ml concentration. However, *V. myrtillus* extract enhanced the kanamycin intake and increased its efficiency against *E. coli* strain. The phytochemical characterization of the extract showed the presence of different groups of phenolics. Based on obtained data, we suggest the aerial parts of the *V. myrtillus* plant as an alternative source of bioactive natural products for food supplements, nutraceuticals, functional foods and medicine.

P-15-004
Bioactive peptides from dairy industry by-products: an innovative nanotechnological strategy and biological effects

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Nowadays, in the context of circular economy, different molecules present in agri-food waste can be reused to give rise to products with beneficial effects on human beings. Food waste-derived bioactive peptides, besides the numerous applications, can be used as ingredient of functional foods and nutraceuticals to prevent or manage chronic diseases. In this work, surface active maghemite nanoparticles (SAMNs) were used to extract bioactive peptides from an agri-food by-product, milk whey of cows affected by mastitis (M). Indeed, the latter must be discarded as a waste, causing serious economic losses to dairy industry. Herein, the isolation, identification, and evaluation of antioxidant and anti-inflammatory effects on human cells of new bioactive peptides from food waste was demonstrated by a nanotechnological strategy. The physical-chemical properties of the peptide components of the core-shell hybrid (SAMN@M) were compared to peptides isolated by solid phase extraction (SPE) and to peptides from the milk of healthy cows. According to HPLC and LC-MS/MS analyses the composition of SAMN biocorona consisted in a small group of acidic peptides, that completely differ from the busy profiles of SPE extracts. The peptides obtained with SAMNs showed to exert protective effects against oxidative stress and inflammation induced by TbOOH and TNF-α, in human cell cultures. Moreover, besides being active as free molecules, this group of acidic peptides display a great biological activity by being firmly immobilized onto SAMN surface. SAMN@peptides cellular uptake was studied following the fluorescent core through confocal microscopy analysis. As a result, the peptides can enter the cells through vesicles mediated mechanism. Thus, a new nanotechnological strategy was proposed to valorize a dairy industry by-product as a source of new specific health-promoting peptides, that can be advantageously employed in the development of nutraceutical products and functional foods.

P-15-005
Transgenic plant organs with human interferon α2b gene serves as platform for purposes of green biotechnology

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Human interferon alpha (huINFα2b) plays a crucial role in maintaining human immunity. It has antiviral properties and is used to treat a number of human diseases. The aim of this work was to obtain transgenic organs of *Amaranthus caudatus* plants expressing the *infα2b* gene, which can be used as future models to study the functional activity of interferon in a cell culture and animal test system. A series of 'hairy root' lines were generated with the target *infα2b* gene and the genes: *rolB*, *bar*, *nptII* and *gfp*. Stable integration of these genes was confirmed during initial selection on growth media with selective agents, by PCR analysis, UV microscopy. Transgenic plant material was thus generated, which can be used to further study the activity of interferon in animal systems and its effect on animal immunity. The obtained plant raw material can potentially be used as a food additive to support human immunity.

P-15-006
Novel topical formulations based on *Salvia haenkenium* extracts and hyaluronan for inflamed skin treatment and dermal regeneration

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Salvia haenkenium (SH), a native plant of Bolivia known as strong inhibitor of senescence and recently exploited in wound healing and for its potential anti-inflammatory properties. Hyaluronan at high and low molecular weight (HCC), explored in different cell models, and recently used in the clinical practice, showed beneficial effects in dermoaesthetic and regenerative injective treatments. In this research work, a formulation based on HCC coupled with SH was tested for its potentiality in counteracting dermal injury. *In vitro* wound healing has been used to demonstrate HCC + SH capacity to improve keratinocytes migration compared to the sole HCC, validated also by remodeling and integrity biomarker modulation (elastin, integrin αV and aquaporins). In addition, an *in vitro* dehydration test displayed the ability to defend the skin from dryness and its combination with SH even increased the protective effect. Moreover, *in vitro* inflammation model (with lipopolysaccharides derived from *E. coli*) was used to evaluate molecular fingerprint of the pathological model and comparing the cell response after treatments. Inflammatory biomarkers (e.g.KRT-6, TLR-4 and NFk-B) and specific cytokines (e.g.IL-6, IL-22, IL-23) proved the effect of

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HCC + SH, in reducing inflammatory mediators. A more complex model, 3D-FT skin, was used to better resembling an *in vivo* condition, and confirmed the efficacy of novel formulation to counteract inflammation like 2D model. All results trigger the interest in the novel formulation based on SH extract and hyaluronan for its potential efficacy as natural anti-inflammatory agent for damaged skin, further prompting hyaluronan healing and regenerative properties. 3D skin model, contribute to achieve a better understanding of the biochemical features of the coupling of hyaluronan of diverse MW to a plant extracts.

P-15-007
Exploring the regenerative and anti-inflammatory properties of oat-derived avenanthramides in human keratinocytes

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Oat (*Avena sativa*) is a widely consumed whole grain, appreciated for its numerous health benefits. Within its complex phytochemical composition, low molecular weight phenolic alkaloid compounds known as avenanthramides (Avns) stand out. These compounds are present in oats at approximate concentrations of 300 parts per million (ppm), and various studies have observed potent antioxidant activity in a variety of cell types. In addition to their antioxidant action, the potential of avenanthramides has been investigated in other aspects related to cellular health, including their possible role in cell regeneration. Previous studies suggest that these molecules may be involved in cellular regeneration processes, although further research is required to fully understand their mechanisms. In this study, we aim to explore in depth the impact of avenanthramides on cell regeneration and their anti-inflammatory effect in human HaCaT keratinocytes. Our results have shown that cells treated with some of the avenanthramides were able to reduce the levels of secreted and messenger RNA of different proinflammatory interleukins such as tumor necrosis factor-alpha (TNF-α), interleukin-6 (IL-6), and interleukin-1 beta (IL-1β), after inducing an inflammatory state with lipopolysaccharide (LPS). These findings demonstrate that avenanthramides have anti-inflammatory effects that can reduce inflammation in the skin, which is important to facilitate wound healing and cell regeneration. To further evaluate the effect of avenanthramides, we determined the levels of cell proliferation in keratinocytes treated with Avns and performed wound closure assays. Together, these results demonstrate that avenanthramides function as anti-inflammatory agents in human keratinocytes, suggesting their potential therapeutic utility in skin regeneration and wound healing.

P-15-008
Effect of the cannabis extract PHEC-66 on melanoma cell function

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Melanoma, characterized by its high metastatic rate and resistance to traditional treatments, is the leading cause of death in skin cancer patients. While it represents ~6% of skin cancer diagnoses, it accounts for >80% of related fatalities. Surgery still remains the best method of treatment, and although a range of therapies (chemotherapy, radiation and immunotherapy) are in use, melanoma survival rates remain low. Hence there is a need for new therapies to treat this debilitating tumour. Cannabinoids are emerging as a new line of natural therapy for various debilitating diseases, such as cancer. Human epidermal melanocytes (HEM) and MM418-C1 (1° $BRAF^{V600E}$), MM96L (2° $BRAF^{V600E}$) and MM329 (1° $BRAF^{WT}$) melanoma cells were used in this study. The effect of the cannabis extract PHEC-66 (which contains ~60% cannabidiol – CBD), along with that of CBD alone on these cells was examined. PHEC-66 reduced the viability of the melanoma cell lines but was less cytotoxic towards HEM cells. A similar observation was observed when the melanoma tumour spheroids were treated with PHEC-66 or CBD. PHEC-66 significantly inhibited the melanoma colony formation above its IC_{50} value, while CBD at the same concentration was less inhibitory. Both PHEC-66 and CBD had a significant inhibitory effect on melanoma cell migration. The morphology of the PHEC-66-treated cells showed that while most cells were undergoing apoptosis some were undergoing necrosis. PHEC-66 signals via the CB1 receptor and triggered apoptosis by increasing the expression of the pro-apoptotic marker *BAX*, while concurrently reducing that of the anti-apoptotic marker *Bcl-2* in these melanoma cells. It also induced DNA fragmentation, halted cell progression at the G1 cell cycle checkpoint as well as substantially elevating intracellular ROS levels. The significance of these findings and of the role PHEC-66 may have as a potential melanoma therapeutic agent will be discussed. *The authors marked with an asterisk equally contributed to the work.

P-15-009
Dual effect of anandamide on myogenic differentiation, and its modulation by sphingosine 1-phosphate

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Myogenic differentiation is a fundamental process involved in skeletal muscle regeneration, where the sphingosine 1-phosphate

(S1P) and the endocannabinoid (eCB) systems – transient receptor potential (TRP) channels included – control key processes such as cell proliferation, survival and migration (1,2). In cultured murine skeletal-muscle C2C12 cells, we have shown a modulatory role of S1P on TRPV1 expression and on the regulation of mitochondrial activity, which is essential for skeletal muscle metabolism (2). Moreover, the stimulation of cannabinoid receptor 1 (CB_1) by endogenous or synthetic cannabinoid agonists, promoted myoblast proliferation while counteracting myoblast differentiation (3). Against this background, we investigated the effect on myogenic differentiation of the eCB anandamide (AEA) by inhibiting its hydrolytic enzyme – fatty acid amide hydrolase (FAAH) – with the specific inhibitor URB597, in the presence of S1P and/or selective antagonists of TRPV1 and CB_1 receptors. Thus, we followed the expression of myogenic markers in murine myoblast C2C12 cells by using quantitative Real Time–Polymerase Chain Reaction (qRT–PCR), western blot and confocal immunofluorescence analyses. Our results suggest a dual role of AEA – both endogenous and applied exogenously – on myogenic differentiation, with a TRPV1-dependent pro-myogenic effect and a CB_1-dependent anti-myogenic effect, respectively. Of note, exogenous AEA prevents S1P-induced C2C12 differentiation and counteracts TRPV1 activation during differentiation, whereas S1P blocks TRPV1 activation by its selective ligands. In conclusion, the dual effect of AEA on myogenic differentiation, and its modulation by S1P, could lead to the discovery of new pharmacological approaches against skeletal muscle disorders. References: 1. Cencetti F. et al. (2020) BBA Mol. Cell Biol Lipids 1865, 158759; 2. Standoli S. et al. (2022) Int J Mol Sci 23, 11103; 3. Iannotti, F. A. et al. (2018) Nat Commun 9, 3950.

P-15-010
2,3-Dehydrosilybin induces nitric oxide formation in cardiomyoblasts

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2,3-Dehydrosilybin (DHS) is a minor component of silymarin, a standardized extract from the milk thistle plant, *Silybum marianum*. Chemically, it belongs to the flavonolignan group and exhibits a wide spectrum of biological effects. Hepatoprotective effects, protection against UV light irradiation, antioxidant, neoplastic, and antiangiogenic activity have been reported for DHS in various experimental models. Moreover, it shows cardioprotective effects both *in vitro* and on isolated hearts. However, the overall mechanism of action is still unknown. Experiments performed on isolated perfused rat hearts showed that after exposure of the heart to DHS, nitric oxide (NO) was present in the perfusate. Due to the heart's complex nature, it was impossible to determine which type of cells/tissue is responsible for the increased NO production. In this study, the H9c2 cell line was used as a model of functional heart tissue, and EA.hy926, or human umbilical vein endothelial cells (HUVEC), represented the endothelium model. Based on the results using a genetically encoded fluorescent NO biosensor, the C-geNOp, it was found that DHS affects NO formation only in H9c2 cell line. Follow-up western blot experiments confirmed that increased NO production is associated with phosphorylation of eNOS at Ser1177, a hallmark of eNOS activation. Although the association of increased eNOS activity with mitochondria and calcium ions is discussed in the literature, DHS did not induce any statistically significant change in intracellular Ca^{2+} in any of the tested models. Acknowledgments: This research was supported by Young Researcher Grant UP JG_2024_022, IGA_LF_2024_011, and the Institutional Support of Palacký University in Olomouc RVO 61989592.

P-15-011
Profiling the distribution of phospholipid fatty acids in the brain in response to the chronic palm oil diet

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Dietary fatty acids are pivotal for optimal neuronal function, influencing the biochemical properties of neurons through phospholipid composition. This study investigates the dynamic interplay between chronic palm oil consumption and the distribution of phospholipid fatty acids in the brain. As palm oil, a common dietary component, is known for its saturated fatty acids linked to cognitive decline, understanding its impact on brain molecular composition is crucial. Adult female C57BL/6 mice were randomly assigned to either a control or an experimental group subjected to a 100-day chronic high-fat diet with 25% palm oil. Employing gas-liquid chromatography, we analyzed both the fatty acid composition of palm oil and the brain phospholipids. Statistical comparisons were conducted using unpaired t-tests. Prolonged palm oil consumption led to significant reductions in palmitoleic acid ($p < 0.05$), vaccenic acid ($p < 0.05$), and docosapentaenoic acids ($p < 0.001$), while linoleic acid ($p < 0.001$) exhibited a noteworthy increase within brain phospholipids compared to the control group. In conclusion, our investigation elucidates the intricate impact of chronic palm oil consumption on the brain phospholipid fatty acid profile. Despite elevated levels of saturated fatty acids in our palm oil, their limited insertion into brain phospholipids suggests alternative pathways or structures for utilization. These findings contribute to our understanding of the complex relationship between dietary habits and neural health, providing valuable insights for further exploration into potential interventions or nutritional modifications to optimize cognitive well-being.

P-15-012
A biotherapeutic formula based on *L. rhamnosus* CRL 1505 bacteria cryoprotected with hydroxyectoine for the alleviation of intestinal inflammation using a 3D and 2D leaky gut model

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Many studies highlighted the role of probiotics in re-establishing the gut microbiota balance, preventing intestinal barrier dysfunction. In fact, they can also contribute to the upregulation of anti-inflammatory genes and the down regulation of pro-inflammatory genes, which are contributing to the development of the intestinal bowel disease (IBD) syndrome. The present study aims to investigate the effect of the compatible solute hydroxyectoine (HOE), to be used as functional cryopreservant but also for its biological properties (Czech et al., 2018), to obtain a new formula containing *L. rhamnosus* CRL 1505. Specifically, *L. rhamnosus* CRL 1505 biomass was obtained from bottle fermentation, on semi defined media, the biomass was mixed with HOE and freeze dried for 18 h. Viability of the freeze-dried formula was evaluated before and after lyophilization. The biological functionality of this formula was also investigated (as live biotherapeutics) on a path-mimetic Leaky Gut Chip that recapitulates increases permeability and inflammation through the stimulation with LPS (Vila et al., 2020). The mRNA expression levels of the inflammatory cytokines (IL-6, IL-1ß, and TNF-α) were analyzed using real-time PCR. Changes in the modulation of (TLR-4 and NF-kB) were assessed by western blot assay, and the effect of HOE/PRO formula on the intestinal epithelial barrier function was also assessed by TEER measurements. Results showed that, when either pure HOE or HOE/PRO formula were administrated on the leaky epithelium, transepithelial electrical resistance (TEER) values of the epithelial barrier were significantly improved, showing a recovery post inflammation. In addition, the production of pro-inflammatory markers including IL-1beta, IL-6 and TNF-alpha that were highly expressed in cells treated with the sole LPS, were significantly reduced. *The authors marked with an asterisk equally contributed to the work.

P-15-013
Saffron floral bio-residues as sustainable resource with potential beneficial effects on the oxidative stress and related diseases

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Crocus sativus L. tepals, waste obtained from saffron flowers, represent a valuable nutraceutical and renewable source of phenolic compounds, with potential applications in the prevention and treatment of oxidative stress and related diseases. Our study aimed to identify the phenolic profile and antioxidant activity *in vitro* of hydromethanolic extracts from Saffron tepals (CsHMTEs) and investigate the influence of harvest time. Additionally, an *in silico* study was conducted to assess the cytotoxic properties of phenolic molecules identified by LC-MS in CsHMTEs. The obtained results revealed that the studied extracts serve as a significant source of phenolic compounds, demonstrating notably high contents of polyphenols (75.57 ± 4.45 µg GAE/mg), flavonoids (46.67 ± 0.99 µg QE/mg), flavonols (43.11 ± 2.1 µg QE/mg), and anthocyanins (2.29 ± 0.08 µg C3G/mg), exhibiting remarkable total antioxidant, radical scavenging, and ferric reducing capacities. Moreover, the *in silico* study showed that flavonols found in CsHMTEs present a strong affinity for the active site of VEGF protein expressed in colorectal cancer. In conclusion, our findings indicate that harvest time did not influence the antioxidant activity of CsHMTEs, suggesting the potential use of saffron flower bioresidues as a renewable resource of phenolic compounds with therapeutic potential. Further biological investigations are warranted for better understanding of the mechanisms underlying the action of these molecules."

P-15-014
Synergistic effects of quercetin and metformin on insulin resistant C3A hepatocytes and L6 myotubes

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Metformin is the most widely used drug for treatment of type 2 diabetes mellitus (T2DM). The natural flavonoid quercetin, which is abundant in many fruits and vegetables, has also shown

significant potential to reduce insulin resistance. Metformin and quercetin both exert their effects through multiple targets and could potentially have synergistic effects. This study compared the effects of metformin, quercetin, and a combination of the two on insulin resistant C3A hepatocytes and L6 myotubes. Insulin resistance was induced using a combination of palmitic acid, fructose, and dexamethasone for 48 h. Cells were concurrently treated with 10 μM quercetin (Q), 0.1 mM metformin (M), or their combination (QM). Insulin sensitivity was assessed by measuring glucose uptake and quantitative fluorescence microscopy was used to monitor phosphorylation levels (activation) of protein kinase B (PKB/Akt). Phosphoenolpyruvate carboxykinase (PCK) levels were used as a measure of gluconeogenesis. Potential insulin sensitizing synergism with QM was observed in both cell lines. In C3A cells, insulin increased glucose uptake in insulin resistant control cells by 3.65 ± 1.9% and by 13.94 ± 2.15% in QM treated cells. This increase was significantly higher than with Q or M alone. Similar trends were seen in L6 myotubes. PCK levels in C3A cells decreased by 15% compared to insulin resistant cells, potentially lowering gluconeogenesis. Insulin-stimulated Akt phosphorylation at Thr308 and Ser473 was increased by 31% and 29%, respectively, in QM treated C3A cells compared to insulin resistant cells and by 17% and 29%, respectively, in L6. The observed increased efficacy of the combination of quercetin and metformin warrants further investigation. Combination treatment may lead to dosage reductions; thereby improving the overall adverse effect profile compared to metformin monotherapy.

P-15-015
Dicaffeoylquinic acids from silver wormwood: antioxidant profile and implications in kidney cancer

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Kidney cancer is the 14th most common cancer worldwide. Natural compounds have been increasingly important in cancer research because of their diverse effects and low toxicity. Dicaffeoylquinic acids (diCQAs) are polyphenolic antioxidant compounds with various biological effects found naturally in silver wormwood (*Artemisia ludoviciana* Nutt.). However, there is no data about diCQAs's effect on kidney cancer development. Our study investigated the antioxidant effect *in vitro* of diCQAs fraction and their anticancer effects in malignant (Caki-2) kidney cells. The fraction of diCQAs from silver wormwood herb extract was separated using column chromatography and then analyzed using the HPLC method. We used ABTS, FRAP and cytochrome c reduction assays to evaluate antioxidant activity. The effect of diCQAs fraction on the mitochondrial respiratory rates was measured in malignant (Caki-2) kidney and healthy (RPTEC) kidney cells by applying Oroboros Oxygraph-2k. Our results revealed that diCQAs fraction is rich 3,4-diCQA (175,9 ± 0,5 mg/g), 3,5-diCQA (378.8 ± 0.5 mg/g) and 4,5-diCQA (101.1 ± 0.6 mg/g). DiCQAs fraction possesses high antioxidant activity and reducing properties (6052 ± 81 μM TE/g) are more potent than antiradical properties (1693 ± 59 μM TE/g). DiCQAs fraction reduced cytochrome c by 55.0 ± 4.0% [1]. Here, we show that diCQAs fraction (63 and 125 μg/ml) inhibited the mitochondrial oxidative phosphorylation rate (with both substrates, glutamate/malate and succinate) in Caki-2 cells by 42.8% and 33.5%, respectively, and increased the mitochondrial respiration rate in the leak state. It is important to note that diCQAs fraction had no effect on healthy RPTEC cells. Our data suggests that diCQAs from silver wormwood suppress mitochondrial oxidative phosphorylation of cancer Caki-2 kidney cells without harming healthy cells and could be promising compounds in cancer research. [1] Previously published in: Kamarauskaite et al. (2021) Antioxidants (Basel) 10(9):1405. *The authors marked with an asterisk equally contributed to the work.

P-15-016
Wild strawberry (*Fragaria vesca* L.) nutraceutical properties on human colorectal cell lines

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The occurrence of colorectal cancer (CRC), third most common cancer with about one million new cases yearly, does not seem to have any relationship with age, sex, or ethnicity; however, epidemiological studies have found a rooted incidence in North America, Australia, New Zealand, and Europe, while it occurs at a much lower rate in rural areas of Africa and Asia, suggesting that also environmental factors may be responsible for the occurrence of this disease. Therefore, the adoption of dietary patterns that introduce food with a chemopreventive role seems to have many beneficial benefits. The wild strawberry *Fragaria vesca* L. is endowed with several bioactive components, such as polyphenols, easily absorbable monosaccharides, vitamins (C, B1, B2, K), and organic acids (malic, citric, and salicylic), that can contribute to cancer prevention. In this study, we tested the nutraceutical potential of wild strawberry extract by using human colorectal carcinoma cell lines as biological systems. The proliferation of SW480 cells, responsible for very aggressive tumor phenotypes, is significantly slowed down upon treatment with low doses of wild strawberry extracts: cell cycle analysis has shown a blockage in the G2 phase, with a concomitant increase in the phosphorylation level of cdc2 at Tyr15, a critical point of cell cycle progression. On the contrary, the mechanism through which wild strawberry extract acts on the cancer E705 cell lines was found to be associated with the induction of programmed cell death: an increase in early apoptosis as well as in late apoptosis/necrosis was found in treated cells. In addition, wild strawberry extract lack of toxicity towards healthy colorectal cells makes *Fragaria vesca* L. a promising chemopreventive nutrient.

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P-15-017
Active substances from *Ganoderma adspersum* extracts stimulate LPS/TLR4 signaling pathway, by using OSMAC cultivation strategy

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Anti-inflammatory therapy is among the effective strategies to suppress the inflammatory response causing the release of a large amount of pro-inflammatory cytokines, after exposure to triggering stimuli. The main problem of today's treatments is the fact that several classes of drugs exhibit adverse side effects. Therefore, it's of great importance to look for active substances with high anti-inflammatory potential and favorable safety. In this context, drug discovery from natural sources offers the opportunity to identify bioactive compounds that can serve as lead agents for designing more effective and less toxic drugs. The 'one strain many compounds' (OSMAC) strategy serves as a straightforward metabolic mining tool for investigating an organism's capacity to produce bioactive secondary metabolites. This is achieved through the alteration of environmental signals during fermentation process by modifying the nutrient composition of a growth substrate or adjusting relevant physical parameters. In our study we applied OSMAC cultivation strategy on the fungal organism *Ganoderma adspersum* by cultivating in 3 different types of liquid media (PDB, SDB, YMB) and each medium was combined with the additives (sawdust, tyrosine, tryptophan, D-glucose monohydrate, arabinose and potassium iodine) resulting in 21 cultivation conditions. Extracts prepared from these cultivations were evaluated for discrepancies in NF-kB/AP-1 pathway activation upon LPS/TLR4 signaling, using THP1-XBlue™ reporter cells. We observe that, by cultivating in YMB medium with various additives, the extracts could significantly down-regulate the NF-kB/AP-1 activation, notably at the same level as prednisolone, used as positive control. Employing an NMR metabolomics analysis, while implementing statistical model, we analyzed the relationships between specific components and observed biological activity. By this approach we prioritized possible metabolic targets for anti-inflammatory treatment.

P-15-018
Impaired mitochondrial quality control in fibromyalgia: mechanisms involved in skeletal muscle alteration

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Fibromyalgia (FMS) is a chronic syndrome characterized by widespread musculoskeletal pain and behavioral symptoms. Given the hypothesis linking FMS etiology to mitochondrial dysfunction and oxidative stress, we investigated the biochemical correlation among these factors by examining specific proteins associated with mitochondrial homeostasis in gastrocnemius muscle. Additionally, this study explored the potential of *Boswellia serrata* gum resin extract (BS), known for its various functions including the potent induction of antioxidant enzymes, to determine protective or reparative mechanisms in muscle cells. *Sprague–Dawley* rats were administered reserpine to induce FMS. These animals exhibited moderate changes in hind limb skeletal muscles, resulting in mobility difficulties. Moreover, notable morphological and ultrastructural alterations were observed, along with the expression of myogenin, oxidative enzymes (citrate synthase and cytochrome-c oxidase subunit II), and oxidative stress markers in the gastrocnemius muscle. Interestingly, BS demonstrated a reduction in spontaneous motor activity difficulties and showed a positive impact on musculoskeletal morphostructural aspects, as well as a decrease in oxidative stress and mitochondrial alterations. Specifically, BS restored PGC-1α and Mfn2 expressions and mitochondrial coenzyme Q10 (CoQ10) levels in gastrocnemius samples. Overall, this study underscores the pivotal role of mitochondrial alteration in FMS and highlights the potent antioxidant effect of BS on these organelles and cells.

P-15-019
Role of mitochondrial dysfunction and biogenesis in fibromyalgia syndrome: molecular mechanism in central nervous system

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A critical role for mitochondrial dysfunction has been shown in the pathogenesis of fibromyalgia. It is a chronic pain syndrome characterized by neuroinflammation and impaired oxidative balance in the central nervous system. Boswellia serrata (BS), a natural polyphenol, is a well-known able to influence the mitochondrial metabolism. The objective of this study was to evaluate the mitochondrial dysfunction and biogenesis in fibromyalgia and their modulation by BS. To induce the model reserpine (1 mg/kg) was subcutaneously administered for three consecutive days and BS (100 mg/kg) was given orally for twenty-one days. BS reduced pain like behaviours in reserpine-injected rats and the astrocytes activation in in the dorsal horn of the spinal cord and prefrontal cortex that are recognized as key regions associated with the neuropathic pain. Vulnerability to neuroinflammation and impaired neuronal plasticity have been described as consequences of mitochondrial dysfunction. BS administration increased PGC-1α expression in the nucleus of

spinal cord and brain tissues, promoting the expression of regulatory genes for mitochondrial biogenesis (NRF-1, Tfam and UCP2) and cellular antioxidant defence mechanisms (catalase, SOD2 and Prdx 3). According with these data BS reduced lipid peroxidation and the GSSG/GSH ratio and increased SOD activity in the same tissues. Our results also showed that BS administration mitigates cytochrome-c leakage by promoting mitochondrial function and supported the movement of PGC-1α protein into the nucleus restoring the quality control of mitochondria. Additionally, BS reduced Drp1 and Fis1, preventing both mitochondrial fission and cell death, and increased the expression of Mfn2 protein, facilitating mitochondrial fusion. Overall, our results showed important mitochondrial dysfunction in central nervous system in fibromyalgia syndrome and the role of BS in restoring mitochondrial dynamics.

P-15-020

Abstract withdrawn.

Impacts of Climate Change on Nutrition and Health

P-16-001
Studies of matrix effects to increase bioavailability of functional bioactive compounds from okara, the soymilk processing byproduct

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Interest towards a sustainable food production and consumption is directed to the exploitation of byproducts currently considered a waste. In last decades, the effects of food on health and the effects of processing on food have been deeply investigated often evaluating the quality and quantity of components without considering the supramolecular organization. The "matrix effect" may deeply influence the release of molecules from food during digestion, absorption, and then utilization for physiological functions in the target tissue. Okara is the byproduct obtained during soymilk and tofu production. Although it is considered a waste it still contains a good quantity of nutrients. In this work different extraction methods were tested to overcome the matrix effect to increase the extraction yield, the bioavailability and bioactivity of functional components. To test the effective extraction of the compounds different assays were carried out. MTT assay was performed using human colorectal adenocarcinoma cells (CaCo2). Protein samples were given to the cells to analyse if they have cytotoxic activity at different concentrations and incubation times. The potential protective effects of proteins and peptides on cell inflammation were also studied using CaCo2, whose immune response was triggered by IL-1β leading to the induction of NF-κB. Since the expression levels of NF-κB mirror the expression of chemokine IL-8, the expression of IL-8 was quantified by qRT-PCR to evaluate the anti-inflammatory effect of peptides. The results show that the bioactive compounds' extractability and bioavailability still present in the by-products may be modulated by a detailed study and choice of extraction conditions. This study was carried out within the Agritech National Research Center and received funding from the European Union Next-GenerationEU (PIANO NAZIONALE DI RIPRESA E RESILIENZA (PNRR) – MISSIONE 4 COMPONENTE 2, INVESTIMENTO 1.4 – D.D. 1032 17/06/2022, CN00000022).

P-16-002
Sustainable recovery of bioactives form buckwheat husk

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Buckwheat is a gluten-free summer pseudocereal with low environmental impact. Buckwheat is also a source of edible fibers, minerals, low-digestibility carbs, biologically valuable proteins, vitamins, and necessary amino acids. Husk is a high-volume byproduct from buckwheat decortication and is rich in bioactives (polyphenols and dietary fibers), that are much less abundant in de-hulled seeds. This report deals with the use of "green" methods for the recovery of water-soluble bioactives from

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buckwheat husk (a high-volume byproduct of buckwheat processing) such as ultrasound assisted extraction (UAE) and microwave assisted extraction (MAE), using water-based media as the extractant. Findings were compared to a standard extraction method (acidified methanol). The profile of bioactive compounds in the different extracts was evaluated in terms of relative abundance and antioxidant activity. Both the overall yield and the concentration of relevant bioactive species were highest in the "green" extracts. "Green" extracts retained substantial effects on chosen inflammatory indicators when tested in a human Caco-2 cell model, confirming their potential for straightforward application in food and non-food items. 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).

P-16-003
Identification of a new lipase in *Corylus avellana*

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The hazelnut (*Corylus avellana* L.) stands out for its high fat content, predominantly composed of triacylglycerols. Widely used in various food products such as candies, cookies, bread and ice cream, hazelnuts find a significant application in the chocolate industry, where approximately 80% of the production is utilized. Despite their versatile uses, the preservation of quality in both raw and processed hazelnuts pose a persistent challenge in the industry, demanding effective solutions. Rancidity development in nuts is influenced by various physical and biological factors, with different classes of enzymes (lipase, lipoxygenase, etc.) playing pivotal roles. Lipases, inherent in the reserve tissues of many oilseed plants, primarily contribute to post-germination oil reserve mobilization. These enzymes are typically inactive in resting and intact seeds, with their activity being contingent upon moisture content. The consequence of lipase activity is the liberation of fatty acids, which undergo oxidation reactions at a faster rate than those esterified in triacylglycerols. This process leads to the generation of volatile compounds responsible for the off-flavours and off-odours commonly associated with hydrolytic rancidity – a particularly pertinent concern in hazelnuts due to their elevated levels of unsaturated fatty acids. The implementation of a protein purification protocol, coupled with a lipolytic enzymatic assay, has led to the isolation of a lipase from hazelnuts. This comprehensive approach has resulted in the identification of a purified protein with a molecular weight of 9 kDa, demonstrating activity towards both synthetic and natural substrates. Intriguingly, this newly discovered enzyme exhibits limited or no homology with canonical lipases, marking it as a distinct and potentially novel lipase. This project may lead to the development of more sustainable industrial technology and to a zero-waste scenario two highly relevant issues for the food industry.

P-16-004
Towards airborne corona viruses detection in outdoor air

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Airborne viruses, such as influenza, corona, and rhinovirus, are responsible for many respiratory infections, some causing the spread of severe acute respiratory diseases, like the recent pandemic of severe respiratory syndrome coronavirus 2 (SARS-CoV-2). The establishment of monitoring methods determinately contribute for the early detection of respiratory viruses in the air and to anticipate mitigation strategies. It was aimed at developing a methodology to detect viruses in outdoor air. Samples were collected using a high-volume cascade impactor (CHEMVOL, Butraco) with 2 stages (PM >10 & PM10) and preserved at −80°C. Total RNA extraction was performed with the Phenol-Chloroform method using TRItidy GTM reagent according to the manufacturer's instructions. The commercial E.Z.N.A.® Total RNA Kit-I was used to RNA purification. real-time reverse transcription PCR was executed to detect the N-gene from the Sarbecovirus family and RdRp gene from SARS-CoV-2 using the ViroReal® Kit SARS-CoV-2 Multiplex. A protein-rich fraction was obtained with ammonium bicarbonate buffer extraction followed by lyophilization. Spike protein was assessed by specific SARS-CoV-2 Antigen Test Kit. The samples from the last week of December 2020, first and second weeks of January 2021, from both PM >10 and PM10, were positive for the N-gene and Cq >33, identifying Sarbecovirus family. The RdRp gene was undetectable, probably due to low virus concentration. The protein extracts from the same periods tested positive for the specific antigen spike protein. In conclusion, all results combined confirm the detection of airborne corona virus and establish the bases for a molecular-based method for virus monitoring in ambient air, eventually providing the base for early alert systems and the implementation of preventive measures to control outbreaks and mitigate future pandemics. This work was supported by FCT—Fundação para a Ciência e Tecnologia, I.P. (projects UIDB/04683/2020 and UIDP/04683/2020).

Membrane Biochemistry

P-17-001
Gas vesicles as acoustic force enhancers

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Gas vesicles represent specialized structures found in certain bacteria and archaea, facilitating control over their buoyancy within aquatic environments. Comprising proteinaceous shells predominantly constituted of structural proteins GvpA or GvpB, these vesicles are characterized by cylindrical shapes with lengths

spanning 100 to 4000 nm and diameters ranging from 45 to 200 nm, and closed ends featuring two cone-shaped caps. This configuration creates an internal volume filled with gaseous phases derived from the surrounding cytosol. The distinctive properties of gas vesicles, particularly their ability to serve as contrast agents in conjunction with MRI and ultrasound, underscore their relevance in non-invasive imaging techniques. We delved into the binding interactions between proteins within the *B. megaterium* cluster and isolated gas vesicles, employing flow cytometry and cryo-electron microscopy. Our results unveiled GvpJ's binding affinity to the vesicles, even in the presence of 6 M urea. In order to enable binding of GVs to mammalian cells we tagged GvpJ with a peptide tag RGD which enables interaction with integrins. As GvpJ is not present on all isolated vesicles we introduced the accessory protein GvpC from the Anabaena gas vesicle cluster to gas vesicles originating from *B. megaterium*. Using GVs with RGD tags we were able to confer gas vesicle binding to integrins, which are cell-specific molecules that allow us to target particular cells using specially designed RGD sequences. After binding GVs to HEK293 cells we were able to improve ultrasound susceptibility of mammalian cells and production of proteins under Ca^{2+} dependent transcription factors. Our research highlights the potential applications of gas vesicles in biomedical and biotechnological fields. By understanding the binding properties and incorporating accessory proteins, we can harness the unique characteristics of gas vesicles for targeted cell manipulation and enhanced therapeutic approaches.

P-17-002
Redox- and photomodulation of mitochondrial potassium channel mitoBKCa in cardiomyocytes and glial cells

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Mitochondria are the main source of ATP in eukaryotic cells, they play a key roles in maintaining Ca^{2+} and ROS homeostasis and also take part in cell life/death signalling. Mitochondrial potassium (mitoK) channels present in inner mitochondrial membrane (IMM) are players in this phenomenon. Opening of mitoK channels has a well-documented cytoprotective effect. MitoK channels are considered as a potential therapeutic and photobiomodulation targets. Infrared (IR) light seems to be very promising, because it penetrates the tissue up to 1 cm and could be absorbed by mitochondrial cytochrome c oxidase (COX). According to our data, COX and a regulatory subunit β of mitochondrial large conductance Ca^{2+}-activated potassium ($mitoBK_{Ca}$) channel can be functionally interrelated. COX has two heme and two copper (Cu_A, Cu_B) active centres, which are responsible for the IR light absorption. Their absorption maxima could be shifted by the redox state of the mitochondrion. To determine the effect of IR light on the $mitoBK_{Ca}$ channel, we performed patch-clamp experiments with illumination system using mitoplasts obtained from two research models: cardiomyocytes isolated from guinea pig heart and the human astrocytoma cell line U87. We observed that the $mitoBK_{Ca}$ channel was redox-sensitive. In response to oxidizing agent $K_3[Fe(CN)_6]$, the channel activity was inhibited. Interestingly, it was restored upon irradiation with 820 nm light. The channel was fully blocked by paxilline, a well-known $mitoBK_{Ca}$ inhibitor. These effects were observed in both models. Our data show that the $mitoBK_{Ca}$ channel is modulated by redox state of IMM and IR light, providing evidence for a new regulation scheme for $mitoBK_{Ca}$ channel in different models. Study was supported by the Polish National Science Centre (grants No. 2019/34/A/NZ1/00352 to AS). *The authors marked with an asterisk equally contributed to the work.

P-17-003
Neuroprotective effect of URG7 protein against stress caused by the neurotoxin 6-OHDA in SH-SY5Y cells

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Neurodegenerative diseases are among the leading causes of disability and morbidity worldwide. These diseases are mainly based on the constant deterioration of neuronal function, leading to brain atrophy. Although different neurodegenerative diseases develop in different brain locations and have distinct etiologies, some cellular and molecular processes are similar such as alteration of proteasome homeostasis, mitochondrial dysfunction and oxidative stress derived from the production of reactive oxygen species (ROS) [Teleanu, D.M et al. (2022) Int. J. Mol. Sci. 23, 11]. Considering the key role of oxidative stress in neurodegenerative diseases, manipulating ROS levels may represent a promising therapeutic option to slow neurodegeneration and alleviate associated symptoms. URG7 (Upregulated Gene clone 7) is an ER-resident protein, the expression of which is upregulated during HBV infection. It is able to modulate the expression of UPR markers towards survival outcomes, reduces ER stress by decreasing the amount of unfolded proteins, and counteracts apoptosis. Such effects have been demonstrated in neuroblastoma cells, i.e. SH-SY5Y, stressed with tunicamycin to generate ER stress [Nigro I. et al. (2023) Int. J. Mol. Sci. 25(1)]. Based on the activity carried out by this protein and to continue characterizing it in the context of proteinopathies, we simulated another phenotype typical of neurodegenerative diseases, namely oxidative stress, using the neurotoxin 6-OHDA. Our results demonstrate that URG7 is able to counteract this stress by activating the Nrf2-ARE pathway (responsible for controlling the expression of genes whose protein products are involved in the detoxification and elimination of reactive oxidants), by increasing the expression of antioxidant enzymes, accordingly attenuating intracellular ROS content, and finally by blocking the apoptotic mechanisms, data overall confirming its stress reliever activity inside the cell.

P-17-004
Single chain fragment variable (scFv) chimeric constructs for outer membrane vesicles (OMVs) surface functionalization

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Most bacteria produce outer membrane vesicles (OMVs) as a result of controlled membrane bulging mechanisms. OMVs are small proteo-liposomes, displaying multiple functions *in vivo*;

they can be easily engineered to tailor a site-specific targeting though surface modification or cargo loading. These features make OMVs optimal candidates for the development of drug delivery systems. Here, *Escherichia coli* OMVs surface functionalization was achieved using the single chain fragment variable (scFv) of the CD19 CAR FMC63 antibody. This antibody fragment is currently used in the construction of chimeric antigenic receptors for immune-checkpoint inhibition therapy. We developed 2 chimeras composed by the monomer of the membrane protein cytolysin A (ClyA), used as a membrane scaffold, fused at the C-terminus to the FMC63 scFv. One of the chimeric constructs also carries the OmpA signal peptide at its N-terminus. The two constructs were recombinantly expressed in *E. coli*, and the chimera's production was verified in both cells and purified OMVs by western blot. The correct surface exposure of the proteins on the outer membranes was confirmed through a Proteinase-K proteolysis assay on OMVs, and an immunofluorescence experiment on *E. coli* cells. Results highlighted those two different fusion constructs to be delivered to the OMVs, with the OmpA leader construct displaying a lower molecular weight at the OMVs level. However, both engineered OMVs were functionally active, as demonstrated by binding experiments carried out through surface plasmon resonance using CD19 protein as binding partner. KD values of $1.22*10^{-8}$ and $3.46*10^{-9}$ were measured for OmpA αCD19 and αCD19 constructs, respectively. Based on these results, our work led to the production of two types of engineered vesicles directed against the same molecular target but with different affinities. Further studies will be needed to shed light on the post-translational modifications occurring to the proteins.

P-17-005
The antidiabetic drug ipragliflozin induces vasodilation of the rabbit femoral artery by activating a Kv channel, the SERCA pump, and the PKA signaling pathway

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Many clinicians recommend sodium-glucose cotransporter-2 inhibitors for type 2 diabetic patients with arteriosclerotic vascular disease. In particular, ipragliflozin, one such inhibitor, lowers the risks of hypoglycemia and abdominal symptoms and can be safely combined with another anti-diabetic drug. Here, we explored the vasodilatory effects of ipragliflozin, an SGLT-2 inhibitor, on rabbit femoral arterial rings. Ipragliflozin dilated phenylephrine-induced pre-contracted rings in a dose-dependent manner. Pre-treatment with the ATP-sensitive K^+ channel inhibitor glibenclamide, the inwardly rectifying K^+ channel inhibitor Ba^{2+}, or the Ca^{2+}-sensitive K^+ channel inhibitor paxilline did not influence the vasodilatory effect. However, the voltage-dependent K^+ (Kv) channel inhibitor 4-aminopyridine reduced the vasodilatory effect. Specifically, the vasodilatory response to ipragliflozin was significantly attenuated by pretreatment with the Kv7.X channel inhibitors linopirdine and XE991, the sarcoplasmic/endoplasmic reticulum Ca2+-ATPase (SERCA) pump inhibitors thapsigargin and cyclopiazonic acid, and the cAMP/PKA-associated signaling pathway inhibitors SQ22536 and KT5720. Neither the cGMP/PKG-associated signaling pathway nor the endothelium was involved in ipragliflozin-induced vasodilation. We conclude that ipragliflozin induced vasodilation of rabbit femoral arteries by activating Kv channels (principally the Kv7.X channel), the SERCA pump, and the cAMP/PKA-associated signaling pathway independent of other K+ (ATP-sensitive K^+, inwardly rectifying K^+, and Ca^{2+}-sensitive K^+) channels, cGMP/PKG-associated signaling, and the endothelium. Our results explain how ipragliflozin exerts protective vascular effects and suggest that patients with hypertension and diabetes would benefit from ipragliflozin.

P-17-006
Second-generation antipsychotic lurasidone blocks the voltage-gated K^+ channels in coronary arterial smooth muscle cells

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Lurasidone is a second-generation antipsychotic drug used to treat schizophrenia, mania, and bipolar disorder. The drug is an antagonist of the 5-HT2A and D2 receptors. Clinical studies have found that lurasidone is associated with fewer side effects (e.g. weight gain and cardiovascular issues) than are most atypical antipsychotics. However, no effect of lurasidone on the voltage-gated K^+ (Kv) channels has yet been identified. Here, we show that lurasidone inhibits the vascular Kv channels of rabbit coronary arterial smooth muscle cells in a dose-dependent manner with an IC50 of 1.88 ± 0.21 μM and a Hill coefficient of 0.98 ± 0.09. Although lurasidone (3 μM) did not affect the activation kinetics, the drug negatively shifted the inactivation curve, suggesting that the drug interacted with the voltage sensors of Kv channels. Application of 1 or 2 Hz train steps in the presence of lurasidone significantly increased Kv current inhibition. The recovery time after channel inactivation increased in the presence of lurasidone. These results suggest that the inhibitory action of lurasidone is use (state)-dependent. Pretreatment with a Kv 1.5 subtype inhibitor effectively reduced the inhibitory effect of lurasidone. However, the inhibitory effect on Kv channels did not markedly change after pretreatment with a Kv 2.1 or a Kv7 subtype inhibitor. In summary, lurasidone inhibits vascular Kv channels (primarily the Kv1.5 subtype) in a concentration- and use (state)-dependent manner by shifting the steady-state inactivation curve. Therefore, lurasidone should be carefully prescribed for schizophrenia patients with underlying cardiovascular diseases.

P-17-007
Inhibition of voltage-gated potassium channel by aripiprazole in rabbit coronary arterial smooth muscle cells

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Aripiprazole, a third-generation antipsychotic, has been widely used to treat schizophrenia. Some studies have revealed aripiprazole-associated cardiovascular effects, including QT prolongation, Torsades de pointes, and blocking of hERG channels. However, the influence of aripiprazole on vascular ion channels, specifically Kv channels, has not been studied. In this study, we

evaluated the effect of aripiprazole on voltage-gated potassium (Kv) channels in rabbit coronary arterial smooth muscle cells using the patch clamp technique. Aripiprazole reduced the Kv current in a concentration-dependent manner with a half-maximal inhibitory concentration of 0.89 ± 0.20 μM and a Hill coefficient of 1.30 ± 0.25. The inhibitory effect of aripiprazole on Kv channels was voltage-dependent, and an additional aripiprazole-induced decrease in the Kv current was observed in the voltage range of full channel activation. The decay rate of Kv channel inactivation was accelerated by aripiprazole. Aripiprazole shifted the steady-state activation curve to the right and the inactivation curve to the left. Application of a repetitive train of pulses (1 and 2 Hz) promoted inhibition of the Kv current by aripiprazole. Furthermore, the recovery time constant from inactivation increased in the presence of aripiprazole. Pretreatment of Kv1.5 subtype inhibitor reduced the inhibitory effect of aripiprazole. However, pretreatment with Kv 7 and Kv2.1 subtype inhibitors did not change the degree of aripiprazole-induced inhibition of the Kv current. We conclude that aripiprazole inhibits Kv channels in a concentration-, voltage-, time-, and use (state)-dependent manner by affecting the gating properties of the channels. In this study, we provided additional information regarding the side effects on vascular Kv currents; therefore, our results should be considered when prescribing aripiprazole to patients with vascular disease.

P-17-008
Evaluation of peptide binding for oligopeptide binding proteins (OppA) in *Borrelia burgdorferi*

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Lyme disease is one of the most frequently diagnosed vector-borne infections globally. The reductive genome of *Borrelia burgdorferi* (Bb) is devoid of several metabolic pathways. Consequently, Bb contains no pathways for the synthesis of amino acids (AA) making it heavily reliant upon AA transporters. Indeed, the BbOpp system has been shown to be essential for Bb viability. The Opp system orchestrates AA acquisition through oligopeptide-binding proteins (OppA), permeases (OppBC), and NBD containing proteins (OppDF). Bacterial OppAs are known for promiscuous binding via the peptide backbone, allowing for a diverse repertoire of peptide binding partners. Previous, studies have shown Bb OppAs bind tripeptide and heptapeptides, but have elucidated little about ligand specificity. Herein, we began a large-scale study to evaluate the peptide binding capabilities of Bb OppAs. Structural (OppA4, PBD: 4GL8) and modeling data (OppA1, 2, 3, 5) show some variations within binding determinants that suggest each OppA may display specific affinities. Our microfluidics spectroscopy data confirm that all recombinant Bb OppAs have mix a-ß mix secondary elements. For high-throughput binding analyses, we have employed thermal shift assays, which suggest that OppA2 is a promiscuous binder showing binding to >80 peptides out of 120 peptides. Whereas OppA1, OppA4 and OppA5 showed positive binding with 22, 14 and 4 peptides respectively demonstrating the selective nature of OppA. Alternatively, OppA3 appears extremely selective as none of the peptides tested have shown positive thermal shift. We have solved the crystal structures of OppA2 and OppA3 at a 2.4 Å and 1.9 Å resolution, respectively. Consistent with the results of our TSA screens OppA2 was loaded with an endogenous tetrapeptide while OppA3 structure was open. We will continue characterizing the binding strategies for OppAs as well as identifying the unique ligands for OppA3.

P-17-009
The essential amino acid transporter LAT1 (SLC7A5) switches to a copper transporter in presence of Cu-histidinate complex

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L-type amino acid transporter 1(LAT1) is a plasma membrane transporter belonging to the SLC7 family and forms a functional heterodimer with the glycoprotein CD98 (SLC3A2). LAT1 has a peculiar expression profile and substrate specificity. Indeed, it is mainly expressed in critical body districts such as the blood–brain barrier (BBB) and placental barrier where it mediates the flux of essential amino acids, such as histidine and leucine, with an antiport mechanism responsible for the harmonization of amino acid pools. Then, we employed a combined approach of in silico, *in vitro*, and *ex vivo* technology to demonstrate that LAT1 can mediate the flux of Cu^{2+}-histidinate, with an unconventional uniport mechanism. By employing molecular docking analysis and site-directed mutagenesis we identified the key amino acid residues of LAT1 binding site responsible for the recognition of $Cu(His)_2$. We, then, showed that crystals of the $Cu(His)_2$ species could be formed under our experimental conditions.[1] Given that in cells copper is mainly present in the reduced state, i.e. Cu^+, and that it is taken up by the specific transporter CTR1 we sought to investigate if LAT1 could recognize also Cu^+ in complex with His. Interestingly enough, we measured the flux of radiolabelled His in the presence of Cu^+ and we also tested the ability of sulphur containing molecule(s) to chelate Cu^+ inhibiting the transport of His via LAT1. These results may furnish an alternative way of Cu^+ cell absorption. Considering that LAT1 is expressed in the BBB and in cancer cells, the LAT1-mediated transport of $Cu(His)_2$ may have profound therapeutic implications: (i) in the treatment of copper imbalance disorders, such as the rare Menkes Disease (ii) in the design of novel anti-cancer drugs, based on $Cu(His)_2$ scaffold, to overcome side effects of Pt-based therapies. Reference: 1 Scanga, R. et al. iScience 26, 107738, doi:10.1016/j.isci.2023.107738 (2023). *The authors marked with an asterisk equally contributed to the work.

P-17-010
Inhibition by atypical antipsychotic paliperidone on voltage-dependent K^+ currents in coronary arterial smooth muscle cells

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Paliperidone, an atypical antipsychotic, is widely used to treat schizophrenia. In this study, we explored whether paliperidone inhibited the voltage-dependent K^+ (Kv) channels of rabbit coronary arterial smooth muscle cells. Paliperidone reduced Kv channel activity in a concentration-dependent manner with a half-maximal inhibitory concentration (IC50) of 16.58 ± 3.03 μM and a Hill coefficient of 0.60 ± 0.04. It did not significantly shift the steady-state activation or inactivation curves, suggesting that the drug did not affect the gating properties of Kv channels. In the presence of paliperidone, application of 20 repetitive depolarizing pulses at 1 and 2 Hz gradually increased inhibition of the Kv current. Further, the recovery time constant after Kv channel inactivation was increased by paliperidone, indicating that it inhibited the Kv channel in a use- (state)-dependent manner. Its inhibitory effects were reduced by pretreatment with a Kv1.5 subtype inhibitor. However, pretreatment with a Kv2.1 or Kv7 inhibitor did not reduce its inhibitory effect. We conclude that paliperidone inhibits Kv channels (mainly Kv1.5 subtype channels) in a concentration- and use (state)-dependent manner without changing channel gating.

P-17-011
Metal selectivity and translocation mechanism characterization in proteoliposomes of transmembrane NiCoT transporters from pathogenic bacteria

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Trace metals are essential for replication and virulence of bacterial pathogens. *Helicobacter pylori* requires Ni(II) to colonize and persist in the acidic environment inside the stomach, exploiting the nickel-containing enzyme urease. The cellular regulation of Ni(II) homeostasis proves to be a vital process for *H. pylori* pathogenicity, and transmembrane (TM) transporters that permit the selective translocation of Ni(II) across the cellular membranes evolved to control cellular Ni(II) uptake and extrusion. NixA, a crucial transmembrane NiCoT Ni(II) transporter, is primarily responsible for Ni(II) uptake across the inner membrane. Despite its key role in Ni(II) acquisition, urase maturation, and virulence, limited information is available on the structure and molecular mechanism by which NixA performs its function. In this work, we successfully expressed and purified NixA and key mutants and reconstituted them in artificial lipid bilayer vesicles (proteoliposomes). Encapsulation of fluorescent sensors responsive to diverse stimuli within the proteoliposomes enabled, for the first time on a NiCoT transporter, real-time monitoring of NixA transport properties and mechanism. Kinetic analysis revealed NixA's high selectivity towards Ni(II) without substrate promiscuity for Co(II)/Zn(II). Ni(II) transport followed saturable kinetics, and substrate translocation was demonstrated to be electrogenic and independent of a proton motive force. Mutation analysis identified key residues involved in substrate recognition and transport, suggesting a three-step transmembrane translocation conduit. These approaches have been extended to other NiCoT transporter subclasses featuring different promiscuity for Ni(II) and Co(II,) unraveling the molecular determinants that control NiCoT selectivity. Overall, the works provides novel approaches to characterize transmembrane transporters responsible for Ni(II) acquisition in prokaryotes at an unprecedented molecular and mechanistic level.

P-17-012

Abstract withdrawn.

P-17-013
Soluble oligomeric assembly formation by Gasdermin D (GSDMD) is essential for its pore-forming functionality

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Gasdermin D (GSDMD) is a crucial member of the Gasdermin family of eukaryotic proteins associated with pyroptosis and inflammation. Activation of inflammatory caspases leads to the downstream activation of GSDMD and then it punches holes in the inner leaflet of plasma membranes, through which various pro-inflammatory cytokines can leak out into the extracellular milieu, causing inflammation in the neighboring cells and tissues. Upon activation, 33 monomers of the GSDMD-N-terminal domain (GSDMD-NTD) oligomerize to form large, oligomeric, transmembrane ß-barrel pores. The present study addresses questions related to the oligomerization propensities of mouse Gasdermin D (mGSDMD) in solution and also reflects upon the importance of soluble oligomeric assembly states in regulating the pore-forming ability of the protein. In this study, we employed biochemical, biophysical, spectroscopy and microscopy-based techniques to analyze the physicochemical properties of mGSDMD and their implications for the pore-forming ability of the protein on the liposome membranes. Our results suggest that despite exhibiting some of the classical amyloid-like features, mGSDMD exits mainly as soluble oligomeric assembly states that are held together by surface-exposed hydrophobic patches. Besides this, the self-associating assembly states of mGSDMD are crucial for the maintenance of its pore-forming functionality. Taken together, our study for the first time, provides novel insights regarding the physicochemical properties of mGSDMD, which in turn play important roles in regulating cell-death induction.

P-17-014
Unveiling the role of thermo TRP ion channels and neuronal excitability in paclitaxel-induced peripheral neuropathy: a molecular and functional perspective

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Paclitaxel-induced peripheral neuropathy (PIPN) is a challenging side effect arising from treatment of several anti-cancer agents that constitutes a major medical and societal problem because there is no effective prevention or treatment method. The direct impact paclitaxel on sensory neuron excitability and potential gender differences remains unclear. We employed a long-term (15 days *in vitro*) primary culture of mice dorsal root ganglion (DRG) neurons to examine how two consecutive administrations of paclitaxel influence the electrical activity of IB4(+) and IB4(-) sensory neurons of male and female adult mice during sensitization and resolution. Paclitaxel was found to enhance spontaneous activity and amplify tonic firing of action potentials in both IB4(-) and IB4(+) neurons. Although these effects vanished 96 h post-initial treatment, they remained consistent after the second administration. Paclitaxel also decreased the current rheobase for action potential firing by expediting the after-hyperpolarization phase. Furthermore, the drug influenced Na^+ ion currents, notably increasing the activity of TRPV1, TRPM8, and TRPA1 channels upon the second dose. Intriguingly, female DRGs neurons exhibited greater sensitivity to paclitaxel-induced sensitization compared to their male counterparts. Our findings suggest that paclitaxel enhances the electrogenicity of sensory neurons by altering the activity of thermoTRPs and unveil sex-related differences. Our *in vitro*, preclinical PIPN model serves as a valuable tool for exploring the dynamics and molecular mechanisms underlying nociceptor sensitization and desensitization by chemotherapeutics and algesic agents, as well as for evaluating modulators of neural excitability with high clinical translational potential. *The authors marked with an asterisk equally contributed to the work.

P-17-015
Exploring the cosmeceutical properties of Tanzanian seaweeds using various extraction methods

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Skin conditions are often neglected as they are mostly deemed non-fatal. However, findings suggest that skin conditions can place a burden on health status and quality of life by having psychological and social consequences. For several decades, marine macroalgae have prospered at providing a source of bioactive compounds which demonstrate a diverse range of therapeutic activities. The aim was to compare the efficacy of different extraction methods for Tanzanian seaweeds (namely *Sargassum oligocystum*, *Turbinaria conoides* and *Ulva fasciata*) using biochemical assays related to cosmeceutical applications. Four extraction methods were performed in which the material-to-solvent (MTS) ratio, extraction period as well as solvent were altered. The methods were as follows: Method 1 – 1:15 (w/v) MTS ratio using 80% ethanol as solvent with 24 h extraction period; Method 2 – 1:5 (w/v) MTS ratio changing the solvent every 8 h over a 24 h period; Method 3 – 1:5 (w/v) MTS ratio changing the solvent every 4 h over a 12 h period and Method 4 – 1:5 (w/v) MTS ratio using glycerine:ethanol (1:1 v/v) as solvent over a 3-day period. The assays chosen to determine the efficacy of the extraction methods included a metal ion chelation assay and a mushroom tyrosinase inhibition assay. In addition, cell-based assays such as nitric oxide inhibition in LPS-activated RAW 264.7 macrophages and a resistance to oxidative stress assay in TBHP-induced HaCaT keratinocytes were included. Method 1 showed the best activity in all assays except for the tyrosinase inhibition assay with the best activity demonstrated by *U. fasciata* for metal ion chelation, *T. conoides* for nitric oxide inhibition and *S. oligocystum* for antioxidant potential. Method 4 displayed the most promising tyrosinase inhibition specifically *S. oligocystum* and *T. conoides*. In conclusion, the best extraction method was established, and the seaweed species possessed beneficial activities suitable for cosmeceutical application.

P-17-016
Diacylglycerol potentiates canonical Wnt signaling via macropinocytosis

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Canonical Wnt signaling, initiated by caveolin-mediated endocytosis of the Wnt receptor complex into plasma membranes, plays a crucial role in development and adult tissue homeostasis. In our previous study [Azbazdar et al. (2023) Mol Oncol. 17, 2314-2336], we identified changes in lipid content through membrane lipidome profiling after Wnt induction and demonstrated that diacylglycerol (DAG) augments Wnt signaling activity. Moreover, we found that the effect of DAG is accompanied by increased levels of the Huluwa protein, which is known to promote Wnt signaling and plays an important role in early development of zebrafish and *Xenopus* embryos. On the contrary, depletion of DAG suppressed signaling activity and inhibition of DAG resulted in decreased proliferation and migration in cells and the zebrafish xenograft model. While DAG has capacity to induce Wnt signaling activity, it also triggers macropinocytosis via the Wnt pathway. By DAG treatment, colocalization of glycogen synthase kinase 3 (GSK3), a negative regulator of Wnt signaling, and CD63, a marker of multivesicular bodies (MVBs) is increased. The expansion of the MVB compartment is a known downstream consequence of Wnt-induced macropinocytosis, triggered by the transcriptional loop of stabilized β-catenin and GSK3 inhibition. Considering all these results, in conclusion, our findings suggest that DAG could serve as an effective diagnostic, prognostic biomarker, or a therapeutic target for the treatment of some cancers, such as hepatocellular carcinoma and colon cancer with enhanced macropinocytosis capacity.

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P-17-017
Critical role of phosphatidylcholine in intracellular transport dynamics

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Cellular physiological functions rely on intricate interactions among biomolecules. While extensive research has been focused on exploring protein-protein and protein-DNA interactions, studies on specific interactions between transmembrane proteins and lipids residing in the same membrane have been profoundly challenging. In this study, we developed a methodology to decipher the precise membrane protein-phosphatidylcholine (PC) interactome within living cells solely. Given the established correlation between dysregulated cellular PC levels and the onset and progression of various human diseases, including cancer and neurodegenerative disorders, understanding these interactions holds profound significance. By external addition to cells of an alkyne-modified choline head group together with a diazirine-modified fatty acid, cells metabolically incorporated these analogs into phosphatidylcholine lipids generating bifunctional lipids that enable identifying and visualizing the full spectrum of PC-protein interacting partners. Endogenous PC molecular species level downregulation significantly affects endoplasmatic reticulum (ER) protein exit and intracellular transport. Thus, our results highlight the pivotal role of PC molecular species as coordinators of intracellular protein trafficking through the early secretory pathway.

P-17-018
Mobility and localization of SPFH-domain proteins in the inner membrane of *Escherichia coli*

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Clustering of lipids and proteins within the biological membranes creates membrane-bound microenvironments with distinct transport and enzymatic activities. SPFH-domain proteins, evolutionary conserved among prokaryotes and eukaryotes, play critical roles in the organization of biomembranes, i.e. in formation of lipid rafts, membrane protein quality control and trafficking. In the *Escherichia coli*, four SPFH-domain proteins, YqiK, QmcA, HflK, and HflC, form mobile and immobile foci that contribute to the heterogeneous organization of the inner bacterial membrane. Here we use the live-cell time-lapse and single-molecule fluorescence microscopy to investigate the dynamic organization of the SPFH-domain proteins in the inner membrane of *E. coli*. We quantitatively analyze the partial enrichment and altered mobility of the SPFH-proteins' clusters at the cell poles compared to the mid-cell region. We also investigate how localization and mobility of the SPFH-proteins' clusters depend on the physiological state of the cell, altered under the stress conditions or antibiotics treatment. Our findings shed the light on the dynamic organization of protein clusters within bacterial membranes and their role in the bacterial adaptation to the environmental stresses.

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P-17-019
Surface decoration of *Escherichia* coli outer membrane vesicles (OMVs) with the antiviral peptide AR-23 fused with bacterial protein Cytolysin-A

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The improvement of drugs specificity and the minimization of their side effects are the aims underlying the development of innovative drug delivery systems. Bacterial outer membrane vesicles (OMVs) are small (20-250 nm) proteo-liposomal natural carriers, easy to engineer. In this work, we decorated the surface of *E. coli* BL21(DE3) OMVs with a chimeric protein. In previous studies, we identified a mutated form of the Cytolysin A monomer (ClyA) as an optimal membrane scaffold for OMVs surface functionalization, having its C-terminus exposed on OMVs surface. A recently identified small antiviral peptide (AR23), which is endowed with significant antiviral and antibacterial activity described in previous studies, was fused to the C-terminus of ClyA. ClyA-AR23 fusion protein was recombinantly expressed in *E. coli* BL21(DE3). To evaluate the presence of the chimera, we performed a proteomic analysis of cell lysates and OMVs. The presence of diagnostic tryptic peptides of ClyA-AR23 protein was confirmed by LC-MS/MS analysis in cells and OMVs after ClyA-AR23 expression and not in wild-type strain. The antiviral efficacy of AR23 peptide and AR23-OMVs was assessed through a viral pre-treatment assay targeting Herpes simplex type 1 virus. The AR23 peptide, AR23-OMVs and native OMVs were incubated with the virus and the mixture was then introduced to Vero CCL-81 kidney cells. AR23-OMVs exhibited a dose-dependent reduction in viral infectivity, achieving a 50% inhibition (IC50) at a concentration of 31.6 μg/ml. Conversely, treatment with native vesicles did not lead to any significant antiviral activity. Our data confirmed that the engineered peptide fused to the Cly-A scaffold maintains its antiviral activity. In conclusion, the development of AR23-OMVs could be of great interest to overproduce an immobilized, bioactive form of the peptide, easy to further mutate, and to couple the specificity of the peptide to other molecular targets present on the surface of the OMVs.

P-17-020
The role of *Saccharomyces cerevisiae* ATPases in adaptation mechanisms to osmotic stress under aerobic conditions

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Understanding the relationship between metabolic regulation and energy balance under osmotic stress conditions is crucial for enhancing yeast adaptability. Proton ATPases maintain electrochemical gradients of key cations or phospholipids across

membranes and play a central role in the regulation of the bioenergetic charge of the cell. The influence of osmotic stress on total and *N, N'*-dicyclohexylcarbodiimide (DCCD)-sensitive ATPase activity of industrial wine (*Saccharomyces cerevisiae* ATCC 9804) and beer (*S. cerevisiae* ATCC 13007) yeast strains was investigated. Cell viability was assessed using the colony-forming unit (CFU) method and ATPase activity was monitored using the Taussky and Shorr method. It was shown that cell viability is reduced under osmotic stress conditions during aerobic growth, particularly, CFU decreased ~2.6- and ~1.7-fold in ATCC 9804 and ATCC 13007 strains, respectively. Thus, ATCC 13007 cells are more stable under osmotic stress conditions, correlating with higher total ATPase activity (435.1 nmol P_{in} ml^{-1} mg^{-1} protein) with no significant changes observed under osmotic stress conditions. In contrast, osmotic stress results in a double reduction of total ATPase activity (271.1 nmol Pin ml^{-1} mg^{-1} protein) in ATCC 9804 strain. DCCD exhibited concentration-dependent inhibition of proton ATPase activity, however, strains differ in DCCD sensitivity: IC_{50} value for ATCC 9804 is 1.3 mM, which is 1.4-fold higher compared to 13007 strain, where IC_{50} is found to be 0.9 mM. In both strains the inhibitory activity of DCCD remained consistent under osmotic stress conditions, with an IC_{50} value of 1.09 mM. Taken together, the data show the regulatory role of ATPases in stress adaptation of yeast, suggesting a new regulatory mechanism for adaptations to osmotic stress. *The authors marked with an asterisk equally contributed to the work.

P-17-021
Relationship between ER (electrorotation) dielectrophoresis (DEP) profiles and membrane-associated region-1-binding protein (Smar1) and focal adhesion kinase (Fak) gene expression

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We evaluated the mRNA expressions of *SMAR1* and *FAK* genes, in two different colon cell lines CCD841& CaCo2 (healthy/adenocarcinoma), to investigate biological pathways involved under different ER measurement spectra generated in high frequency DEP experiments. The CCD841 and CaCo2 cell lines were grown in their respective media [1]. The expression of *SMAR*1 and *FAK* genes was evaluated via RTqPCR, with gene expression normalized to the β-actin. For dielectrophoresis experiments, cells were washed and resuspended in an appropriate buffer [1]. Cells were then subjected to ER in an engineered plug-and-play chamber using a prototypal DEP chip, administering a frequency range of 104107 Hz, and evaluating their velocity (ω) in radians per second. The expression of *FAK1* did not show differences between the two cells lines (p value = 0.9594) this is an epiphenomenon that shows nonmechanical stress during the High Frequency. *SMAR1* showed a statistically down regulated mRNA expression in CaCo2 (p value = 0.0008). In ERDEP, cancer cells exhibit more marked rotational velocity variations (Slope Coefficent: +0.6918) as frequency changes, opposed to healthy cells that are more resilient to velocity changes (Slope Coefficent: +0,5972). Cancer cells have a higher ω than healthy ones. We observed an inverse relationship between the expression of *SMAR1* and the changes in ER velocity. CaCo2, with the lowest expression of *SMAR1*, reverse their direction of rotation first (60MHz,8.33 ω); CCD841 later reverse their rotation at a slower speed (70MHz,7.96 Ω). The presented study adds a step ahead in the comprehension of mechanisms driving the correlations between gene expressions and electrorotation, potentially adding novel knowledge on CTCs cells [2] useful for their characterization and isolation in early diagnosis. Previously published in: 1. Bonacci et al. (2023) Translational Oncology, 28, 101599. 2. Musso et al. (2022) Int. J. Mol. Sci., 23, 12052

P-17-022
Novel gap junction inhibitor potency is connexin 43 phosphorylation-dependent

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The examination of chemical modulators targeting the gap junction intercellular communication (GJIC) has unveiled promising therapeutic potential for a spectrum of communication-dependent disorders, encompassing epilepsy, cardiac arrhythmia, cancer, stroke, and essential tremor. In our recent research, the monoterpene α-pinene was identified as a GJ inhibitor whose potency was allosterically regulated by Ca^{2+}/calmodulin-dependent protein kinase II (CaMKII). In the current study, we used dual whole-cell patch-clamp techniques, western blotting, and selective kinase inhibitors to demonstrate the involvement of other kinases, including atypical protein kinase C, Pyk2, and cyclin-dependent kinase, in modulation of α-pinene effects on connexin 43 (Cx43) GJs. Western blot analysis revealed differential effects on the phosphorylation levels of serines 265, 279, 282, 368, and 373. Besides α-pinene, other GJ inhibitors, such as octanol, carbenoxolone, mefloquine, flufenamic acid, glycyrrhetinic acid, and sevoflurane, were examined; however, only the potency of sevoflurane was found to be Cx43 phosphorylation-dependent. The non-selective kinase inhibitor staurosporine exerted a modest effect on α-pinene-induced inhibition of Cx36, Cx40, Cx45, and Cx47 GJs, typical to neurons and/or cardiac myocytes exhibiting high electrical activity and phosphorylation levels. Our data suggest that the allosteric modulation of GJ chemical gating is protein kinase-, cell type-, and connexin isoform-dependent.

P-17-023
Vaccinia virus controls apoptosis via intramembrane interactions with cellular Bcl2 proteins

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Intracellular membranes are indispensable for orchestrating a wide range of biochemical processes within the cell. Among these

processes, apoptosis, a well-known form of programmed cell death, is primarily regulated by the Bcl2 protein family. Notably, some DNA viruses have evolved protein homologs of cellular Bcl2s (cBcl2s) to modulate apoptotic pathways, facilitating viral replication. The F1L protein of *Vaccinia virus* serves as a prominent example of these viral Bcl2 homologs, exhibiting structural similarities to Bcl2 and operating at the mitochondria to suppress apoptosis. F1L presents a transmembrane domain (TMD) at its C-terminal end that anchors the protein into the outer mitochondrial membrane. It is known that F1L establishes TMD-TMD interactions with some cBcl2s. However, the significance of these intramembrane interactions in the apoptotic control remains poorly understood. Our approach integrates both empirical and computational methodologies. Initially, using a BlaTM assay, we delineated the TMD-TMD interaction network, revealing interactions of F1L with the TMDs of the pro-apoptotic proteins Bak, Bax, and Harakiri, along with the anti-apoptotic protein BclxL. Next, the TMHOP and PREDDIMER servers were employed to analyze the interactions surface in the TMD-TMD interactions between F1L and cBcl2s, while molecular dynamics simulations were then employed to further investigate the oligomerization states of the interacting partners. Finally, to validate the proposed interaction models, we examined the impact of various mutations within the F1L TMD on interactions and the development of its anti-apoptotic function. This validation was achieved through a combination of BlaTM assays and flow cytometry analysis. Our comprehensive approach provides valuable insights into the molecular mechanisms governing the interplay between the *Vaccinia virus* F1L and cBcl2s TMDs as apoptosis modulators.

P-17-024
Interaction of tick-borne encephalitis virus capsid protein with liposomes and nucleic acid

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Tick-borne encephalitis virus (TBEV) poses a significant threat as a neurotropic pathogen causing neurological diseases such as encephalitis and meningoencephalitis. Despite the availability of vaccines, the TBEV is on the rise. The formation of the viral particle occurs at the endoplasmic reticulum membrane, where one of the important steps of the TBEV life cycle is the recruitment of viral genomic RNA (gRNA) by the capsid protein (TBEVC) and subsequent nucleocapsid formation. However, the detailed mechanism of this process is not well understood. Our focus is on TBEVC, whose 3D structure we have solved by NMR spectroscopy [previously published in: Selinger M et al. (2022) J Biol Chem 298]. Our goal was to understand the mechanism of TBEVC and viral gRNA interactions, assembly of nucleocapsid-like particles, and budding into the endoplasmic reticulum. Here, we prepared several mutants of TBEVC and investigated their interaction with liposomes and selected segments of gRNA. We used three types of liposomes, neutral (PC), charged (PSPC 1:2), and those simulating the endoplasmic reticulum (ER) membrane. Their interactions with full-length wild-type and mutant TBEVC were analyzed using flotation experiments, microscale thermophoresis, and microcalorimetry. The results of this study could aid in designing strategies to intervene in important stages of the virus life cycle. This could ultimately contribute to the development of targeted therapy against TBEV. This study was supported by the Czech Science Foundation (Registration No.: 22-25042S).

P-17-025
The effect of entire deletion of hydrogenase-1 and 2 on proton flux during utilization of varied glucose concentrations at pH 7.5

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Hyd-1 and Hyd-2 exhibit distinctions in terms of how they are expressed, their ability to function in the presence of oxygen, and the composition of their subunits when integrated into the cytoplasmic membrane [1]. The impact of hydrogenase-1 and hydrogenase-2 deletions (HDK100 and HDK200, respectively) on total proton flux (J_{H+}) in *E. coli* was investigated during fermentation of low (2 g L^{-1}) and high (8 g L^{-1}) glucose. In the HDK100 strain lacking whole Hyd-1, no significant differences in total J_{H+} was observed when supplementing assays with low glucose compared to the parental MC4100 strain. However, a significant reduction (35%) in DCCD-sensitive J_{H+} was noted. When high glucose was supplemented total J_{H+} decreased by ~20% and DCCD sensitive J_{H+} increased by 40%. HDK200 mutant exhibited J_{H+} levels similar to the wild type under both glucose conditions, with DCCD-sensitive J_{H+} increase by 35% under low glucose and 60% under high glucose supplementation. When cells were grown in a presence of high glucose concentration and supplemented in assays with low glucose only in HDK100 mutant, total J_{H+} was higher compared to the wild type, with no other significant differences observed in both HDK100 and HDK200 mutants in DCCD-sensitive J_{H+}. However, when high glucose was added, an increase in total and DCCD-sensitive J_{H+} was observed in both mutants, moreover the contribution of F_OF_1 increased by 70–80%. Thus, while Hyd-1 appears to be more directly involved in membrane-associated proton transport, especially during switch from low to high conditions, Hyd-2 seems to exert a modulatory influence on DCCD-sensitive proton flux pathways, regardless of glucose concentration. Reference: Pinske, C., Jaroschinsky, M., Sargent, F. et al. (2012) BMC Microbiol. 12, 134. doi.org/10.1186/1471-2180-12-134.

P-17-026
Atypical effects of lipophilic natamycin derivatives on lipid membranes

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Nosocomial fungal infections are one of the leading causes of death among hospitalized patients with suppressed immunity. In 2022, the World Health Organization published a list of priority

fungal pathogens (WHO FPL), highlighting the discrepancy between the level of attention paid to research and development (R&D) in the field of fungal infections and their perceived importance for public health. Among the three main classes of antifungal drugs – azoles, echinocandins, and polyene antibiotics – the latter are characterized by the lowest risk of drug resistance, making them a promising basis for the creation of new-generation antifungal drugs. The antifungal polyene antibiotic natamycin is widely used as a preservative in the food industry and is also used for the topical treatment of mycoses of the skin and mucous membranes. Recently, we reported water-soluble natamycin amides as potential antifungal agents for treating systemic mycoses and noted that an amide with a C-14 lipophilic side chain showed strong fungicidal activity *in vitro* [Previously published in: Tevyashova AN et al. (2023) ACS Infect Dis 9, 42–55]. Our further studies showed that introducing long alkyl chains (*N*-(2-((2-decyl)amino)ethylnatamycin and N-((1-tetradecylpiridin-1-ium-3-yl)methyl)amide of natamycin) into the structure of natamycin led to a change in its interaction with lipid membranes. A comparative study on polyene-induced leakage of calcein from unilamellar vesicles composed of POPC/ERG vs POPC/CHOL revealed that these derivatives, in contrast to other natamycins, were capable of causing calcein leakage at concentrations close to the MIC value and were twice as selective for POPC/ERG vesicles. *N*-((1-tetradecylpiridin-1-ium-3-yl) methyl)amide of natamycin was superior to the original antibiotic in terms of activity against biofilms formed by *Candida* spp., which suggests it deserves further research. This work was supported by the Russian Science Foundation project number 21-74-20102. *The authors marked with an asterisk equally contributed to the work.

P-17-027
Increased intracellular SVCT2 expression in amyloid beta treated cells, as a model of Alzheimer's disease

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Ascorbic acid (AA) is a reducing agent present in all living beings. It plays a key role in antioxidant defense and acts as a cofactor in numerous cellular functions. Cell transport is needed to acquire vitamin C into different cells and tissues. AA is also relevant in different organelles. For instance, mitochondria, as a source of reactive oxidant species, must have a strong AA antioxidant support. It has been previously reported that AA mitochondrial uptake is mediated by a mitochondrial form of the sodium vitamin C co-transporter SVCT2. We have functionally characterized mitSVCT2 in cancer cells, and we have suggested a role for this protein in oxidative stress survival in this disease. In this work we analysis intracellular AA transporters in other oxidative stress linked group of diseases: neurodegenerative disorders. To determine the expression of SVCT2 in the context of Alzheimer's disease (AD), we exposed mice-derived hippocampal neuronal cells to Aβ oligomers and tested SVCT2 expression. Interestingly, we found augmented levels of intracellular SVCT2 in higher Aβ concentrations. However, we fail to detect any of this protein within the mitochondria. We also used the neuronal-like cell line CAD (mouse origin) exposed to Aβ oligomers to test if we could replicate what we observed in neurons. Overexpression was related to Aβ increase, and most of SVCT2 colocalized with endoplasmic reticulum with low colocalization index with mitochondria. We finally performed different analysis to test oxidative stress response to Aβ in these cells to understand if SVCT2 increase might be linked to a pro-oxidant environment. We conclude that SVCT2 in mouse is detected in an intracellular compartment that is not mitochondria and it increases in response to Aβ oligomer exposure. SVCT2 also exhibits different locations in different species, tissues, and disease models. We discuss the implications of intracellular AA in health and disease. *The authors marked with an asterisk equally contributed to the work.

P-17-028
N-terminal cleaved form of cyclophilin D in human and murine tissues: structural and functional characterization

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Cyclophilins are ubiquitous proteins endowed with peptidyl-prolyl cis-trans isomerase (PPIase) activity. Among the 17 CyPs encoded in the human genome, cyclophilin D (CyPD) is the only one found in mitochondria. One of its best characterized functions is the regulation of the permeability transition pore (PTP), a Ca^{2+}-dependent, multi-conductance channel whose long-lasting openings can lead to cell death. We demonstrated that CyPD interacts with the oligomycin sensitive conferral protein (OSCP), a subunit of F-ATP synthase, which is currently the most promising candidate for PTP formation. Here we report that two forms of the mature CyPD can be identified in various animal and human tissues. Mass spectrometry analysis showed that one form corresponds to the full-length protein (FL-CyPD), while the other is ~1 kDa shorter at the N-terminus (ΔN-CyPD). Nuclear magnetic resonance (NMR) spectroscopy revealed that recombinant human FL-CyPD and ΔN-CyPD are structurally identical except for the highly flexible N-terminal tail (which is absent in the truncated form). Protein-protein interactions studies showed that the N-terminal tail, which contains most Ser residues modified by phosphorylation, has a strong influence on CyPD binding to OSCP, affecting in turn CyPD-dependent channel formation by F-ATP synthase. To characterize the protease(s) involved in the cleavage of FL-CyPD giving rise to ΔN-CyPD and to identify metabolic conditions that may lead to generation of the truncated form, we performed interactomics studies in human embryonic kidney cells (HEK 293) in order to identify potential partners of CypD. Overall, our data provide evidence for the presence of a N-terminally cleaved form of CyPD that behaves differently from FL-CyPD, and open new insights on regulation of the PTP. *The authors marked with an asterisk equally contributed to the work.

P-17-029
Pyrazinamide and pyrazinoïc acid efficacy against *Mycobacterium* correlate with their ability to cross lipid membranes, release protons and disrupt intrabacterial pH homeostasis

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Tuberculosis (TB), caused by *Mycobacterium tuberculosis* (Mtb) remains one of the main causes of death due to a single infectious agent worldwide. Pyrazinamide (PZA) is a front-line prodrug that plays a key role in TB chemotherapy; however, its mode of action remains poorly characterized. Our group and others have recently proposed that PZA's active form, pyrazinoic acid (POA), acts as an acid dependent compound that disrupts intrabacterial pH homeostasis, but the exact molecular mechanism underlying this process remains unclear. Herein, we combined bacterial genetics, antimicrobial susceptibility testing, bioelectrochemistry experiments and phenotypic assays to investigate PZA/POA mode(s) of action against Mtb and several non-tuberculous mycobacteria (NTM). Using these approaches, we confirmed that the surrounding acidic pH is an important factor driving PZA/POA antibacterial efficacy against Mtb. By capitalising from a genetically-encoded pH biosensor, we demonstrated that POA antibacterial efficacy was correlated with a dose-dependent intrabacterial pH homeostasis disruption. Experiments using the patch-clamp method of artificial lipid membranes and pyranine-loaded liposomes revealed that POA is not protonophore as previously suggested but rather acts as a weak acid that diffuses across the bacterial membrane and quickly collapses intrabacterial pH homeostasis upon protons releasing. Finally, we showed that several NTM species were intrinsically resistant to both PZA and POA regardless of the environmental pH with minimal inhibitory concentration (MIC) superior to 1 mg/ml *in vitro*. Such resistance was associated with a minimal and/or an inexistant alteration of their intrabacterial pH homeostasis, suggesting the existence of a detoxification pathway that prevents PZA/POA pH-disruptive activity and antimycobacterial efficacy.

P-17-030
Alterations of lipid membrane composition and antioxidant potential of mouse erythrocytes exposed to hypergravity

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To sustain safe human space exploration, it is important to understand how the different effectors, including gravitational force, influence organisms. Altered levels of gravity affect the physiological function of multiple tissues, cells, and organs in living organisms, and might cause integrated changes in the lipid membrane composition, inducing greater sensitivity to oxidative stress. The evaluation of the effects of hypergravity is a fundamental step toward complete knowledge of the physiological response to altered gravity. This study aimed to investigate in vivo the effects of hypergravity on lipid phenotype and metabolism in mouse erythrocytes. Animals were housed in the ASI MDS facility, designed for rodents on the ISS and adapted for the LDC centrifuge, to expose mice to 3xg. Vivarium and MDS-like cage animals were compared as controls. After 30 days a tissue-sharing protocol was performed to analyze all the tissues; we purified and analyzed the red blood cells. The membrane lipid phenotype was assessed by GC and HPLC systems and the hemolyzed fractions were used to test antioxidant enzyme activities. Our results show that hypergravity induced fatty acid composition changes in 3xg mice compared to control and an increase in cholesterol content. To evaluate the impact of fatty acid composition on the level of oxidative stress, we calculated the peroxidability index, which was significantly increased under 3xg condition. Furthermore, we have analyzed the endogenous antioxidant activity and GSH peroxidase was significantly increased in 3xg mice. This study demonstrates that hypergravity induces metabolic changes in lipid composition and antioxidant system of erythrocytes. Our results will be integrated with tissue and metabolic data by other researchers of the team. Further studies will be necessary to identify possible countermeasures to ensure an adequate level of crew health and safety during long-duration space missions.

P-17-031
Unraveling the mechanisms of lipid-mediated protein sorting at the ER

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Protein sorting in the secretory pathway is crucial to maintain cellular compartmentalization and homeostasis. While coat-mediated sorting is well understood, the involvement of membrane lipids in driving protein sorting during secretory transport remains a longstanding and unresolved question. To address this question, we have investigated in the yeast *Saccharomyces cerevisiae* how a special type of lipid-linked cell surface proteins, the GPI-anchored proteins, are differentially exported from the endoplasmic reticulum (ER). Our findings reveal that ceramide plays a pivotal role in clustering and sorting GPI-anchored proteins into specialized ER exit sites. Here we provide a better comprehension of the potential mechanism for this ceramide-based sorting process. After realizing that both GPI and free membrane C26 ceramides drive together clustering and sorting of GPI-APs into selective ER exit sites (ERES), we studied by molecular dynamic simulation the location of C26 ceramides around the cytosolic transmembrane domain (TMD) of the GPI-AP receptor (p24 complex). By assays *in vivo* we observed that the cytosolic TMD of the receptor is required for GPI-AP clustering and sorting. These findings suggest that it is not the lipid alone but transmembrane proteins that initiate sorting by locally reorganizing and concentrating specific lipids. We propose that specific emergent biophysical properties associated with concentrated lipids around the transmembrane domains eventually drive lateral segregation and sorting of proteins.

P-17-032
Wnt trafficking in the early secretory pathway

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The secretion of Wnt signaling proteins is essential to control cell proliferation, cell polarity and cell fate determination during embryonic development and tissue homeostasis. Wnt secretion also contributes significantly to the pathogenesis of human diseases such as cancer, diabetes and congenital malformations. Although much focus has been placed on understanding intracellular Wnt signal transduction in signal-receiving cells, the molecular mechanisms that control the secretion of Wnt proteins in signal-producing cells are still unclear. Lipidation of newly synthesized Wnt proteins in the endoplasmic reticulum (ER) confers a unique mode of membrane association within the lumen of secretory organelles that may lead to specialized trafficking through the secretory pathway. Here, we used the RUSH (retention using selective hook) system and super-resolution microscopy to investigate whether the association of lipidated Wnt proteins with specific membrane lipids regulates their trafficking from the ER to the Golgi apparatus. We generated a construct with Wnt linked to SBP (streptavidin binding protein), and another one which encodes for streptavidin with KDEL ER location signal. So, Wnt remains in ER after synthesis, but when we add biotin to the medium, it is released from streptavidin and continue in the secretory pathway. At different times of biotin treatment, Wnt is located in different ratio in the ER and the cis-Golgi. After treatment with biotin in different conditions, cells are fixed and an immunofluorescence assay is carried out for fluorescence confocal microscopy analysis. In the results obtained, we found that depletion of the sphingolipid ceramide accelerates the exit of Wnt-RUSH from ER exit sites and its arrival at the Golgi. Since ceramide depletion increases membrane fluidity, our results suggest that ceramide may control ER export of lipidated Wnt proteins by forming compact and rigid lipid membrane domains. *The authors marked with an asterisk equally contributed to the work.

P-17-033
Reciprocal regulation between very-long chain sphingolipids and phosphatidylserine maintains the plasma membrane lipidome and proteome

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The hydrophobic acyl-chains affect how lipids behave in membranes and interact with other lipids and membrane proteins. Sphingolipids (SLs) are abundant lipids in the outer leaflet of the plasma membrane (PM) and, in contrast to glycerophospholipids, they are rich in very long-chain acyl-chains. It is not fully understood how very long-chain sphingolipids (VLC-SLs) differ in functions from long-chain sphingolipids. Using CRISPR-Cas9, we generated mutant HeLa cells lacking CERS2, the enzyme required for the synthesis of VLC-SLs. Lipidomics revealed that CERS2 mutant cells have a drastic reduction in VLC-SLs and a specific reduction of the phosphatidylserine (PS) species 18:0/18:1, a major inner leaflet lipid in many cells. To test if PS affects SL reciprocally, we generated cells lacking PS synthesis by mutating PSS1 and PSS2, the two enzymes required for PS synthesis. Lipidomic analysis revealed that the blockade of PS synthesis specifically reduces saturated VLC-SL levels. This suggests a reciprocal regulation between saturated VLC-SLs and 18:0/18:1 PS. Using lipid-binding proteins fused to fluorescent proteins, we found that PS localization at the PM is impaired in CERS2 mutants, suggesting that the reciprocal regulation maintains the PM lipidome. Furthermore, proximity-dependent biotinylation and proteomics analysis revealed that CERS2-dependent synthesis of VLC-SLs shape the PM proteome. In CERS2 mutants, we observed a downregulation of various PM proteins such as caveolin-1, which is known to require PS for caveolae formation, or the small GTPase ARF6, known to regulates endocytic recycling membrane trafficking. We hypothesize that the saturated VLC-SLs and 18:0/18:1 PS have trans-bilayer interactions, which retain PS in the PM and prevent its endocytosis, while helping PS-dependent protein recruitment. These interacting lipids could create functional domains in the PM, which are reminiscent of lipid rafts but have more defined compositions.

P-17-034
Analysis of unilateral Walker A and A-loop mutants indicate that a single active catalytic site is sufficient to promote transport in ABCB1

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Human ABCB1 (P-glycoprotein, Pgp) is an exporter type ABC protein that can expel numerous chemically unrelated xeno- and endobiotics from cells using the energy of ATP hydrolysis. When expressed in tumor cells, ABCB1 may cause multidrug resistance contributing to the failure of chemotherapy. ABCB1 contains two transmembrane domains (TMDs) that collectively form a substrate binding pocket and two nucleotide binding domains (NBDs) that build up two composite nucleotide binding sites (NBSs). The conserved tyrosine in the A-loop of NBSs aligns the adenine ring of the bound ATP, while the Walker A lysine interacts with the α and β phosphate of ATP. The integrity of both NBSs is generally believed to be needed for transport and it is also supposed that the NBSs hydrolyse ATP in a strictly alternating order. Here we demonstrate that ABCB1 variants carrying bilateral mutations in the above residues are completely inactive, while unilateral exchange of these residues is compatible with both ATP hydrolytic activity and transport function. Characterization of the single mutants revealed an about 10-fold reduction of the apparent ATP binding affinity compared to wild-type (Wt). Stabilization of the post-hydrolytic complex by phosphate mimicking anions, such as vanadate also occurred at higher ATP concentrations in the mutant variants. Although the basal catalytic activity was strongly reduced in accordance with the

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decreased ATP binding affinity of the single mutants, the degree of ATPase stimulation by verapamil was almost identical to that of the Wt, showing that substrate-stimulation of the ATPase activity is preserved in the single mutants. Location of the mutation in the N or C terminal NBD did not affect the extent of ATPase stimulation. Taken together, in contrast to prevailing views, unilateral NBD mutants retain a significant uphill transport activity, suggesting that the Wt catalytic site can hydrolyse ATP in repeated cycles without hydrolysis at the other NBS.

P-17-035
Interaction of Mason-Pfizer monkey virus matrix protein with calmodulin and its role in the retrovirus life cycle

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Calmodulin, a ubiquitous cellular protein, plays a key role in the regulation of numerous cellular processes by undergoing significant conformational changes in response to calcium cation binding. Its involvement in the life cycles of various viruses has been studied in detail. The concentration of calmodulin is significantly increased in human immunodeficiency virus (HIV-1) infected cells compared to uninfected cells. The structural polyprotein Gag of HIV-1 interacts with calmodulin, specifically through its N-terminal domain called the matrix protein (MA). This interaction induces a conformational change known as a myristoyl switch in the myristoylated MA, which leads to the exposure of the N-terminal myristoyl group and facilitates binding of the MA to the plasma membrane. We have described analogous interaction and myristoyl switch also for Mason-Pfizer monkey virus (M-PMV) MA. Furthermore, we demonstrated that calmodulin promotes binding of MA M-PMV to cytoplasmic membrane-mimicking liposomes and facilitates myristoyl switch on membrane vesicles. This shows that these late phases of the two retroviruses exhibit similar characteristics despite differences in their morphogenesis, M-PMV assembles in the cytoplasm while HIV-1 assembles at the plasma membrane. We are currently focusing on studying the interaction between MA and calmodulin and its effect on M-PMV life cycle in infected cells. Describing the role of this interaction in the late stages of the retrovirus life cycle will help to understand the regulation and propose potential targeting of these steps at the molecular level. The research was supported by the Czech Science Foundation (grant No. 22-19250S) and by the project National Institute of virology and bacteriology (Programme EXCELES, ID Project No. LX22NPO5103) – Funded by the European Union – Next Generation EU.

P-17-036
Air pollution and COVID-19: possible role of lipid rafts

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The overlap between the geographical distribution of Coronavirus Disease 2019 (COVID-19) outbreaks and pollution levels suggests a strong correlation between exposure to atmospheric particulate matter and the severity of SARS-CoV-2 infection. 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. The membrane metalloprotease ADAM17 mediates the proteolytic shedding of ACE2, playing a fundamental role in the triggering of the infection. Other proteins, such as COX-2 and HO-1 or interleukins, such as IL-8 and IL-6, are involved in the regulation of inflammation and oxidative stress following air pollution exposure and also in cytokine storms related to COVID-19. Recent studies highlight the fundamental role of membrane micro-domains called lipid rafts in facilitating viral entry, as SARS-CoV-2 is capable of binding to ganglioside GM1, a marker of lipid rafts. Therefore, in this work, an *in vitro* model of A549 pulmonary alveolar cells was selected to evaluate the effects of sub-toxic (1.6 μg/ml) and a ten times higher exposure to diesel exhaust particles (DEP) on lipid rafts isolated from a membrane-enriched fraction. The treatment with DEP 1.6 μg/ml proved to be optimal for our study. No significant alterations are observed in the levels of COX-2, HO-1, and also in the release of IL-8 and IL-6. However, it is evident a shift of ACE2 protein into lipid rafts, followed by a significant increase in imADAM17. The localization of ACE2 near GM1 appears to be critical for regulating the entry of SARS-CoV-2. This suggests that living in the most polluted areas induces a pulmonary condition that predisposes to serious outcomes from COVID-19. In our opinion, 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 the use of synthetic analogs of gangliosides capable of inhibiting the viral entry.

P-17-037
Proton motive force generation in *Dehalococcoides mccartyi* strain CBDB1 through intracellular proton uptake during organohalide respiration

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Proton translocation across the cytoplasmic membrane is a fundamental physiological process in all organisms. *Dehalococcoides mccartyi* strains of the strictly anaerobic *Chloroflexi* phylum use H_2 as sole electron donor to respire with organohalides in a process referred to as organohalide respiration (OHR). This process involves the reduction of diverse organohalides, catalyzed by a corrinoid-dependent reductive dehalogenase (RdhA) that is part of a membrane-bound OHR complex, which consists of at least seven proteins: RdhA and its anchoring protein RdhB, hydrogenase subunits HupL and HupS, ferredoxin-like protein HupX, OmeA, and the membrane-bound subunit OmeB, notably lacking quinones and cytochromes. Despite the recognition of its constituents, the precise functional mechanisms remain elusive. Employing a dehalogenase-based enzyme activity assay with deuterium-labelled water across diverse experimental designs, we obtained evidence indicating the substitution of the halogen atom in the halogenated electron acceptor with a proton from the cytoplasm. This observation indicates that the OHR complex is coupling extracellular electron flow through periplasmic subunits to the endergonic translocation of protons from the cytoplasm against the proton gradient to the halogenated electron acceptor across the cytoplasmic membrane. Using computational tools, we

identified two proton-conducting channels within the *in silico* OmeB structure of the OHR complex using AlphaFold2. However, one channel in OmeB is linked to a putative proton-conducting path within the RdhA. Our findings indicate that RdhA and its halogenated substrate serve as both electron and proton acceptors. These insights deepen our understanding of the proton translocation mechanism within the OHR complex of strain CBDB1, and to a better understanding of energy conservation in *Dehalococcoides* strains, unveiling a straightforward quinone-independent, protein-based mode of energy conservation in anaerobes.

P-17-038
Cholesterol interaction and effects on the membrane transporter SLC22A4

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A crucial role of cholesterol in regulation of membrane transporter function recently emerged. Several transporters for amino acids such as SLC1A5, SLC38A9 and SLC7A5 or for organic cations such as SLC22A2 and SLC22A5 were shown to be modulated by cholesterol. The effect of this lipid has been investigated on another member of the SLC22 family: SLC22A4 also known as OCTN1. This transporter is involved in the non-neuronal cholinergic system and in inflammation. The effect of the lipid has been studied by either removing cholesterol from the native membranes by using MβCD or by adding cholesterol to the cholesterol-free SLC22A4 over-expressed in *E. coli*. The transport activity has been detected by measuring the uptake of [3H]-acetylcholine in proteoliposomes. Cholesterol removal impaired, whereas its addition stimulated transport. To predict cholesterol-binding sites of SLC22A4, canonical CRAC/CARC cholesterol-binding motifs were identified [Pochini L et al. (2020) Int J Mol Sci 21(3), 1091]. The homology model of SLC22A4 was constructed using the structure of SLC22A3 as a template. This allowed us to locate the predicted binding sites in the protein structure. Indeed, most of the cholesterol binding sites corresponded to transmembrane segments of the protein. Blind molecular docking confirmed that cholesterol molecules cluster near the identified CRAC/CARC motifs. More accurate docking simulations were then performed to characterize these potential sites in terms of binding energy. Moreover, we have investigated the relationships between cholesterol interaction with OCTN1 and the dependence of transport of organic cations by the membrane potential *in vitro* (unpublished results).

P-17-039
Membranous mechanisms of antibacterial action of Spirulina-derived silver nanoparticles on kanamycin-resistant *Escherichia coli*

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Antibiotic resistance is one of the top global public health problems. In this regard, there is increasing interest in developing new strategic approaches to prevent drug resistance. Green synthesized nanoparticles (NPs) with antibacterial activity can be successfully used as a new approach. A prominent feature of employing NPs for bacterial growth repression is their distinct influence on various biochemical processes. In this work, the effect of Ag-NPs, biosynthesized using cyanobacteria *Spirulina platensis* IBCE S-2, on kanamycin-resistant *Escherichia coli* pARG-25 strain (Microbial Depository Center, NAS, Yerevan, Armenia) was studied. To explain the action mechanisms of *Spirulina*-derived NPs, the H^+-translocating F_oF_1-ATPase activity of *E. coli* membrane vesicles was determined. Kanamycin was used as a positive control. *Spirulina*-derived Ag-NPs exhibited a concentration-dependent inhibitory effect on the growth properties of kanamycin-resistant *E. coli*. Furthermore, these NPs showed minimal inhibitory effect at a concentration of 5 μg/ml. The interaction of Ag-NPs with membrane proteins, such as F_oF_1-ATPase, can cause an alteration in bacterial membrane permeability. The ATPase activity of kanamycin-resistant *E. coli* membrane vesicles was suppressed by *N,N'*-dicyclohexylcarbodiimide (DCCD), an inhibitor of H^+-translocating systems, by ~25%. In the presence of 5 μg/ml *Spirulina*-derived Ag-NPs a further decrease in DCCD-sensitive F_oF_1-ATPase activity up to ~70% was observed. In summary, *Spirulina*-derived Ag-NPs inhibit the growth of kanamycin-resistant *E. coli*; this effect can be the result of the interaction of NPs with H^+-translocating ATPase, leading to changes in bacterial membrane permeability and cell death. The work was supported by the Higher Education and Science Committee of RA, in the frames of the research project № 21T-1F179.

Cellular Organelles

P-18-001
Dual specific mitochondria targeting drug delivery system

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Targeted drug delivery (TDD) that uses anticancer drugs, increases the efficacy and specificity of drug delivery to abnormal cells reducing the off-target toxicity, and thereby improves quality of medical treatment. TDD systems additionally equipped with a permanently fluorescent or turn-on switchable fluorescent dye enable real-time monitoring of drug delivery and drug release, respectively, which is extremely important for controlled cancer treatment. The near-infrared (NIR) dyes absorbing and emitting within 650–900 nm spectral region is advantageous for monitoring in the body due to deeper light penetration, reduced photo-damage to tissue, and minimal interference with background autofluorescence of biomolecules. Essential and very

desirable targets for drug delivery inside the cells are mitochondria, which play a principal role in programmed cell death. In order to target mitochondria, we have synthesized novel fluorescent NIR cyanine dyes incorporating triphenylphosphine (TPP+) group. The specificity of these dyes to mitochondria was evidenced by fluorescence imaging in the example of SKBR3 (HER2 positive) and MD-AMB-231 (HER2 negative) breast cancer cell lines. Furthermore, recently we have designed new NIR dyes with primary hydroxyl group for anticancer drug (as pro-drug) conjugation to study real time monitoring of drug delivery and release. As well to increase dual specificity of the scaffold anion group is attached for antibody conjugation for successful delivery of our pro-drug into mitochondria of desired cancer cells.

P-18-002
Changes in oligomeric structure of muscle fructose 1,6-bisphasphatase influence internal structure of mitochondria by regulating its interaction with mitofilin

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Mitochondria serve as the powerhouses of the cell by producing ATP. The cellular network of these organelles undergoes dynamic changes that are crucial to maintaining the proper quality of these organelles. In cardiac myocytes, appropriate intracellular distribution of mitochondria is essential for sequestration and release of Ca^{2+}, regulating (together with ER cisterns) its local and global concentration. The organelles' trafficking is also responsible for meeting the cellular region-specific energy requirements and for the efficient removal of excess lactate from the cytoplasm. Fructose 1,6-bisphosphatase 2 (Fbp2) is a glyconeogenic enzyme and a multifunctional protein, whose cellular function depends on its oligomeric state – dimeric or tetrameric. Quite recently, we have shown that in the HL-1 cardiomyocyte cells, chemically induced tetramerization of Fbp2 results in a disturbance of tubulin network, significant restriction of mitochondrial trafficking, and intensification of mitophagy. Here, we provide a piece of evidence that this intensification results from disruption of the internal structure of mitochondria. Studies using electron microscopy reveal that tetramerization of Fbp2 leads to alteration in the morphology of mitochondrial cristae. Results of proteomic studies and proximity ligation assays suggest that at the basis of the phenomenon lies the interaction of dimeric Fbp2 with Mic60/mitofilin – the core component for the cristae organizing system (MICOS). Disruption of this interaction by reduction of the cellular pool of available Fbp2 dimers (using chemically induced tetramerization or partial silencing of Fbp2 expression) is sufficient to induce defects in the organization of inner mitochondrial membrane. These results emphasize the significance of oligomerization for regulating the physiological role of Fbp2 in the cell, and suggest involvement of the protein in cardiac diseases originating from mitochondrial defect.

P-18-003
Optogenetically engineered calcium oscillation-mediated DRP1 activation promotes mitochondrial fission and cell death

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Mitochondrial dynamics regulate the quality and morphology of mitochondria. Calcium (Ca^{2+}) plays an important role in regulating mitochondrial function. Here, we investigated the effects of optogenetically engineered Ca^{2+} signaling on mitochondrial dynamics. More specifically, customized illumination conditions could trigger unique Ca^{2+} oscillation waves to trigger specific signaling pathways. In this study, we found that modulating Ca^{2+} oscillations by increasing the light frequency, intensity, and exposure time could drive mitochondria toward the fission state, mitochondrial dysfunction, autophagy, and cell death. Moreover, illumination triggered phosphorylation at the Ser616 residue, but not the Ser637 residue of the mitochondrial fission protein, dynamin-related protein 1 (DRP1), via the activation of Ca^{2+}-dependent kinases, CaMKII, ERK, and CDK1. However, optogenetically engineered Ca^{2+} signaling did not activate calcineurin phosphatase to dephosphorylate DRP1 at Ser637. In addition, light illumination had no effect on the expression levels of the mitochondrial fusion proteins, mitofusin (MFN)-1 and MFN2. Taken together, this study provides an effective and innovative approach to altering Ca^{2+} signaling for controlling mitochondrial fission with a more precise resolution than pharmacological approaches in the temporal dimension.

P-18-004
Influence of statins on mitochondrial biogenesis

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Statins are ones of the most frequently prescribed drugs in the world. They lower cholesterol level and reduce risk of cardiovascular disorders by blocking a key enzyme in the malonate pathway. This pathway is also responsible for the synthesis of coenzyme Q, which is crucial for the proper functioning of the respiratory chain and the production of ATP by mitochondria. Brain cells are most susceptible to mitochondrial disorders due to their high energy requirements. We examined the effects of atorvastatin and simvastatin on mitochondrial biogenesis in *Rattus norvegicus* astrocytes and in the brains of young (3-month-old) and old (20-month-old) animals treated with statins for seven weeks. We used qPCR to determine mitochondrial copy number and examined the enzyme activities of citrate synthase (CS) and lactate dehydrogenase (LDH). Our studies showed that both statins stimulated mitochondrial biogenesis in astrocytes but not in brains of young animals. Mitochondrial DNA copy numbers were elevated by ~20% in astrocytes treated with both statins. Interestingly, in the brains of old statin-treated animals we observed an effect opposite to that observed in astrocyte cells. In old atorvastatin-treated rats, mitochondrial DNA copy number in the brain was reduced by ~26% compared to saline-treated control animals. No changes in the activity of CS and LDH enzymes were observed in any of the experiments. To explain this phenomenon, we will turn to mitochondrial staining experiments

in astrocytes. Interestingly, the reduction in the number of mitochondrial DNA copies in the brains of old animals may indicate that the effect of statins on mitochondrial biogenesis is age-dependent. This work was funded by Polish National Science Centre project 2020/37/B/NZ1/01188.

P-18-005
Understanding nucleolar stress management through the lens of CHIP ubiquitin ligase

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Our research explores the complex dynamics of the heat shock response, explicitly focusing on the role of the nucleolus – a unique nuclear biomolecular condensate recognized as a refuge for damaged proteins, aiding in their post-stress refolding. Central to our study is ubiquitin ligase CHIP, which moderates the cellular balance of protein folding and degradation in tandem with chaperones. We uncovered the nuanced relationship between CHIP, HSP70, and nucleophosmin (NPM1) in maintaining nucleolar proteostasis. Following heat shock, CHIP translocates to the nucleoli, remaining operative within its granular portion to fortify the NPM1 and HSP70 complex. However, excessive CHIP, much like HSP70 inhibition, impacts the availability of NPM1 and HSP70 to misfolded proteins, subsequently obstructing their elimination. Moreover, we found that CHIP depletion can reshape nucleoli morphology, obstructing post-stress ribosome formation. Our findings elucidate a new mechanism where CHIP balances nucleolar proteostasis capacity and ribosome assembly. This process, steered by CHIP-regulated expression, advances our understanding of cellular stress responses. *The authors marked with an asterisk equally contributed to the work.

P-18-006
Vps27 is essential for mitochondrial respiration and lifespan extension in Sit4 deficient cells

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Sit4 is the catalytic subunit of a PP2A-like Ser/Thr protein phosphatase implicated in the regulation of several cellular processes in *Saccharomyces cerevisiae*, such as cell cycle, carbohydrate and lipid metabolism, nutrient signalling, vacuolar and mitochondrial function, and chronological lifespan. In this study, we performed a proteomic analysis of vacuolar membranes from wild type and *sit4*Δ cells. Our results revealed changes in *sit4*Δ vacuolar proteins that are mostly associated with late endosome to vacuole transport, vacuole fusion, microautophagy, polyphosphate metabolism and metal ion transport. Notably, the levels of the endosomal sorting complex required for transport-0 complex (Vps27-Hse1) increased on *sit4*Δ vacuolar membranes and *SIT4* showed a negative genetic interaction with *VPS27*, as *sit4*Δ*vps27*Δ double mutants exhibited a shortened lifespan. The analysis of protein trafficking and vacuolar sorting pathways showed that Vps27 did not increase *sit4*Δ lifespan by improving autophagy or the cytoplasm to vacuole targeting (Cvt) and carboxypeptidase Y (CPY) pathways. However, Vps27 was required for iron homeostasis and mitochondrial derepression in *SIT4* deleted cells, as *sit4*Δ*vps27*Δ mutants exhibited high iron levels and a very low oxygen consumption rate. These results suggest a cross-talk between Sit4 and Vps27 in the regulation of mitochondrial fitness and lifespan extension in yeast. This work was funded by national funds through FCT – Fundação para a Ciência e a Tecnologia, I.P., under the project UIDB/04293/2020. T.S.M. (SFRH/BD/136996/2018) and C.P. (IF/00889/2015) were supported by FCT.

P-18-007
Assessment of mitochondrial network morphology as a tool for fast estimation of potential mitotoxicity of experimental treatments and expression of recombinant proteins

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The mitochondrial network undergoes constant morphological rearrangements, which affect mitochondrial function and turnover. It is an important part of cellular adaptation to metabolic changes, as well as a way to offset mitochondrial dysfunctions. We used the analysis of mitochondrial network morphology for fast screening for potential mitotoxicity of investigated compounds in bronchial epithelial cells BEAS-2B and lung cancer cell line A549. We also applied this approach in HeLa cells expressing split GFP based reporter fusion proteins directed into mitochondria, in order to select the constructs possibly neutral for mitochondrial physiology. The analysis was based on the confocal images of mitochondrial network visualized with different fluorescent probes, in viable as well as in fixed cells. We applied openly available ImageJ plugins to determine the degree of mitochondrial network fragmentation, the complexity of network structure, and the average thickness of mitochondrial tubules. There was a clear variability in mitochondrial morphology between the investigated cell lines. Thus, for each cell type, we determined the profile of mitochondrial morphology changes in response to insults of known effects on mitochondrial physiology, such as starvation or mitochondrial depolarization. Within the obtained landscape of mitochondrial shape descriptors, we analysed the influence of the tested experimental conditions on mitochondria. Combining the high-speed spinning disc confocal microscopy with semiautomated image analysis allowed for fast screening of multiple conditions without requiring a high number of cells. The study was supported by Polish National Science Center grants no. 2019/34/E/NZ1/00321 and 2021/43/B/NZ5/01646.

P-18-008
A fiber type-specific response to resveratrol impinges on retrograde communication regulating mitochondrial biogenesis in old rat soleus muscle

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Skeletal muscle (SM) performs its function, obtaining the energy for muscle fiber contraction through ATP hydrolysis, carried out by the myosin adenosine triphosphatase (ATPase) activity. There are three distinct fiber types: fast-glycolytic (type IIa), fast-oxidative glycolytic (type IIb), and slow-oxidative (type I). The slow-oxidative fiber is mostly present in slow muscles such as the soleus (Sol). Aging affects several functional features of SM, as well as Sol, at level of fibers, substrate availability, phosphocreatine repletion, and especially number and functionality of mitochondria. The polyphenol resveratrol (RSV), through promotion of mitochondrial biogenesis, can counteract the age-related loss of muscle mass and strength/function in SM. This work aimed to analyze the effects of a six-week administration of RSV on mitochondrial biogenesis, quality control and dynamics in the Sol of 27 month-old male rats. In RSV rats no change in the SIRT1, a decrease in the PGC-1α and PRXIII proteins were found. A novel correlation between protein amounts of PGC-1α and PRXIII was demonstrated. In RSV rats no change in the mtDNA relative content, an increase in the frequency of the oxidized base OH8dG in mtDNA and an increase in the DRP1 protein were detected. A correlation between PGC-1α and the frequency of OH8dG, as well as between PRXIII and the frequency of OH8dG were also found. Such results suggest that a short-term RSV administration in aged Sol impinged on the oxidative fiber-specific retrograde communication from mitochondria to the nucleus, influencing the expression of PGC-1α, PRXIII and DRP1. The absence of mitochondrial biogenesis stimulation by RSV did not counteract the age-related mitochondrial decline, oxidative stress and enhanced mtDNA oxidative damage. Studying the potential differential responses induced by RSV in both oxidative and glycolytic SM could allow a more reliable translation into human applications.

P-18-009
Defeating ischemia–reperfusion injury using selected spiropiperidine compounds

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Various forms of programmed cell death exist, among them apoptosis facilitated by mitochondrial permeability transition. This process involves specific alterations in the inner mitochondrial membrane (IMM), resulting in the influx of solutes into the mitochondrial matrix and the loss of mitochondrial structural and functional integrity. Central to this phenomenon is the mitochondrial permeability transition pore (mPTP), a complex comprising proteins that create a channel within the IMM. The functional state and formation mechanism of mPTP are regulated by both negative and positive modulators. While numerous components and regulators of mPTP have been identified, only a handful exhibit specific interactions, and none have been clinically adopted for cardioprotective or nephroprotective purposes in ischemia–reperfusion injury (IRI) treatment. Current strategies predominantly rely on broad-spectrum molecular therapies, showing modest efficacy in organ protection post-reperfusion, while clinical trials targeting specific drug candidates have yielded disappointing results. Through a blend of molecular modeling and chemical innovation, we have uncovered potent spiropiperidine-based compounds designed to target mPTP, bolstering cellular resilience following reperfusion. Validation through meticulous *in vitro* experiments on HeLa and human renal cells signals a breakthrough. Next step of our journey will be in vivo investigations, charting a promising path forward. *The authors marked with an asterisk equally contributed to the work.

P-18-010
VDAC expression and gene regulation for mitochondrial function

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Voltage dependent anion-selective channel (VDAC) are a family of pore-forming protein embedded in mitochondrial outer membrane regulating the exchange of metabolites with the cytosol. Three isoforms have been evolutionary originated in mammals sharing high similarity in sequence and structure. However, much evidence suggests different biological roles in normal and pathological conditions for each isoform (1). Bioinformatics approaches highlighted regulatory signatures of promoter core, transcription factors binding sites, epigenetic features of VDACs gene, which were validated experimentally. All three VDAC genes share common molecular pathways involved in controlling cell growth, proliferation, differentiation, apoptosis and bioenergetics metabolism. Moreover, we found specialized and differentiated mechanisms of gene regulation for the three isoform genes. VDAC1 is the essential gene for mitochondrial functional adaptation in metabolic stress condition confirming its crucial role in cell life and death balance. VDAC2 has a pivotal role in the regulation of development of specialized tissues and the organogenesis process mainly related to nervous system genesis. VDAC3 is also involved in organogenesis, and in particular in the development of germinal tissues and sex determination (2). The methylation profile of VDAC genes promoter regions revealed a mechanism of gene expression control inhibiting transcription factors activation (unpublished).Investigation on VDACs genes expression and regulatory mechanisms revealed interesting implication that justify the evolution of three specialized VDAC isoforms. References: 1. De Pinto V (2021) Biomolecules 11, 107. 2. Zinghirino F et al. (2021) Front Physiol 12, 708695.

P-18-011
TCA cycle in Trypanosomatidae: isocitrate dehydrogenases

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Isocitrate dehydrogenase (IDH) is an enzyme converting isocitrate to α-ketoglutarate in the canonical tricarboxylic acid (TCA) cycle. There are three different types of IDH documented in eukaryotes. Our study points out the complex evolutionary history of IDHs across kinetoplastids, where the common ancestor of Trypanosomatidae and Bodonidae was equipped with two isoforms of the IDH enzyme: the $NADP^+$-dependent IDH1 with possibly dual localization in the cytosol and mitochondrion and $NADP^+$-dependent mitochondrial IDH2. In the extant trypanosomatids, IDH1 is present only in a few species suggesting that it was lost upon separation of *Trypanosoma* spp. and replaced by the mainly $NADP^+$-dependent cytosolic IDH3 of bacterial origin in all the derived lineages. In this study, we experimentally demonstrate that the omnipresent IDH2 has a dual localization in both mitochondrion and cytosol in at least four species that possess only this isoform. The apparent lack of the NAD^+-dependent IDH activity in trypanosomatid mitochondrion provides further support to the existence of the non-canonical TCA cycle across trypanosomatids and the bidirectional activity of IDH3 when operating with $NADP^+$ cofactor instead of NAD^+. This observation can be extended to all 17 species analyzed in this study, except for *Leishmania mexicana* which showed only low IDH activity in the cytosol. The variability in isocitrate oxidation capacity among species may reflect the distinct metabolic strategies and needs for reduced cofactors in particular environments.

P-18-012
Remodeling of mitochondrial function in human lung cells affected by nano-plastics

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Plastics are integral to contemporary living. The escalating volume of plastic waste poses an environmental challenge. Waste degrades into micro- or nano-particles (MPs or NPs). Notably, NPs have been identified globally in both urban and remote atmospheres. This distribution underscores the global nature of NPs-related air pollution. Inhaled NPs has been associated with health issues (asthma, allergies). Furthermore, inhaled NPs exhibit the capacity to penetrate cellular barriers. This study represents an innovative exploration into the impact of inhaled plastic on human health. Specifically, our focus is on assessing the stress induced by NPs on mitochondria, the central hub of cellular metabolism pivotal for adapting to stress conditions. Our research aims to elucidate the effects of NPs pollution on mitochondrial function, the activation or modulation of mitochondrial stress signalling pathways. Since lungs are exposed as first, we are utilizing bronchial epithelial cells (BEAS-2B and A459)– healthy and cancerous lines, respectively. Our experimental model involves long-term exposure to low doses of NPs. This model simulates the conditions of everyday exposure to NPs in the air. Our findings indicate that NPs accumulates in/near mitochondria during short-term exposure and within lysosomes during long exposure (weeks). Demonstrating alterations in mitochondrial dynamics and physiology, our research investigates the fate of NPs in the cell over time. Moreover, the comparative analysis of cancerous and healthy lines provides valuable insights into the fundamental biology of these cells, considering their distinct metabolic. This comprehensive investigation contributes to our understanding of the intricate interplay between NPs pollution, mitochondrial adaptation, and cellular responses, thereby shedding light on potential implications for human health. The project is financed by OPUS 22 – 2021/43/B/NZ5/01646.

P-18-013
Autophagy beyond degradation: drugs impairing the autophagic flux alters the release of extracellular vesicles

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Extracellular vescicles (EVs) are membrane enclosed nanostructures released by all cell types and retrieved in every fluid of the body. When the degradative capacity of the autophagic/lysosome pathway is impaired by the administration of two commonly used autophagy inhibitors, bafilomycin A1 (Baf A1) and chloroquine (CQ), EV release is altered in a drug-dependent manner. BafA1 and CQ both induce the release of EVs enriched in autophagy markers in several cell types, highlighting a general and not cell type-specific feature. Interestingly only BafA1 induced the release of a higher number of small EVs, i.e. about 4 times higher. Density gradient ultracentrifugation showed that Baf A1 induced the release of less dense EVs. Their average size was smaller, as determined by nanoparticle tracking analysis. The lipid composition of EVs was consistent with an enrichment of the general lipid content in BafA1 treated samples compared to controls, according to the LC/MS analysis of the EV lipid content. We concluded that both drugs impaired autophagosome-lysosome fusion by raising lysosomal pH, prompting the release of EVs carrying autophagy markers, but only Baf A1, that inhibits the ER calcium pump SERCA, was able to increase the number of released EVs, suggesting that ER calcium released may play a role in the event. So, although they are commonly used with the same purpose to inhibit autophagy, these drugs have different effect on EV secretion. EVs carrying autophagic markers could be retrieved in EVs from plasma of healthy subjects. The role of these EVs in blood pathological conditions, characterized by impairment of the lysosomal/autophagic pathway, confirmed that this type of EVs is present in biological fluids and may be possibly useful as biomarker of lysosomal dysfunction. This work has been funded by the European Union – NextGenerationEU under the Italian Ministry of University and Research (MUR) National Innovation Ecosystem grant ECS00000041 – VITALITY (to Carla Emiliani).

P-18-014
Endothelial cell contacts remodelling as an element of cellular response to proinflammatory factor

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Excessive stimulation of endothelium with proinflammatory stimuli [lipopolysaccharide (LPS)] may reduce cell viability. However, a recent study indicates potentially adaptive mechanisms which prevent endothelial cell damage and death from stress-inducing stimuli leading to a gradual activation of mitochondrial biogenesis and substantial changes in mitochondrial network organisation. In human aortic endothelial cells (HAECs) exposed to LPS transient changes in mitochondrial network organisation precede cell adaptation to this stimulus. Mitochondria localized closer to the perinuclear region and more fragmented (fission) after 6 h treatment partially resembled control cells at the end of the experiment (after 24 h) [1]. Our recent findings show that long-lasting treatment of human umbilical vein endothelial cells (HUVEC) with TNFα substantially increases the number of mitochondria localized in close vicinity of cell boundaries or even directly on the intercellular tubular connections. Moreover, in endothelial cells exposed to harmful stimuli, the number of intercellular connections visible as intensely bright green clusters of the cytoskeleton is more abundant. We believe that the formation of cell-to-cell communication routes may indicate an activation of the vasculoprotective mechanism in response to the mild-stress condition (stimulation with pro-inflammatory agents like LPS or TNFα). Although the mitochondrial network remodelling was previously confirmed, our goal was to investigate the importance of intercellular junction formation as an element of cellular reaction to proinflammatory factors, which could contribute to the protective response of vasculature against pathological stimuli. Reference: 1. Stępińska O, Dymkowska D et al. (2022) Int J Biochem Cell Biol. 151, 106292. doi: 10.1016/j.biocel.2022.106292. This work was supported by the National Science Centre, Poland. Grant no. 2021/41/B/NZ5/03599.

P-18-015
How to build a secretory cell: lessons from endometrial stromal cell decidualization

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Virtually all biological functions rely on specialized cells secreting proteins, constitutively or in response to external stimuli. The decidualization of endometrial stromal cells (EnSCs) is a clear example of inducible secretory differentiation: upon hormonal stimulation, these cells transform into protein factories, devoted to the production and secretion of all the factors needed for a successful embryo implantation[1]. During decidualization, EnSCs undergo radical changes in their transcriptome[2], which result in a coordinated enlargement of secretory pathway organelles and a dramatic increase in the secretory capacity[1]. We recently demonstrated that the organelle reshaping during decidualization is not limited to secretory pathway organelles, and the mitochondrial network is completely reorganized during the differentiation program. Indeed, key regulators of mitochondrial biogenesis are activated early in decidualization, resulting in a rapid induction of mitochondrial gene expression from both the nuclear and the mitochondrial genomes. This results in a significant increase of mitochondrial mass, and in the enhancement of the mitochondrial respiratory metabolism. At the same time, the architecture of the mitochondrial network changes during EnSC decidualization, as mitochondria elongate and establish more contacts with the endoplasmic reticulum. These results likely underlie the tight connection between the increased secretory activity and the consequent changes in energy requirements during EnSC decidualization: radical metabolic rewiring is clearly needed to achieve an efficient secretory phenotype. Together, our results provide novel, interesting insights into the process of EnSC decidualization, which is not only a beautiful model to investigate the physiology of protein synthesis and secretion, but also a crucial aspect of human reproductive health. References: 1. Anelli T et al. (2022) Traffic 23, 4–20. 2. Pittari D, Dalla Torre M et al. (2022) Front Cell Dev Biol 13, 10, 986997.

P-18-016
N-terminal signal peptides on mature protein transport

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Intracellular organelles have complex transport systems and unique enzyme groups. It is critical to understand the phenomenon and function maintained in each organelle. Protein synthesis in most eukaryotic cells is mediated by N-terminal signal peptide (SP). The early-synthesized SP identified by signal recognition particle are inserted into the endoplasmic reticulum (ER) through the translocons. This process initiates the exocytosis pathway for intracellular protein transportation. After the ER insertion, SPs are typically cleaved from the mature protein. However, due to a wide diversity in amino acid sequences of SPs, it may be inadequate to consider SPs are only involved in the insertion into the ER. In addition, proteins harboring SPs, including those lacking other distinct localization signals, undergo translocation to various organelles after ER localization, resulting in a wide range of protein final destinations. Considering the possibility that SP may be involved in mature protein transportation after SP cleavage, in addition to ER insertion, we aimed to evaluate the effects of SPs on intracellular trafficking of proteins. In this study, we fused human origin SP and viral origin SP each with green fluorescent protein (GFP) expressing vectors, later expressed in HEK293 cells. Subcellular localization was then evaluated based on GFP fluorescence through observation. As a result, a difference in GFP emitting locations based on changing various SP regions in SP-GFP fusion proteins was observed. GFP fluorescence showed to localized (1) in nucleus and cytoplasm, (2) only in ER. Therefore, our inquiry suggests that SP regions can potentially influence mature protein transport. We will present the correlation between the physicochemical properties of SP sequences and their respective subcellular localizations.

P-18-017
Nuclear lipid droplets exit to the cytoplasm: a novel mechanism of non-nuclear pore-dependent material exchange

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In cellular biology, the extrusion of large molecular complexes from the nucleus has consistently represented a considerable challenge. In this investigation, we reveal a hitherto unexplored mechanism facilitating the efflux of nuclear lipid droplets (nLDs) from the nucleus directly into the cytoplasm, a process integral to comprehending cellular processes and nuclear dynamics. Through high-throughput sequencing, immunoprecipitation, and immunofluorescence experimentation, we demonstrated that the type I nucleoplasmic reticulum engages with nLDs, enabling the transfer of the dynamic protein Rab8a to the nLDs and fostering their translocation to the nuclear membrane. Phosphorylation of LMNA/C at the S392 site, adjacent to the nLD-nuclear membrane contact site, induces local depolymerization of lamins, creating an aperture that enables gradual nuclear exit of nLDs. Following this, nLDs, carrying DNA fragments, are released into the cytoplasm through budding and subsequently undergo autophagy-mediated degradation. Furthermore, we demonstrate that RhoA-PKC signaling enhances nLD movement and LMNA/C phosphorylation, while its inhibition impedes nuclear nLD efflux. Our groundbreaking investigation illuminates non-nuclear pore-dependent mechanisms of material exchange and offers fresh perspectives on nuclear metabolic pathways.

P-18-018
Exposure to vinclozolin leads to oxidative stress and brain mitochondrial dysfunction

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Vinclozolin (VCZ) is a widely utilized fungicide predominantly employed in agriculture for fruit and wine production as a pesticide. The effects of VCZ exposure on many organs have been extensively studied, but nothing is known about how it affects the brain. This study looked into how exposure to VCZ affected brain tissue's oxidative stress and mitochondrial dysfunction. For 28 days, oral gavage of VCZ dissolved in saline (100 mg/kg) was administered to C57BL/6 mice. In comparison to control animals (CTR), VCZ treatment altered the mRNA expression levels of mitochondrial markers such as Sirt1, Sirt3, PGC-1α, TFAM, Nrf1, VDAC-1, and Cyt c in brain tissue. The results also revealed significant biochemical alterations such as an imbalance between antioxidant and oxidant enzymes; specifically, the treatment of VCZ decreased glutathione (GSH) levels, superoxide dismutase (SOD) and catalase (CAT) activities, while raising lipid peroxidation and total antioxidant capacity (T-AOC) levels. Furthermore, DNA oxidation (higher PARP-1 immunostaining) and apoptosis (higher TUNEL + cells, higher Bax mRNA expression, and lower Bcl-2 levels) were seen in the brain tissues from the VCZ group. The accumulation of PINK1 and Parkin in mitochondria was associated with an enhanced mitophagic pathway, as demonstrated by western blot and immunohistochemistry studies. Furthermore, the upregulation of the autophagic pathway was correlated with the enhanced expression and colocalization of neurotrophic factors LC3 with GFAP and Neun. VCZ acts on the modulation of processes that ensure mitochondrial homeostasis, as well as biogenesis, fusion, fission and mitophagy. Overall, as this study is now published, long-term exposure to VCZ was shown to exacerbate oxidative stress and impair mitochondrial homeostasis in brain regions.

P-18-019
Unlocking the secrets of iron: ferritinophagy and ferroptosis in skin cells

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Ferritinophagy is a crucial process in maintaining of the iron balance by selective degradation of ferritin, the primary intracellular iron storage protein. Recently, the attention has been focused on the understanding of its relationship with ferroptosis, which is a form of cell death driven by iron-dependent lipid peroxidation. Understanding ferritinophagy's role in cellular responses to iron perturbations offers insights into different sensitivity of cells to a given type of death and therefore, into novel treatment strategies, particularly in cancer therapy. The aim of the presented work was to evaluate the sensitivity or resistance of tested cell lines to ferroptosis, by exploring the role of the ferritinophagy in this process. This relationship is of key importance in the treatment of cancer. We tested sensitivity or resistance of the HaCaT and 1205Lu cell lines by studying *PROM 2, NCOA4, ACSL4* gene expression, the level of lipid oxidation, database analysis, and literature. The *PROM2* is expressed solely in cell presenting resistance to ferroptosis, *NCOA4* is the marker for autophagy and *ACSL4* is the marker of lipid peroxidation. Normal keratinocyte cells, HaCaT, showed resistance to erastin-induced ferroptosis, by activating the expression of *PROM2*, increasing the expression of *NCOA4* and decreasing of *ACSL4*. When 1205Lu cells, melanoma cells from the primary tumor, were sensitive to ferroptosis, because we observed lack of expression of *PROM2* or *NCOA4*, increase in *ACSL4* expression, and visible lipid oxidation were observed. This work was supported by grants 02/040/BK_24/1056 from Silesian University of Technology in Gliwice, Poland.

Redox Biochemistry

P-19-001

Abstract withdrawn.

P-19-002
Biochemical characterization and structural insights into *Mycobacterium tuberculosis* dihydroorotate dehydrogenase as innovative therapeutic target

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Over the years, the pyrimidine biosynthesis pathway (PBP) has been studied as a potential source of therapeutic targets due to its involvement in crucial cellular mechanisms such as DNA and RNA synthesis. Among the six enzymes involved in the *de novo* PBP, dihydroorotate dehydrogenase (DHODH) has been identified as a promising pharmaceutical target for the treatment of several disorders, including infective diseases[1]. This encompasses both more conventional pathogen-directed therapies (PDT) and innovative host-directed therapies (HDT). Given the strong interest of our laboratory in antitubercular research pipeline, we directed our focus towards *Mycobacterium tuberculosis* DHODH (*Mt*DHODH). While *human* DHODH (*h*DHODH) has been extensively studied as a crucial player for maintaining the fitness of the host, *Mt*DHODH still lacks biochemical and structural insights. In the present study, we propose the first biochemical characterization and the original crystal structure of the full protein that enabled us to systematically screen our *in-house* chemical library, leading to the identification of the first selective *Mt*DHODH inhibitor, setting the stage for further development in the *hit-to-lead* process[2]. Additionally, since *Mycobacterium tuberculosis* is an obligate intracellular pathogen, it heavily relies on host nucleotides for its survival and replication. Therefore, in the context of MTB-infected-macrophages, we are currently investigating the effect of blocking pyrimidine biosynthesis in a dual *scenario* targeting both *Mt*DHODH and *h*DHODH, simultaneously. Our findings demonstrate that a plant-derived compound, previously reported to be active on *h*DHODH[3], also exhibits activity on *Mt*DHODH, suggesting its potential use in a balanced combination of PTD and HDT, which may result in a severe nucleotide deficiency and pyrimidine starvation. References: 1. Sainas S *et al.* (2022) *J Med Chem* 65, 12701–12724. 2. Alberti M *et al.* (2023) *FEBS Lett* 597, 2119–2132. [3]Appendino G *et al.* (2004) *J Nat Prod* 67, 2108–2110.

P-19-003
Hydroxyapatite-based nanoparticles modulate the oxidative stress and DNA damage of bone cells

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Hydroxyapatite (HA) is a bioceramic material – based on calcium and phosphate and that can be doped with different ions to tune its biological activity – and, together with collagen, is the main component of our bones. Recently, it was reported that some HA-based particles can promote genotoxicity, nevertheless little is known about the effect of the bioconjugation of HA with other polymers – as happens in the bone where HA nanoparticles are present on collagen fibers – especially in combination with different doping ions. In the present contribution, we report the biological activity of HA-based nanoparticles doped with different ions – including magnesium, iron, and copper – and bioconjugated with chitosan, a natural polymer that shares some peculiar molecular properties with collagen. The biochemical activity of the resulting nanoparticles towards osteoblasts-like cells (MG63 and HOBIT, Human OsteoBlast-like Initial Transfectant) and osteocytes (MLOY4) was investigated by considering the cell viability, proliferation, and production of reactive oxygen species (ROS) by using metabolic assays and a general oxidative stress indicator. On the other hand, confocal microscopy images were acquired to analyze cell morphology and detect possible γH2A.X foci and DNA:RNA hybrids corresponding to DNA damage. These aspects were also deepened by COMET assay and western blot analyses of H2A.X and PRXSO3 expression. These latter tests indicated that the presence of iron within the HA lattice stimulates ROS production promoting DNA damage and this effect is enhanced by the presence of chitosan suggesting that it favors the nanoparticles' internalization by cells and modulates their biological activity. Considering the overall results, HA-based nanoparticles can modulate oxidative stress and DNA damage and these aspects should be considered when these components are used to devise materials intended for biological applications.

P-19-004
Novel clostridial formate dehydrogenases for carbon dioxide valorization

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Atmospheric carbon dioxide (CO_2) concentration has been increasing since pre-industrial times, having a negative impact on the environment. For this reason, the development of strategies to reduce and even valorize carbon dioxide is a key challenge for our society. Since CO_2 has always been an abundant molecule in the atmosphere, Nature developed several effective pathways for its fixation. An important role in this kind of processes is played by metal-dependent formate dehydrogenases. In particular, these class I enzymes are able to catalyze the reversible two-electron reduction of CO_2 into formate which is considered an alternative industrial building block to fossil fuel-based molecules. The biodiversity among these enzymes is huge; thus, it is important to characterize as many as possible. In this work novel clostridial metal-dependent formate dehydrogenases are explored as biocatalyst for CO_2 reduction. Formate dehydrogenases from *Clostridium drakei* and *Clostridium scatologenes* were identified based on their sequence similarity with highly efficient formate dehydrogenases from *C. carboxidivorans*[1] and *C. ljungdahlii*[2]. The enzymes were heterologously expressed in *E. coli*, purified with affinity chromatography, characterized and tested on their ability to reduce CO_2 and to interact with metal oxides. These results contribute to increase the palette of formate dehydrogenases involved in CO_2 conversion, with the prospect of improving their performances and making their application closer and closer. Previously published in: 1. Alissandratos et al. (2013) Appl Environ Microbiol 79, 741–744. 2. Singh et al. (2018) ACS Catal 8, 12, 11085–11093. This work was produced during and with the support of the Italian national interuniversity PhD course in Sustainable Development and Climate Change and the project NODES which received funding from the MUR–M4C2 1.5 of PNRR funded by the European Union-NextGenerationEU (Grant Agreement No. ECS00000036).

P-19-005
New horizons in the investigation of oxygen-resistant [FeFe]-hydrogenases

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Bacterial [FeFe]-hydrogenases represent promising biocatalysts for high efficiency production or consumption of hydrogen in bio-electrolysers or bio-fuel cells. So far, the [FeFe] hydrogenase from *Clostridium beijerinckii*, named CbA5H, seems to be the only one with a self-protection mechanism against irreversible damage caused by oxygen and other oxidants[1,2]. Herein two new [FeFe]-hydrogenases, not previously studied, were selected through sequence alignment based on conserved regions and three key amino acid substitutions identified so far. The new enzymes were successfully produced in *E. coli* through co-expression of maturases, followed by aerobic purification and characterization. Both hydrogenases demonstrated consistent production of molecular hydrogen, as detected by gas chromatography, highlighting the enzyme's tolerance to O_2. Moreover, spectroscopic measurements showed, upon exposure to oxidants, such as the aerobic atmosphere, thionine or DCIP, the characteristic signal, called H_{inact}. In parallel, the QM/MM and molecular dynamics approach provided "direct" information that led to model the structural rearrangements related to the mechanism. Identifying and characterizing novel [FeFe]-hydrogenases presents an opportunity to fill the existing gaps in information about these enzymes. Moreover, those biocatalysts could address the growing demand for producing H_2 at high rates and low costs. These results contribute to the expansion of a particularly robust enzyme library, with the potential to identify new critical residues responsible for this distinctive trait. References: 1. Winkler M et al. (2021) *Nat Commun* 12, 756. 2. Morra S et al. (2016) *Biochemistry*, 55, 42:5897–5900. The work is produced while attending the PhD SDC at IUSS Pavia and funded by the European Union NextgenerationEU – PNRR. DM118/2023-M4C1-Inv. 3.4 – Transizioni digitali e ambientali and the 2022 PRIN project "MORF: Molecular determinants of Oxygen Resistance in a unique [FeFe]-hydrogenase".

P-19-006
Studying redox biology of cancer by using patient-derived organoids (PDOs) and compartment-specific genetically-encoded redox biosensors

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Redox regulation in tumor cells plays a significant role in drug resistance and cancer aggressiveness. The development of genetically-encoded redox biosensors has represented a great improvement in the redox field as they enable the analysis of the production of redox species in specific subcellular compartments in real-time, both in living cells and in whole organisms. We have applied this technology to the preclinical model of patient-derived organoids (PDOs). Organoids are three-dimensional structures derived from stem cells that closely recapitulate tissue architecture and cellular composition and that can be maintained long-term while retaining genetic stability. The cell phenotypic heterogeneity in PDOs, determined by different transcriptional landscapes, allows the study of differential drug response within PDOs and inter-PDOs. We have transduced PDOs from tumor and healthy samples with lentivirus carrying genetically-encoded redox sensors for hydrogen peroxide (H_2O_2, roGFP2-Orp1) and for the glutathione redox potential (E_{GSH}; Grx1-roGFP2) in different subcellular compartments. We have also generated chemo-genetic models of PDOs expressing DAAO-Hyper7, which consists in a D-amino acid oxidase (DAAO, which catalyses the oxidative deamination of exclusively D-amino acids with the production of H_2O_2) and Hyper7, a sensible H_2O_2 biosensor. This chemo-genetic tool allows to induce H_2O_2 production in specific subcellular compartments whilst simultaneously providing a ratiometric fluorescent readout for H_2O_2 levels. We have

studied oxidant production by different cell populations within the PDOs and oxidant production by PDOs treated with standard chemotherapy and several common anti-tumor drugs. Our results show that expression of redox sensors in PDO is a promising tool to study the role of redox species in cancer progression and drug resistance.

P-19-007
Investigating the redox state of stress-induced pancreatic tumor cells

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Cancer cells can reprogram their metabolism supporting tumor formation and propagation, synergistically exploiting increased reactive oxygen species (ROS) production along with overexpression of ROS scavenger mechanisms. This leads to activation of several pro-tumorigenic cell signaling and survival pathways and altered intercellular signaling. Conversely, many chemo- and radio-therapy approaches are based, directly or indirectly, on ROS production to exert their cytotoxic activity. Redox status of the cell is thus a pivotal player in steering tumor cell metabolism toward proliferation or apoptosis. A clear understanding of its dysregulation is thus crucial in determining key targets for improving drug efficacy by means of combined therapy. In this view, both expression and post-translational modification of enzymes involved in redox metabolism must be considered. On these premises, we selected response of two pancreatic ductal adenocarcinoma cell lines differing to various stress inductors, to better define the antioxidant defenses put in place. Cell lines were first assessed for viability and ROS production after exposure to different concentrations of stress inductors. Those conditions leading to effective cell response (i.e. activation of anti-oxidant defense pathways with measurable variation in cell redox metabolism) were further investigated at different levels. A panel of genes involved in redox balance was assessed for their expression levels in stress-induced and not stress-induced cells by RT-qPCR. Catalytic activity of enzymes involved in redox status control was evaluated, along with a quali-quantitative determination of thiol, ROS, and carbonyl derivatives. Overall, this comprehensive investigation of metabolic reprogramming in stress-induced cells highlights the most relevant players in detoxification and chemoresistance and is useful as a general strategy to develop more effective combined therapies.

P-19-008
$1,25(OH)_2D_3$ treatment alleviated the ethanol induced kidney damage

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Chronic ethanol (EtOH) consumption causes structural and functional changes in liver and kidneys. Oxidative stress, acetaldehyde (AA) accumulation, AA-induced advanced glycation end products (AGEs) and inflammation play a role in EtOH toxicity. $1,25(OH)_2D_3$ has antioxidant, antiinflammatory and antiglycation abilities and exerts renoprotective properties. Contrarily, vitamin D deficiency (VDD) may enhance EtOH toxicity in kidneys as in the liver. However, there is no knowledge about the effect of both $1,25(OH)_2D_3$ treatment and VDD on EtOH-induced renal toxicity. In this study, Wistar rats were fed on VDD diet for 12 weeks. EtOH (5–20%) was applied in drinking water for the last 8 weeks. One group of rats were injected with $1,25(OH)_2D_3$ (5 g/kg; twice a week) in this period. Renal reactive oxygen species (ROS), thiobarbituric acid reactive susbtance (TBARS), advanced oxidation protein products (AOPP), AGEs and glutathione (GSH) levels, superoxide dismutase (SOD), GSH-peroxidase (GSH-Px) and myeloperoxidase (MPO) activities were examined. Serum urea and creatinine levels increased in EtOH group. Renal ROS, TBARS levels and SOD activity also elevated. GSH levels and GSH-Px activity diminished in EtOH-rats. Renal function markers in serum and renal ROS, TBARS levels and SOD activity decreased, but GSH levels and GSH-PX activity increased in EtOH rats by $1,25(OH)_2D_3$ treatment. On the other hand, in EtOH-applied VDD group, significant increases in renal TBARS, GSH levels were detected, but other parameters remained unchanged as compared to ETOH group. $1,25(OH)_2D_3$ ameliorated EtOH-induced histopathological findings such as glomerular degeneration and necrobiotic cells. In addition, there was no change in histological appearance between VDD + EtOH and EtOH groups. Our results indicate that $1,25(OH)_2D_3$ treatment alleviated EtOH-induced renal toxicity by modulating prooxidant-antioxidant balance. Moreover, VDD diet did not cause an additive effect on EtOH-induced renal toxicity.

P-19-009
Modulation of human VDAC3 expression under oxidative stress conditions in HeLa cells

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VDAC (voltage dependent anion channel) is a family of pore forming proteins located in the outer mitochondrial membrane of all eukaryotes. They mediate metabolites exchange across the organelle and also participate in a wide range of pathways thanks to the interaction with cytosolic enzymes and both pro-apoptotic and anti-apoptotic factors [1]. Among the three mammal isoforms named VDAC1, VDAC2 and VDAC3, knowledge about isoform 3 is limited and its biophysical characteristics are still debated. Clues within literature suggest VDAC3 acts as a sensor of mitochondrial ROS level through the redox modifications of its cysteine residues [2]. Reina et al. [2] confirmed the protective role of VDAC3 upon oxidative insults, demonstrating the heightened protein level in cells treated with ROS-inducers and the extreme vulnerability of VDAC3-knock-out cells to free radical damage. However, the molecular mechanism of VDAC3 up-regulation remains elusive. To answer this question, we investigated herein VDAC3 mRNA levels upon exposure to sub-lethal doses of drugs that trigger mitochondrial superoxide accumulation (i.e. rotenone and menadione) in HeLa cells by focusing on the activity of a 600 pb region of the protein promoter. Data obtained with real-time PCR analysis and luciferase assays confirmed that VDAC3 transcript is strongly influenced by the mitochondrial redox status and that specific transcription factor binding sites directly involved in the oxidative stress response are

located in the promoter sequence examined. Those interesting findings represent a breakthrough in understanding the regulatory mechanisms involved in the expression of VDAC3 gene, emphasizing once again the extreme importance of cysteines in protein function. References: 1. Guarino F et al. (2020). Biochim Biophys Acta Bioenerg 1861, 148289. 2. Reina S et al. (2022). Redox Biol 51, 102264.

P-19-010
A role for the signaling molecule H_2O_2 in skin regeneration

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In the past, H_2O_2 was solely considered a toxic waste in cells, a relic of the times in which oxygen appeared. Nowadays, this concept has reverted, and it is widely accepted that H_2O_2 also acts as a signaling molecule in different intracellular pathways, including proliferation, survival, and migration. Interestingly, all these routes must be timely orchestrated during skin re-epithelization, as actively dividing cells residing in inner layers must on one side differentiate and move upwards to reconstitute the tissue and on the other maintain the undifferentiated niche. Hence, we decided to explore a role for H_2O_2 in the process using an immortalized keratinocyte cell line (HaCat cells) expressing the H_2O_2-specific HyPer7 probe in the cytosol. By simultaneously recording the response of the cells to a mechanical-like injury while monitoring their intracellular redox environment, we detected two oxidation waves at distinct times that respectively correlated with features of proliferative and migratory phases in the culture. Moreover, we were able to manipulate these phases by switching the differentiation stage of the cells, observing that the intracellular oxidative milieu shifted accordingly. Importantly, these findings open a new perspective on H_2O_2 significance in skin regeneration and suggest that topological control of redox-mediated pathways plays a fundamental role in coordinating the process. *The authors marked with an asterisk equally contributed to the work.

P-19-011
Bisphosphonates zoledronate and alendronate induce aerobic metabolism adaptations in endothelial cells

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Osteoporosis is one of the most common bone metabolism disorders in post-menopausal women. One of the first-line drugs used in anti-resorptive treatment are nitrogen-containing bisphosphonates. Their mechanism of action is to block the mevalonate pathway and inhibit osteoclast-mediated bone resorption. One of the products of mevalonate pathway is coenzyme Q – an important antioxidant and mitochondrial respiratory chain electron carrier. During intravenous treatment endothelial cells are the first to be exposed to these drugs. We studied the effects of chronic 6-day exposure to two common bone-preserving nitrogen-containing bisphosphonates, alendronate and zoledronate, on the endothelial function and aerobic metabolism of cultured human endothelial EA.hy926 cells. Our results indicate that in endothelial cells, 6 days of exposure to bisphosphonates induced a significant lowering of coenzyme Q10 level as well as lowering cell viability in higher concentrations. At a given concentration, the effects of zoledronate were much stronger than those of alendronate. Both bisphosphonates show increase in mitochondria biogenesis in endothelial cells. Additionally, we observed significant increase in the expression of inflammatory marker proteins, i.e., ICAM1 and IL6, in zoledronate-treated cells. Bisphosphonate-treated cells also showed significant reductions in HIF1α, which is a marker of hypoxia, and a 6-fold increase in KDM6A expression which functions as a direct cellular oxygen sensor. Zoledronate and alendronate also induced an increase in mitochondrial biogenesis markers while there was a significant decrease in the mitochondrial fission marker (MFF) indicating bisphosphonate-driven change in mitochondria turnover. This work was funded by Polish National Science Centre project 2020/37/B/NZ1/01188 and minigrant ID-UB 054/13/SNP/0002.

P-19-012
Attenuation of c-Jun/AP-1 dependent signalling and ROS production in LPS-stimulated macrophages via a thiol compound reduces pro-inflammatory cytokine production and NLRP3 inflammasome expression

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Macrophages (MΦ) play a pivotal role in initiating, maintaining, and resolving inflammation through the production of cytokines. Thiol homeostasis represents a key factor in the development of a well-balanced immune response, as evidenced by the activation of the NF-kB and AP-1 transcription factors in response to alterations in cellular redox potential and intracellular stresses, accompanied by excessive reactive oxygen (ROS) production. Additionally, the redox-dependent regulation of the NLRP3 inflammasome, whose assembly and activation results in the release of IL-1β and IL-18, has also been described. In this study, we explored the effects of a thiol molecule, called I-152, able to release N-acetyl-cysteine (NAC) and β-mercaptoethylamine (MEA) on the LPS-induced inflammation in THP-1 MΦ by evaluating the expression of inflammation-related cytokines, the involved transcription factors, NLRP3 inflammasome activation and ROS level. Firstly, I-152 metabolism was studied via HPLC in THP-1 MΦ, and the 0.25 and 10 mM concentrations were selected for the following experiments to explore the compound's anti-inflammatory activity. To this aim, MΦ were treated with I-152 for 2 h and then stimulated with LPS; the pro-inflammatory cytokines were evaluated at mRNA and protein level using respectively real time PCR and multiplex immunoassay systems, the main transcription factors involved in the inflammatory response were evaluated through western immunoblotting while ROS levels were determined through microscopy observation and flow cytometry measurements. A dose-dependent action of I-152 on AP-1 and NF-kB signaling emerged. IL-1β and IL-18

secretion was impaired due to the direct influence on the NLRP3 inflammasome and reduced ROS. Our study suggests that the control of the inflammatory response in MΦ can be achieved through modulation of the intracellular redox state by thiol molecules and different effects are obtained depending on the employed doses.

P-19-013
Thiol-breaking compounds inhibit platelet aggregation and regenerate mercaptoalbumin, protecting against oxidative stress

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Albumin (HSA) is the most abundant circulating protein and plays a pivotal role in maintaining the redox state of the plasma. Several studies have shown a relationship between HSA redox status and the severity of some diseases, highlighting that its oxidation can contribute to their progression. Three HSA proteoforms have been identified based on the redox state of cysteine 34, including the reduced proteoform (HSA-SH), non-mercaptoalbumin-1 containing a disulfide with small thiols such as cysteine (HSA-Cys) and non-mercaptoalbumin-2, the higher oxidized proteoform. We demonstrated that N-acetylcysteine (NAC), an antioxidant molecule able to break down protein disulphides generating free thiols, inhibits platelet aggregation by restoring HSA-SH [previously published in Eligini S et al. (2022) Antiox 11(3), 445]. Moreover, the amide derivative of NAC (AD4/NACA), a thiol with improved lipophilicity and bioavailability compared to NAC, as well as the thioredoxin-mimetic (TXM) peptides TXM-CB3, TXM-CB13, and TXM-CB30, significantly inhibited platelet aggregation induced by collagen, being more effective than NAC. These compounds also reduced the release of TxB2 and 12-HETE and increased the clotting time of the whole blood. Furthermore, all of them increased the free sulfhydryl groups in plasma and regenerated HSA-SH. Indeed, by mass spectrometry techniques, we observed that all these molecules significantly decreased the HSA-Cys levels with a concomitant increase of HSA-SH levels in plasma, recovering its antioxidant activity. These data suggest that these peptides might become useful for the prevention and treatment of oxidative stress conditions associated with platelet activation.

P-19-014
Ochratoxin A induces hepatic and renal toxicity in mice through increased oxidative stress, mitochondrial damage, and multiple cell death mechanisms

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Ochratoxin A (OTA) is a widespread food toxin produced by *Aspergillus ochraceus* and other molds. In this study, we developed and established acute OTA toxicity conditions in mice, which received daily oral doses of OTA between 0.5 and 8 mg/kg body weight for up to 7 days and were subjected to histological and biochemical analysis to characterize renal and hepatic damage. Oral administration of OTA for 7 days resulted in loss of body weight in a dose-dependent manner and increased the levels of serum biomarkers of hepatic and renal damage. The kidney was more sensitive to OTA-induced damage than the liver. In addition to necrosis, OTA induced hepatic and renal apoptosis in dose- and time-dependent manners. Especially, a high dose of OTA (8 mg/kg body weight) administered for 7 days led to necroptosis in both liver and kidney tissues. OTA dose-dependently increased the oxidative stress levels, including lipid peroxidation, in the liver and kidneys. OTA disrupted mitochondrial dynamics and structure in hepatic and renal cells, leading to the dysregulation of mitochondrial homeostasis. OTA increased transferrin receptor 1 and decreased glutathione peroxidase 4 levels in a dose- and time-dependent manner. These results suggest the induction of ferroptosis. Collectively, this study highlighted the characteristics of acute OTA-induced hepatic and renal toxicity in mice in terms of oxidative stress, mitochondrial damage, and multiple cell death mechanisms, including necroptosis and ferroptosis.

P-19-015
Targeting mitochondrial homeostasis with small molecules: from environmental contaminants to inhibitors of nucleotide recycling

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Mitochondria constantly sustain cell energetic needs and maintain redox homeostasis. Under stress conditions mitochondria may also be the major responsible for the overproduction of reactive oxygen/nitrogen species (ROS/RNS), thus leading to a decreased antioxidant defense and promoting oxidative/nitrosative stress (OS/NS). Prostate cancer is featured by a peculiar energetic metabolism, that makes mitochondria highly susceptible to alterations caused by endogenous or exogenous factors. Environmental contaminants (EC) are responsible for detrimental effects due to their high diffusion/bioaccumulation. Agrochemicals, such as Imidacloprid (IMI) and Phenmedipham (Phen), and the plastic component Bisphenol A (BPA), are able to exert endocrine disruption activity and induce mitochondrial alterations with ROS/RNS dysregulation. Interestingly, dysregulation of ribonucleotides re-cycling, the latter exerted by the mitochondrial ribonuclease REXO2 (degrading 2–5 nucleotides RNA

fragments) leads to altered mitochondrial energy profiling and genome stability. We developed specific inhibitors of this enzyme to promote mitochondrial dysfunction in prostate cancer cells (DU145/PC3, showing different level of malignancy and bioenergetics deficit). A set of dinucleotide analogues has been screened *in vitro* and in cells to assess REXO2 inhibition activity. The EC involvement in OS/NS has been investigated and their effect on ROS production and mitochondrial membrane potential (MMP) has been characterized by taking advantage of specific fluorescent probes (JC-1/DCF-DA/MAK145); furthermore, the oxygen consumption of cells in the presence of EC or REXO2 inhibitors has been assessed. The results will be discussed in the frame of an alteration of mitochondrial homeostasis and function highlighting a potential for REXO2 as a new biomarker for mitochondrial physiology or as a target for anticancer therapies.

P-19-016
Better understanding of cellular oxidative stress via LC-MS analysis of selected markers

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Oxidative stress represents a harmful state to the organism, resulting from an unbalanced production of reactive oxygen species (ROS) and the organism's antioxidant capacity. While ROS play crucial roles in pathogen defense and other physiological processes, their prolonged and uncontrolled presence can be responsible for the development of diseases such as atherosclerosis, Alzheimer's and Parkinson's diseases among others. ROS activity leads to structural damage of cells and tissues at the molecular level, producing non-functional or further reactive and dangerous intermediates. To effectively monitor oxidative stress, a complex set of 19 analytes somehow associated with oxidative stress was selected. This includes intermediates of oxidative reactions (guanine-, guanosine-, tyrosine-derivates), antioxidants involved in the degradation of reactive molecules (ascorbic and dehydroascorbic acid, oxidized and reduced glutathione), or prostaglandins as products of lipid oxidation contributing to the organism's inflammatory response. A protocol including cell cultivation, extraction, liquid chromatography and mass spectrometry was established for the determination of these analytes in samples. The LC-MS/MS method consists of two separations, for polar substances on HILIC and for less polar analytes on reversed C18 phase. The HeLa cells (untreated and stressed) were used as matrix for method validation. Its applicability extends to the determination of oxidative stress markers across various cell lines and tissue samples. This research was supported by Young researcher Grant UP JG_2024_022, IGA_LF_2024_011 and the Institutional Support of Palacký University in Olomouc RVO 61989592.

P-19-017
Endothelial pro-inflammatory mechanisms of micronanoplastics during atherosclerosis

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Micronanoplastics (MNPs) are emerging as a potential risk factor for cardiovascular disease. Exposure to MNPs is inevitable due to their omnipresence in the environment and their impact on human health is a critical emerging issue for public health. Direct evidence of the occurrence of MNPs in atheroma and their association with cardiovascular outcomes has emerged recently (N. Engl. J. Med. 390, 900–910, 2024). However, knowledge gaps on the adverse effects and the molecular mechanisms activated by MNPs in endothelial cell dysfunction during atherosclerosis still exist. To this end, human aortic endothelial cells (TeloHAEC) were treated with polyethylene (PE) and polyvinyl chloride (PVC) 70 μg/ml for 48 h. The effects of PE and PVC on cell viability, mitochondrial dysfunction and cell death pathways were explored using CCK-8, XF HS Seahorse Bioanalyzer, flow cytometry, enzyme-linked immunosorbent and western blot analyses. Under PE and PVC stimulus, data showed that endothelial cells underwent to inflammatory pathways ($p < 0.01$), adhesion molecule expression ($p < 0.01$), mitochondrial stress ($p < 0.001$), cell cycle arrest in G1 phase ($p < 0.01$), apoptosis ($p < 0.01$), and lysosomal-autophagic process induction ($p < 0.01$). These cellular events were followed by downregulation of NAD-dependent deacetylase sirtuin-6 (SIRT6) and p62 protein levels ($p < 0.01$). Taken together these results contribute to the further risk assessment of MNPs on human health, serving as a basis for future studies on the physiological threat of MNPs in promoting cardiovascular disorders mediated by endothelial dysfunction.

P-19-018
The mitochondrial bioenergetics modulation of melatonin can rescue aortic endothelial cells from hypoxia/reoxygenation injury

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The molecular mechanism of action of the biogenic compound known as melatonin has been explored at the mitochondrial level [1]. The positive effect on mitochondrial function was linked to a protective effect on hypoxia/reoxygenation (H/R) injury on aortic endothelial cells acting as a radical scavenger and improving the energy cell metabolism. The data suggest that melatonin desensitized the mitochondrial permeability transition pore (mPTP) opening, a phenomenon linked to different forms of regulated cell death. The F_1F_o-ATPase might be activated by Ca^{2+} triggering the mPTP [2] and the enzyme was identified as the biological

target of melatonin. In particular, the purified hydrophilic F_1 domain in the presence of Ca^{2+} as cofactor was inhibited by melatonin. A mutual exclusion test between melatonin and 4-chloro-7-nitrobenzofurazan showed an overlapping binding site on the catalytic region of the F_1 domain. Moreover, the anion superoxide (SOX) production stimulated by antimycin A added to energized mitochondria with pyruvate/malate or succinate was decreased in the presence of melatonin. At the cellular level, the viability and the impaired cell metabolism under H/R injury were improved in the presence of melatonin. All the bioenergetic parameters, *i.e.* basal respiration, maximal respiration, spare respiratory capacity, and ATP production, were increased in the presence of melatonin compared with H/R alone. In addition to this, the melatonin counteracted the SOX generated by H/R condition. On balance, melatonin has F_1F_o-ATPase as a molecular target in mitochondria exploiting an inhibitory effect on mPTP formation. The results highlighted the antiradical activity of melatonin on SOX production in mitochondrial dysfunctions linked to H/R injury rescuing oxidative cell metabolism and viability of cells. References: 1. Lei X et al. (2023) Front Pharmacol 14, 1332567. 2. Nesci S (2022) Biochimie 198, 92–95.

P-19-019
New insights into the role of cysteines in VDAC function, structure and mitochondrial import

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Located in the outer mitochondrial membrane of eukaryotes, the voltage dependent anion channel (VDAC) is the main modulator of mitochondrial metabolism as it regulates the organelle cross-talk with the rest of the cell [1]. In addition to the numerous pathways involving VDAC, a role in redox homeostasis has been recently proposed for isoform 3 whose cysteine residues would be responsible for the protein ability to perceive changes in the amount of mitochondrial reactive oxygen species [2]. Cysteine content is indeed a notably distinctive feature of the three VDAC isoforms that, on the other hand, share conserved sequences, similar structures and the same gene organization. Herein, we provide a thorough analysis of the oxidative modifications of VDAC cysteine thiols determined by mass spectrometry in view of their consequences on the structure and activity of isoforms 1, 2 and 3. Accordingly, VDAC1 cysteines have no specific relevance to pore-conductance neither to oligomerization nor apoptosis induction. Same residues are crucial for folding, stability and overall architecture of isoform 2, although again, they do not significatively contribute to the protein propensity to insert into membrane bilayers. On the contrary, removal of cysteine residues completely reshapes the controversial biophysical characteristics of isoform 3 by turning small-sized and ungated channels into canonical high-conducting pores and, concurrently, confirms these amino acids are indispensable for VDAC3 to counteract ROS-induced oxidative stress. In agreement with these findings, our latest studies revealed cysteines are directly involved in the mitochondrial import of VDAC3, but not VDAC1 and VDAC2. Results and implications are discussed. References: 1. De Pinto V et al. (2022) Front Physiol 13, 871586. 2. Reina S et al. (2022) Redox Biol 51, 102264.

P-19-020
Vascular homeostasis after exposure to sheep polymerized hemoglobin – developments in semi-artificial oxygen carriers

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In search of the semi-artificial oxygen carriers as blood substitutes, polymerized sheep hemoglobin (SpolyHb) was noticed as a valuable candidate in terms of oxygen delivery, redox impact as well as multiorgan toxicity. However, its effect on vascular homeostasis remains incompletely understood. To address this gap, we conducted an *in vivo* experiment utilizing Wistar rats (8/group) subjected to hemorrhagic shock conditions and perfused with SpolyHb. For comparison, another group received i.v. Dextran 40, while the Control group underwent hemorrhagic shock without fluid perfusion. SpolyHb and Dextran 40 exposure was limited to 24 h. Subsequently, animals were deeply anesthetized (ketamine-xylazine), and samples of the abdominal aorta (2–2.2 cm) were collected for electron microscopy, iNOS immunohistochemistry, and assessment of inflammation status via ELISA for IL-6, IL-10, IL-1beta, IL-1alpha, and prostaglandin E2. Kinetic assays were conducted for catalase and peroxidase, and levels of reduced and oxidized iron were estimated using colorimetric methods. Matrix metalloproteinases were assessed using a gelatin zymography protocol. In summary, exposure to SpolyHb led to a reduction in proinflammatory cytokines, matrix metalloproteinases and an increase in IL-10, while Dextran 40 maintained vascular inflammation similar to the control group. Both exposed groups (Dextran 40 and SpolyHb) exhibited a significant increase in oxidative stress. However, ultrastructural investigations revealed only slight endothelial damage in the hemorrhage group, while Dextran and SpolyHb did not have prominent detrimental effects on the vascular endothelium. Following exposure to SpolyHb, highlights of the vascular effects included no hypertension, slight endothelial damage with slight iNOS changes, and a decrease in vascular inflammation mediated by IL-10. This research received support from a GTC grant awarded by Babes-Bolyai University (Grant No. 32939/22.06.2023).

P-19-021
Differential role of carbon source on bioenergetic properties of *Thermus scotoductus* K1 cells during aerobic metabolism

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Discovery of microbial physiology in high thermal conditions accompanied with bioenergetic analysis are important studies. This study illuminates the investigation of the peculiarities of directed proton transport in *Thermus scotoductus* K1 strain in presence of glucose, lactose and starch cultivated in aerobic conditions. Specific growth rate was determined with different (1, 2 and 4 g/l) concentrations of sole carbon sources. Membrane potential, ΔpH (difference between $pH_{in/out}$) were measured in harvested cells growing in presence of 2 g/l sole carbon sources. *T. scotoductus* displayed inhibited specific growth rate ~4.8-fold in presence of 4 g/l glucose compared to 1 and 2 g/l at pH 8.5, however starch and lactose high concentrations did not inhibit the bacterial growth. It was shown that ion and substance permeability during lactose utilization was the lowest compared to glucose and starch, showing low membrane potential, and negative ΔpH, whereas in the presence of glucose and starch intracellular pH was more alkaline than extracellular pH showing the ability of the cells to have unique energy conserving strategy. The highest membrane potential and ΔpH observed in presence of glucose. Taken together it can be concluded that carbon source types and concentrations can have different effect on *T. scotoductus* K1 metabolism, which result in unique bioenergetic properties providing potential exploration of microbes' behavior in high thermal conditions. *The authors marked with an asterisk equally contributed to the work.

P-19-022
Extracellular vesicles as shuttle of harmful molecular signals derived from cigarette smoke extract-treated cells

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Extracellular vesicles (EVs) are considered a tool used by cells to spread signals or to eliminate unnecessary/detrimental materials. Oxidative stress affects the release of EVs which, in turn, might exert detrimental or beneficial effects depending on their modified molecular cargo. In order to study the effect of oxidative stress conditions on the amount of released EVs and on their modified molecular cargo, EVs were isolated by cell culture media of human bronchial epithelial cells (BEAS-2B) treated with burned (b) and heated (h) cigarette smoke extract (CSE), as it is well known that several toxic effects induced by CSE exposure are, at least in part, due to the induction of oxidative stress. Firstly, we demonstrated that either hCSE and bCSE reduced cell viability and induced an increase of intracellular ROS and carbonylated protein levels, in a dose-dependent manner. Both treatments also induced changes in the expression levels of antioxidant enzymes. Exposure for 24 h at 1% hCSE and 10% bCSE, concentrations increasing intracellular ROS and carbonylated proteins levels but only slight modified cell viability, induced an increased content of oxidized proteins in EVs. CSE exposure induced also changes in phospholipid fatty acid composition of released EVs. These results suggest that oxidative stress induced by CSE was able to prompt the release of EVs with an altered biochemical cargo, that might exert harmful effects on target cells. Notably, CSE-treated cells can remove oxidized toxic proteins via EVs in order to maintain cellular homeostasis. Results reported in this study not only increase knowledge on the effects of oxidative stress conditions on the released EVs but might represent a starting point for their use as biomarkers for CSE-induced lung damage. This work has been funded by the European Union- NextGenerationEU under the Italian Ministry of University and Research (MUR) National Innovation Ecosystem grant ECS00000041 – VITALITY (to Carla Emiliani). *The authors marked with an asterisk equally contributed to the work.

P-19-023
Plumbagin: potential role in the co-treatment of Duchenne muscular dystrophy

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Plumbagin is a secondary metabolite of the 1,4-naphthoquinone class found in several plants of the Plumbaginaceae family. It is used in traditional Asian and Australian medicine and has been investigated in several studies for its antitumor, antioxidant, anti-inflammatory, antifungal and neuroprotective effects [1,2]. Duchenne muscular dystrophy (DMD) is a genetic disease characterised by progressive muscle degeneration and weakness due to changes in a protein called dystrophin, which helps to keep myocytes intact. Unfortunately, a definite cure has not yet been identified. Corticosteroids are commonly used because they delay the progression of the disease, but they lead to severe side effects, therefore there is a pressing need for an alternative therapeutic strategy for DMD. Since oxidative stress and inflammation are common features of skeletal muscle in DMD, this study aims to investigate the potential effects of plumbagin on the expression of various enzymes involved in the antioxidant defence system in dystrophic mdx mice, the most known murine model of the disease. The results show that mice treated with plumbagin have a higher antioxidant defence system and decreased inflammation compared to untreated mdx mice. Therefore, we investigated the effect of concomitant treatment with plumbagin and fluoxetine, a selective serotonin reuptake inhibitor (SSRI), known to have antioxidant/inflammatory properties. In particular, plumbagin

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and fluoxetine are able to activate the Nrf2 signalling pathway, which plays a central role in the antioxidant/inflammatory response and muscle regeneration. The combination of these two molecules was tested to verify possible additive/synergistic activities and the results are promising. References: 1. Padhye S et al. (2012) doi: 10.1002/med.20235. 2. Petrocelli G et al. (2023) doi: 10.3390/life13061303. This work was supported by the Association Française contre les Myopathies AFM-Telethon (Grant number 23172). *The authors marked with an asterisk equally contributed to the work.

P-19-024
The effects of FLASH radiotherapy on *in vitro* lung cancer model: an insight into ROS implication

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FLASH radiotherapy (FLASH-RT) represents an innovative cancer treatment, delivering irradiation (IR) at dose/rates significantly higher than conventional radiotherapy (CONV-RT)[1]. The literature controversy results on the comparison between the two IR methods could be influenced by the use of accelerators not specifically dedicated to producing beams with Ultra-High-Dose per Pulse needed to trigger the FLASH effect. Being in possession of a unique device able to change the mode of erogation from CONV- to FLASH-RT, without modifying the experimental set up, allowed us to conduct a more accurate studythat had been done before. One of the major hypotheses to understand the molecular mechanisms of IR effects considers oxygen and the modulation of the cell RedOx state[2]. Human lung adenocarcinoma (A549) cell line was used as *in vitro* model to study the effects of the two IR modalities and to investigate oxidative stress conditions following IR treatments. Cells were irradiated with CONV- or FLASH-RT at different doses (from 0 to 24 Gy) with an electron linear accelerator, to evaluate cells' radiosensitivity using survival and clonogenic assays. Based on cells' radiosensitivity, two doses were chosen, where the difference in survival between CONV- and FLASH-RT was the highest, to assess firstly ROS production as kinetic curve and, after 72 hs, cell death, cell cycle assessment, expression of cytoskeletal proteins, and DNA damage. Findings revealed a radiosensitivity spectrum between 2 and 5 Gy, with chosen doses for deeper analysis set at 2.5 and 5 Gy. Cells showed lower ROS production and higher mortality when exposed to FLASH- compared to CONV-RT; vimentin's expression was instead significantly lower both in CONV- and FLASH-RT. References: 1. Gao Y et al. (2022) J Appl Clin Med Phys 23(10), e13790. 2. Hughes JR et al. (2020) Int J Mol Sci 21(18), 6492. Thanks to Fondazione Pisa and PNRR Tuscany Health Ecosystem, Spoke 1 "Advanced Radiotherapies and Diagnostics in Oncology" CUP I53C22000780001.

P-19-025
Biochemical implications for human health after perfluorooctanesulfonic acid and glyphosate exposure

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Perfluorooctanesulfonic acid (PFOS) is a fluorinated chemical compound previously used in various industrial products but is currently banned in several countries due to its environmental impact. Glyphosate (GLY) is a widely used herbicide subject to controversies regarding its safety and restrictions in some geographical areas. Both are considered environmental contaminants with a significant spread into food chain and impact on human health. To investigate the potential synergistic impact of these composts, two types of neuronal cell lines were employed: C6 (murine astrocytoma) and SHSY-5Y (murine neuroblastoma). The aim was to evaluate biochemical markers of inflammation as well as oxidative stress, and to observe proinflammatory cytokines release of IL-6 and INF-g, as well as anti-inflammatory cytokine like IL-10. The results show that both PFOS and GLY have intrinsic toxicity in neuronal cells, modulated by increased oxidative stress, and together, they exhibit a synergistic effect amplifying the overall impact. To better understand the influence of these environmental contaminants in the immune response to an inflammatory stimulus, induced by lipopolysaccharide (LPS), we used in the second experiment, a murine macrophage cell line RAW 264.7. Our results suggest that, even at concentrations that are not toxic, both compounds have the ability to enhance the inflammatory response increasing intracellular ROS and altering the cytokines involved in the inflammatory process such as IL-6, IL-10 and INF-g. When these two compounds are combined, their effects increase, suggesting a synergistic effects in modulating the inflammatory response. This study could provide a starting point for further research with the aim of better understanding if, how and when these contaminants might affect the human nervous and immune systems.

P-19-026
A novel laccase from *Trametes polyzona* with high performance in the decolorization of textile dyes

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In recent years, thanks to an increased awareness of the potential of biodiversity and to the versatility of biomolecular tools, the use of eco-friendly biocatalysts has increased further its biotechnological interest. Laccases are considered versatile biocatalysts since they catalyze the oxidation of various aromatic and related compounds with the concomitant reduction of oxygen to water as the only by-product. Laccases have gained a prominent role in different industrial fields for very diverse purposes, ranging from food additive and beverage processing to biomedical diagnosis, from pulp delignification to textile dye transformations. Furthermore, their ability to transform complex xenobiotics makes them useful in enzymatic bioremediation and detoxification. For these

reasons, new laccases are continuously sought as their different characteristics can match specific industrial requirements. To identify novel laccase activities of potential industrial interest, we tested several poorly characterized white-rot fungi that were screened on substrates known to be oxidized by laccases. We focused on the best candidate, *Trametes polyzona*, which was further studied for laccase production. Moreover, to describe potential traits of interest of single enzymes, we overexpressed a putative *T. polyzona* laccase encoding gene in the yeast *Saccharomyces cerevisiae*. Here we will illustrate the identification, biochemical characterization, and application of laccase 2 from *Trametes polyzona* (TP-Lac2). Specifically, the ability of TP-Lac2 to decolorize textile dyes was evaluated in the presence of natural and synthetic mediators. Overall, the laccase-mediator system consisting of TP-Lac2 and the natural mediator acetosyringone showed interesting potential as an environmentally friendly alternative for wastewater treatment in the textile industry. *The authors marked with an asterisk equally contributed to the work.

P-19-027
Reactivity studies of dinitrosyl iron complexes (DNIC) towards one electron oxidant

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Dinitrosyl iron complexes (DNIC) are rapidly formed in cells exposed to nitric oxide (•NO) and are considered the most abundant •NO intracellular metabolite [1]. However, there is little information regarding their properties and biological fate. In this work, the reactivity of DNIC was investigated towards oxidants such as the $K_3Fe(CN)_6$ (a molecule used as a 1-electron oxidant model). Such reactivity is relevant, as it can demonstrate whether DNICs generate nitrosothiols under conditions of oxidative stress. The reaction between 70 μM binuclear DNIC and 140 μM $K_3Fe(CN)_6$ was monitored using UV-Vis spectroscopy, showing the reduction of Fe(III) center, generating $[Fe(CN)_6]^{4-}$. The evaluation of nitrite (NO^{2-}) formation was performed through Griess method. After 2 h reaction, the concentration of NO^{2-} reached a plateau, revealing two molecules of NO^{2-} for each mole of DNIC degraded. Considering that each DNIC molecule contains four NO molecules and only two NO were converted into NO^{2-} in the reaction with $K_3Fe(CN)_6$, high performance liquid chromatography was applied for the identification of other products. The chromatogram exhibited a low intensity peak at 15.5 min retention time, whose UV-Vis spectrum presented a band with absorbance at 336 nm, characteristic of s-nitrosoglutathione (GSNO). Over the course of 2 h reaction, the intensity of this peak increased, reaching a maximum concentration (37 ± 3) μM of GSNO. Thus, the use of ferricyanide as a model oxidant proved that DNICs are capable of reacting with 1-electron oxidant and still generate nitrite ions and nitrosothiols as reaction products. Reference: 1. Hickok JR et al. (2011) Free Rad Biol Med 51(8), 1558–1566.

P-19-028
Iron regulatory protein 2: key player in iron homeostasis and ferroptosis

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IRP2, iron regulatory protein 2, is a protein present in eukaryotic organisms that plays a crucial role in controlling the iron levels in cells. It belongs to the iron homeostasis regulatory system and acts as an iron-binding factor in cells. The high concentration of iron contributes to the generation of an excess of reactive oxygen species (ROS) in cells, which can disrupt DNA and protein molecules. Iron is involved in ferroptosis, a type of programmed cell death resulting from the excessive accumulation of oxidation products. Iron is important in redox reactions, influencing various oxidation states of cofactors, anti-oxidative etc. Keratinocyte wild type (WT), HaCaT and GPX4-knockout (KO) obtained by CRISPR/Cas9 method were used for the study. Cells were incubated for 24 h with a ferroptosis inducer erastin, at two doses 5 and 10 μM. The relative markers gene expression level, *TFRC, DMT1* and *SLC40A1* was evaluated by RT-qPCR, while ROS was measured through the flow cytometry. The iron level was determined by the iron-specific Prussian blue. The level of expression of the *TFRC* gene in WT cells decreases with the dose of erastin. In GPX4 KO cells no expression was observed at a lower dose whereas an increase was observed at a higher dose which resulted in the accumulation of iron in the cell. The expression of the *SLC40A*1 gene in HaCaT wild type cells increased after erastin addition, while in GPX4 KO it did not change. *DMT1* gene expression in WT cells decreased at both doses while in GPX4 KO decreased slightly at both doses. CRISPR modification resulted changes in marker gene expression level, but no impacted cell viability. An increased of *IRP2* expression was observed in wild-type cells, which may be a feedback-like response to the low concentration of the iron ions in cells. In the KO mutant cells, *IRP2* expression was silenced, due to excess iron accumulation. The work was supported by Project-Based Education-PBL No. 54/2020 and 55/2020 of the Rector of the SUT.

P-19-029
Functional characterization of the nitrobindin domain of the human THAP4 protein and preliminary analysis of nitrobindin expression profiles in *Danio rerio* embryos and tissues

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Nitrobindins (Nbs) form a new class of evolutionary conserved ferric heme-proteins characterized by a 10-stranded anti-parallel β-barrel fold. Although their physiological role(s) is still unclear, it has been postulated that Nbs are involved in both NO/O_2 and RNS and ROS metabolism. Nbs derived from diverse organisms have been characterized by our research group from both the structural and functional viewpoints. Interestingly, in human cells Nb has been described as a domain of the human protein named THAP4[1,2]. THAP4 is composed of an *N*-terminal modified zinc finger domain able to bind DNA and a *C*-terminal Nb domain. To define the role of THAP4 in RNS detoxification, silencing experiments have been performed in breast cancer MCF-7 human cells. Besides, transcriptomic analyses have been performed in both wild-type and silenced cells after treatment with the NO-donor DETA-NONOate. Moreover, THAP4 interactors have been identified by mass-spectrometry. Overall, results obtained suggest a potential role of THAP4 in interferon-induced anti-inflammatory response, in the detoxification from ROS and RNS, and in the cellular response to stress. To further dissect the Nb role *in vivo*, preliminary studies were performed in zebrafish embryos and in adult tissues. The levels of zebrafish Nb were analyzed by qRT-PCR and whole mount RNA *in situ* hybridization. Data obtained demonstrated that Nb levels were significantly higher in tissues exposed to hypoxic conditions (e.g., brain, gonads, swim bladder, eyes, and gills), supporting the notion that Nb exerts protective effect towards ROS and RNS. Remarkably, Nb levels resulted higher in elderly fishes, thus suggesting that Nb may be somehow implicated also in aging. Experiments aimed at investigating the possible role of Nb in aging are ongoing, using human cells derived from patients affected by progeria. References: 1. De Simone G et al. (2020) Antioxid Redox Signal 33, 229–246. 2. De Simone G et al. (2024) J Inorg Biochem. 250, 112387-112398.

P-19-030
Characterization of recombinant cystathionine γ-lyase (CGL) from *Klebsiella pneumoniae*: insights into H_2S production

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Hydrogen sulfide (H_2S) is increasingly recognized as a key component of bacterial pathogens defense mechanisms against host responses, although its role may not be universal across all bacterial species. Enzymatically produced H_2S primarily originates from two pyridoxal-5′-phosphate (PLP)-dependent enzymes involved in L-cysteine metabolism: cystathionine β-synthase (CBS) and cystathionine γ-lyase (CGL). The opportunity to selectively inhibit one of these enzymes holds significant therapeutic potential. In the search for novel druggable targets, this study comprehensively characterized the purified recombinant CGL enzyme from the human pathogen *Klebsiella pneumoniae* (KpCGL) using a combination of biochemistry and biophysics techniques. *Klebsiella pneumoniae* is a well-known Gram-negative multidrug-resistant superbug, commonly associated with hospital-acquired infections necessitating the urgent development of new pharmacological interventions. Our findings revealed that native KpCGL exists as a tetramer in solution composed of four identical subunits, each containing one PLP molecule. Kinetic analysis combined with product identification through LC-MS/MS demonstrated that KpCGL efficiently catalyzes the hydrolysis of L-cistathionine through γ- and β-elimination mechanisms, along with the H_2S production from L-cysteine and/or L-homocysteine. Comparisons with other CGL enzymes provided valuable insights into the regulation of this H_2S-producing enzyme and unveiled significant structural and mechanistic differences between human and bacterial enzymes. These findings offer significant insights into the evolution of CGL enzymes across different pathogens and provide novel opportunities for developing specific inhibitors targeting KpCGL.

P-19-031
Direct effects of hypoglycemic agent – K_{ATP} channel blocker glybenclamide on the functions of cardiac mitochondria

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The main biological effects of glybenclamide are related to the lowering of blood sugar levels. However, its effects on the cardiovascular system are controversial. In our study, we investigated the effects of K_{ATP} channel blocker glybenclamide on the respiration rate of isolated rat heart mitochondria, oxidizing pyruvate and malate (6 + 6 mM). Respiration rates were registered by means of Clark-type oxygen electrode in KCl respiration medium at 37°C. Our results showed that glybenclamide (10-200 μM) in a concentration dependent manner increased pyruvate-malate supported

State 2 respiration rate of isolated rat heart mitochondria (53.8 ± 2.5 nmol O/min/mg protein, RCI 8.4 ± 0.1, n = 4) by 17.5–145%, whereas it decreased the State 3 respiration rate (450.7 ± 25.6 nmol O/min/mg protein, RCI 8.4 ± 0.1, n = 4) by 9.8–56.5%. Mitochondrial adenine nucleotide translocase (ANT) inhibitor carboxyatractyloside completely abolished glybenclamide-induced uncoupling of pyruvate and malate oxidizing rat heart mitochondria. Furthermore, the uncoupling effect of glybenclamide was significantly reduced in the presence of oligomycin (1 μg oligomycin/mg mitochondrial protein) and substrates of ANT — 200 μM of ADP or ATP, possibly due to a competition of glybenclamide with adenine nucleotides. ANT plays a crucial role in the energy transfer between mitochondria and the cytosol, as it facilitates the movement of ATP, produced in the mitochondria through oxidative phosphorylation, across the membrane in exchange for cytosolic ADP. Thus, ANT may be the important target of hypoglycemic agent – K_{ATP} channel blocker glybenclamide, and further research on their interactions might help to elucidate how glybenclamide influences the cardiovascular system.

Enzyme Engineering and Biotechnology

P-20-001
Molecular approaches to unravel the enigma of dimethylarginine dimethylaminohydrolase 2

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Dimethylarginine dimethylaminohydrolase (DDAH) catalyses the conversion of asymmetric dimethylarginine (ADMA) to L-citrulline and dimethylamine. As known inhibitor of nitric oxide synthase, ADMA plays an important role in the regulation of the intracellular NO levels, and represents as an independent risk factor for cardiovascular and overall mortality in the general population [1]. Two DDAH isoforms have been identified in humans, namely DDAH1 and DDAH2. While the role of DDAH1 in the metabolism of ADMA has been widely demonstrated, that of DDAH2 has been largely debated. We participated to a large international research consortium that, taking advantage of *in silico*, *in vitro*, cell culture, and murine models, demonstrated that DDAH2 is incapable of metabolising ADMA. Nonetheless, the ADMA-independent function(s) of DDAH2 remains unknown. Indeed, a possible role in angiogenesis, regulation of mitochondrial function and insulin secretion has been claimed [2], but no definitive proof has been provided. To try to unravel this enigma, we focused our attention on the obtainment of DDAH2 in the purified form. Since the protein shows high tendency to aggregation, through *in silico* analyses we compared the putative structure of hDDAH2 with that of homologous proteins endowed with higher solubility, and we evaluated the chemical-physical properties of the protein, in particular the distribution of hydrophobic and charged patches on the surface. We then constructed DDAH2 artificial variants through a rational engineering strategy and performed small-scale expression tests in *E. coli* using a variety of experimental conditions. We were able to set the optimal protocol for the recovery of a significant amount of the protein in the soluble fraction of the lysate. These results represent the necessary premise for the future biochemical characterization of the enzyme and the screening of potential small-molecule ligands and/or protein interactors.

P-20-002
Towards deciphering the catalytic mechanism of *Rhizobium etli* inducible L-asparaginase ReAV

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ReAV, the inducible Class-3 L-asparaginase from the nitrogen-fixing bacterium *Rhizobium etli*, is potentially interesting as a novel antileukemic agent, as it shows no sequence or structure similarity to known enzymes with this activity, and must, therefore, have a completely new mechanism of L-asparagine hydrolysis. The crystal structure of ReAV [1] shows a homodimeric protein with an α/β protomeric fold formed by two tightly packed domains. The conspicuous active site region encompasses a zinc cation, curiously coordinated by Cys135, Lys138, Cys189 and a water molecule, as well as two Ser-Lys tandems (Ser48-Lys51, Ser80-Lys263) with an unusually hydrated Ser48 residue. A pan-genomic analysis of all available bacterial genomes [2] revealed that the catalytic site residues are strictly conserved in all Class 3 L-asparaginases. The created sequence profile also indicated a few adjacent residues, such as Arg47, His139, Tyr156 and Asp187, that are preserved in 99% of the orthologs. We applied site-directed mutagenesis together with enzymatic assays and X-ray structure analysis to unravel the role of the selected active site residues in catalysis. Eleven alanine muteins, namely R47A, S48A, K51A, S80A, C135A, K138A, H139A, Y156A, D187A, C189A, K263A, were created in this campaign, and almost all of them lost the ability to hydrolyze L-asparagine, confirming our predictions about the significance of these residues in substrate hydrolysis. We were able to crystallize ten of the ReAV variants and solve their crystal structures, revealing some intriguing structural changes in the active site area as a result of the mutations. Taken together, the results provide a number of useful clues about the catalytic mechanism of the ReAV enzyme, allowing one to cautiously postulate a possible enzymatic scenario. Work supported by National Science Centre (NCN, Poland) grant 2020/37/B/NZ1/03250. References: 1. Loch et al. (2021) *Nature Commun.* 12, 6717. 2. Zielezinski et al. (2022) *Sci Rep.* 12, 15797.

P-20-003
Co-expression of quintuple reverse transcriptase mutant with host transpeptidase in *Vibrio natriegens*

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We produced a mutant reverse transcriptase into the cultivation medium by increasing the amount of host transpeptidase,

specifically penicillin-binding protein 5/6. Changing the amount of low-molecular-weight penicillin-binding proteins likely alters cell morphology. It allows intracellular proteins to be secreted from the cell without using signal sequences [previously published in: Kormanová Ľ et al. (2023) World J Microbiol Biotechnol. 39, 277]. The extracellular secretion of heterologous proteins simplifies the purification process and maintains the solubility of aggregation-prone proteins within the growth medium. Reverse transcriptases are used to prepare complementary DNA for RNA virus detection, analysis of cell expression, ribosome display, etc. The Moloney Murine leukemia virus reverse transcriptase with 5 mutations was obtained by compartmentalized ribosome display [previously published in: Baranauskas A et al. (2012) Protein Eng Des Sel. 25, 657–668]. We utilized *Vibrio natriegens* prophage-free strain as a heterologous expression system with robust protein synthesis capability and high growth rate. Using electroporation, we transformed cells with 2 recombinant plasmids pJexpress404-RTmut and pRSFDuet-PBP5/6. After recombinant selection by restriction analysis of plasmid DNA, we produced reverse transcriptase in 50 ml of LB3 medium at 37°C for 4 and 24 h. Western blot analysis demonstrated the presence of reverse transcriptase in the extracellular environment only when co-expressed with transpeptidase. In addition, we found the amount of intracellular reverse transcriptase to be lower after 24 h of production compared with 4 h of enzyme production, likely indicating reverse transcriptase instability. Further, it is necessary to quantitatively compare the levels of extracellular enzyme with intracellular reverse transcriptase. The research was supported by the Slovak Research and Development Agency grant APVV-21-0215 and APVV-19-0196.

P-20-004
Mechanistic basis of the distinct enzymatic properties of allotype 10 of ER aminopeptidase 1

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Endoplasmic reticulum aminopeptidase 1 (ERAP1) is an intracellular aminopeptidase that holds important functions in the human immune system, generating mature antigenic epitopes for presentation to cytotoxic T cells. It is characterized by high polymorphic variation, that underlies the variability of adaptive immune responses between individuals. ERAP1 has several common allotypes, which are characterized by combinations of single nucleotide polymorphisms (SNPs) in nine specific positions and have been associated with predisposition to cancer, infections, and autoimmunity. Allotype 10 (HAP10) was initially considered to be a low-activity outlier, based on its activity on a typical model substrate. This function has been associated with its protective role towards some autoimmune diseases.[1] However, a follow-up study suggested that it is most likely an allotype with distinct substrate specificity.[2] Aiming to better understand the mechanistic basis of these properties, we focused on the SNP positions 349V and 725Q which are unique for HAP10, by performing an array of kinetic and thermodynamic analyses. Our results show that while these positions are key in determining allotype 10 enzymatic activity they have limited effects on some of its unique properties including its allosteric kinetic behaviour which is likely the result of synergism with other allosteric sites. Overall, our work suggests that allotype 10 is not just an inactive variant of ERAP1 but rather carries distinct catalytic properties and is optimized for trimming larger, while sparing smaller substrates, thus contributing to the ER stability of mature antigenic epitopes. References: 1. Arakawa et al. (2021) J Immunol 207 (9), 2235–2244. 2. Hutchinson et al. (2021) J Biol Chem 296, 100443.

P-20-005
Establishing high-throughput screening of engineered polyhydroxyoctanoate (PHO) depolymerase variants using novel PHO model compounds

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Polyhydroxyoctanoate (PHO) is a biocompatible microbially produced polyester with elastomeric properties currently limited by its high production cost and poor biodegradability in open environments. Enzymatic recycling of post-consumer PHO offers a bio-cyclable route to PHO utilization and poses a solution from both ecological and economical aspects. Enzyme engineering enables industrial efficiency in polymer degradation. Therefore, we have created a mutant library for PHO depolymerase from Pseudomonas fluorescens GK13 (PfPHOase) using error-prone PCR. To advance the speed of identification of PfPHOase variants with improved PHO degradation rates high-throughput screening was performed spectroscopically with in-house synthesized p-nitrophenyl esters of 3-hydroxyalkanoate monomer (3-HA monomer) and 3-hydroxyalkanoic acid dimer (3-HA dimer)[1]. Further, the degradation of PHO polymer for best-performing PfPHOase variants was assessed on commonly employed emulsified PHO-agarose plates. The obtained results indicate the positive correlation between the degradation of novel PHO model compounds with degradation of PHO polymer. Novel model compounds have been successfully employed for the identification of beneficial mutations for the advancement of enzymatic PHO degradation. Reference: 1. Nenadovic et al. High-throughput assay for PHO-depolymerases. 2024, under review. The present study was conducted as a part of Bio Innovation of a Circular Economy for Plastics – BioICEP. This project has received funding from the European Union's Horizon 2020 research and innovation programme under grant agreement No 870292.

P-20-006
Identification of plastic-active enzymes via an activity- and metagenomic-based approach

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Over the past decades, there has been a substantial increase in global plastic production, reaching a remarkable 400 million tons

per year. Inadequate waste management practices pose a significant threat to the environment as plastic waste accumulates in various ecosystems, degrading into microplastics. In this context, microbes thriving in natural or industrial environments contaminated with plastic pollutants may serve as reservoirs of plastic-active enzymes. Hence, this study aimed to investigate microbial consortia isolated from a microplastic-polluted industrial niche, i.e., a municipal wastewater treatment plant sludge. The latter was used as the microbial inoculum for enrichment batch cultures in a medium containing post-consumer polyethylene terephthalate (PET) or polylactic acid (PLA) at 37°C and 50°C. Following a 100-day enrichment period, both biofilm-forming and planktonic communities were isolated and underwent an agar-based functional screening to assess enzymatic activity related to polyester hydrolysis, using emulsifiable triglyceride substrates (e.g., tributyrin and coconut oil) as well as polyesters, such as polyhydroxybutyrate (PHB) and polycaprolactone (PCL). We successfully identified 148 isolates exhibiting esterase activity towards tributyrin. Among these, 25 also displayed lipase activity towards coconut oil, while 3 isolates were able to hydrolyze PHB and PCL. Shotgun metagenomic sequencing was carried out to gain insights into the composition of the microbial communities as well as to identify potential polyester-active enzymes. Enriched consortia include members of the orders Actinomycetales, Bacillales, and Rhizobiales that comprise known plastic-degrading bacteria species. Moreover, from the metagenomic dataset, we retrieved a total of 25 putative PHB depolymerases. Among these, we selected a novel extracellular PHB depolymerase for *in vitro* characterization, confirming activity against PHB at 30, 37, and 50°C.

P-20-007
Enzymatic degradation of thermoset plastics

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Approximately 400 million tonnes of plastics are produced each year and only a minority is recycled. Of these, thermoset plastics in particular constitutes a challenge, as they are highly resistant to remolding and recycling due to their crosslinked structure. The current disposal methods of incineration or landfilling pose environmental and economic challenges. In nature, various microorganisms such as fungi and bacteria express enzymes that can degrade biopolymers such as lignin, cutin, proteins, lipids, etc. These enzymes may have the potential to cleave the bonds in synthetic polymers or could be engineered to do so. Enzymatic degradation of thermosets offers a green and efficient alternative to recover the monomers for reuse. We have identified bacteria from environments enriched with thermoset plastics that can degrade common thermosets and isolated the enzymes involved using an activity-based fractionation approach. We aim to use these enzymes as templates for protein engineering to develop a robust and scalable process for thermoset plastic recycling.

P-20-008
Production of recombinant CGTase with potential to create intriguing sensory products for food industry

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Cyclodextrin glycosyltransferase (CGTase) is an enzyme commonly found in many microorganisms. It belongs to the α-amylase family of glycoside hydrolases. CGTase catalyzes the conversion of starch and related substrates at the α-1,4-glycosidic bond, leading to the formation of a glycosyl intermediate. The reaction is followed by the formation of a new intramolecular α-1,4-glycosidic bond at the non-reducing end of the substrate, resulting in the formation of cyclodextrins. In addition to cyclization, these enzymes have other activities, but cyclization being the most prominent and important. In our work, we focused on the heterologous production of CGTase originating from *Thermoanaerobacter* sp. in the *E. coli* expression system. The first steps were the construction of expression vectors with the gene for the target protein. In the next steps, we focused on the production itself, where we took into account not only the selection of strains but also the cultivation conditions in order to achieve the highest amounts of soluble protein to be able to start purification of the protein. Purification is one of the most important steps in the production of recombinant proteins, since if CGTase is obtained in sufficient quantity and quality, it can be used in biotransformations to obtain products with interesting properties. This work was founded by APVV-17-0333, and APVV-0161.

P-20-009
Effect of cultural conditions on β-glucanase production by *Aspergillus niger*

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β-Glucans are the major non-starch polysaccharides (NSP) fractions in grain, which increase digest viscosity, reduce the digestibility of nutrients and decrease the feed efficiency and growth performance of broilers. These antinutritive carbohydrates are undesirable, as they reduce digestion and absorption of all nutrients in the diet, especially fat and protein. Besides, NSP can accelerate small intestinal fermentation by modulating the intestinal microflora, which may be detrimental to nutrient digestion and absorption for chickens. One of the solutions of this critical issue is the introduction in the poultry diet of β-glucanase enzyme which plays a crucial role in hydrolysis of the β-glucans. β-Glucanases hydrolyze the 1,4- or 1,3-linkages in glucans disrupting the integrity of endospermic cell wall. In this regards, the search for new potent fungi overproducing β-glucanase with industrially significant properties is of great interest. The aim of this study was determination of cultural conditions (pH, to)

providing maximum enzyme production. Among 12 mold fungi *Aspergillus niger* appeared to be the best β-glucanase producer (0.95 U/ml). The highest enzyme activity was obtained at pH of 5.5 and temperature of 35°C. This research was supported by the Scientific Committee of the Ministry of Education and Science of the Republic of Kazakhstan (grant №AP19674476).

P-20-010
Zinc as a booster of *Rhizobium* asparaginases activity

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L-Asparaginases are enzymes that catalyze the hydrolysis of L-asparagine to L-aspartate and ammonia. The members of Class 3 L-asparaginases, ReAIV and ReAV, encoded in the genome of the nitrogen fixing bacterium *Rhizobium etli*, have a unique active site comprising a peculiar but highly specific Zn^{2+} binding site [1, 2]. The aim of this work has been to establish the role of Zn^{2+} cations in the catalytic reaction of *Rhizobium*-type asparaginses. Nessler reaction showed that micromolar Zn^{2+} concentration improves the L-asparaginase activity of ReAIV and ReAV by about 32% and 56%, respectively. The K_m/V_{max} values determined in the presence of optimal Zn^{2+} level (1.0 and 2.5 μM, respectively, for ReAIV and ReV) were 1.5 mM 770 s^{-1} and 2.1 mM 603 s^{-1}. Using ITC titrations, we also investigated the dissociation constants of Zn complexes of both isoforms. The results revealed that Zn^{2+} is bound more strongly by ReAIV (K_d ~1.2 μM) than by ReAV (K_d ~ 3.3 μM). The data clearly show that the optimum Zn^{2+} concentrations for both enzymes are close to the K_d values of their zinc binding. The improvement of K_m by the addition of extra Zn^{2+} suggests that the zinc ions are involved in substrate recognition. Work supported by National Science Centre (NCN, Poland) grant 2020/37/B/NZ1/03250. 1. Loch JI et al. (2021) Nat Commun 12, 1–11. 2. Loch JI et al. (2023) Acta Crystallogr D 79, 775–791.

P-20-011
The mysterious phosphorylation of eukaryotic Class 3 L-asparaginases

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L-Asparaginases are enzymes that play a significant role in medicinal and biotechnological applications, in particular as therapeutics for the treatment of acute lymphoblastic leukemia or antibacterial and antifungal targets in agriculture. The only structures of Class 3 asparaginases from *Rhizobium etli* (*Re*AV and *Re*AIV) were solved recently [1,2], revealing a unique fold with some resemblance to serine β-lactamases and glutaminases. In this work, we selected Class 3 asparaginases from five fungal pathogens for functional and structural characterization, including *Colletotrichum fructicola* (*Cf*A) and *Fusarium oxysporum* (*Fo*A) enzymes. The enzymes were cloned and expressed in bacterial and yeast systems as His-tagged proteins. Using X-ray crystallography, we were able to successfully solve the crystal structures of *Cf*A and *Fo*A. In both structures we detected unexpected phosphorylation of the putative Ser51 nucleophile. Mass spectrometry confirmed that all investigated fungal but not Rhizobial asparaginases were phosphorylated at the equivalent positions. Additionally, we performed mutagenesis of the phosphorylated Ser residues. The enzymatic activity of fungal asparaginases is much lower compared to ReAIV and ReAV, indicating that the phosphorylation significantly affects the enzyme activity by abating substrate affinity. Our analysis of the mysterious phosphorylation of eukaryotic Class 3 asparaginases in *E. coli* unveils an intricate regulatory layer, offering a novel insight into the interplay between prokaryotic host environments and eukaryotic enzyme expression. This knowledge holds promise for improved biotechnological applications and expands our understanding of post-translational modifications in the context of recombinant protein expression. Work supported by National Science Centre (NCN, Poland) grant 2020/37/B/NZ1/03250. 1. J.I. Loch et al. (2021) Nature Commun. 12, 6717. 2. J.I. Loch et al. (2023) Acta Cryst D79, 775–791

P-20-012
Improvement of prodigiosin production in an *E. coli* system

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Prodigiosin, a red pigment natural product produced by bacteria like *Serratia marcescens*, has garnered attention for its diverse therapeutic potentials. This compound is distinguished by its unique tripyrrole ring structure. It exhibits promising anticancer properties, making it a subject of interest in cancer research. In addition, prodigiosin has also shown antimicrobial effects. The unique characteristics of prodigiosin make it a fascinating biomolecule in the fields of pharmacology and medical research. The biosynthetic gene cluster of prodigiosin has been sequenced and the biosynthetic pathway was proposed. In the entire biosynthetic process of prodigiosin, the final step involves a condensation reaction conducted by MAP and MBC, with the key enzyme, PigC. In previous studies, prodigiosin has been heterologously produced using an *E. coli* system. Herein, we successfully expressed the biosynthetic gene cluster of prodigiosin from *Serratia marcescens* via *E. coli* system and tried to improve the yield of prodigiosin. The key factors that may influence the yield, such as carbon source, temperature, and shaking speed were tested. Furthermore, we utilized high-performance liquid chromatography (HPLC) for further purification and quantification. The optimal conditions for the production of prodigiosin in *E. coli* were investigated.

P-20-013
Crystal structure of Gh-TDH in complex with dsDNA reveals unprecedented nuclease structure-activity relationship

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Grimontia hollisae is a marine bacterium, the primary pathogenic factor of which is thermostable direct hemolysin (TDH). Previous research has identified TDH as displaying hemolytic, enterotoxic,

cardiotoxic, and hepatotoxic activities. Our earlier study reported the translocation of TDH into the nuclei of hepatocytes. To delve into the significance of this phenomenon, we characterized the nuclease activity assay of TDH with DNA. Intriguingly, our investigations revealed an unexpected intrinsic 3′–5′ exonuclease activity associated with TDH. Remarkably, TDH is the first hemolysin with nuclease activity. In addition, we determined the binary structure of TDH in complex with designed DNA at a resolution of 1.37 Å. Based on the crystal structure if Gh-TDH-DNA complex, we performed alanine-scanning mutagenesis focused on the putative DNA-binding sites. We proposed that Lys88 acts as a general base for DNA cleavage. Furthermore, using bio-layer interference, we determined that the K88A mutation retained the ability to bind DNA, exhibiting a KD value comparable to that of the wild-type Gh-TDH at the micromolar level. Strikingly, the protein motif responsible for the 3′–5′ exonuclease activity of Gh-TDH differs significantly from the known exonucleases, implying that TDH undergoes a distinct mechanism for DNA cleavage. Our findings provide structural insights into the remarkable DNA-binding region of TDH and unveil the first hemolysin with 3′–5′ exonuclease activity.

P-20-014
Oxidative cyclization in cyclopiazonic acid biosynthesis

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Cyclopiazonic acid (CPA) is a pentacyclic indole natural product and a nanomolar inhibitor of sarcoplasmic/endoplasmic reticulum calcium-adenosine triphosphatase (SERCA). CpaO is a FAD-dependent oxidoreductase and was proposed to catalyze dehydrogenation of β-CPA to form a pentacyclic compound; however, the enzyme catalytic mechanism about the unusual intramolecular ring closure is still a mystery. Here, we used a combination of biochemical characterization, mutagenesis analysis, and structural biology approaches to unravel the catalytic mechanism of CpaO. The crystal structures of CapO in apo form and in complex with the substrate β-CPA and the product α-CPA, respectively, shed light on the substrate binding site and the catalytic mechanism. *The authors marked with an asterisk equally contributed to the work.

P-20-015
Artificial bifunctional chimeras of myeloperoxidase and glucose oxidase: antimicrobial, topological and enzymatic properties

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Substrate channeling and increased local substrate concentration are key advantages in nature to promote the proximity of two enzymes. We studied the bacterial myeloperoxidase enzyme, which produces microbicidal compounds (i.e. bleach) derived from halogenated substrates [1]. The enzyme requires hydrogen peroxide to produce these compounds, and glucose oxidase was used as the H_2O_2 supplier [1]. To optimize the system and the coupling between the two active sites of these enzymes, chimeras were produced. To produce a bifunctional oxidase/peroxidase enzyme by genetic and protein engineering, the open reading frames encoding glucose oxidase from *Penicillium amagasakiense* and myeloperoxidase from *Rhodopirellula baltica* were fused. This has enabled us to acquire a library of chimeric proteins with or without linkers of varying length, amino acid type, charge or flexibility. Firstly, a robust protocol for the production and purification from inclusion bodies was developed. Next, the enzymes were enzymatically characterized. The activities of the individual active sites were carried out and the activity of chimeras was performed with different concentrations of the two substrates, glucose and NaCl, in order to control the chlorination activity of the enzymes. Then, microbicidal assays were performed on *Escherichia coli* American Type Culture Collection number 25922 to test the enzyme's stability. We studied also the different chimera at low-resolution (AFM, TEM, cryofracture, SAXS).We showed that linkers are very important for correct folding and activity of the glucose oxidase and are able to classify them in function of their efficiency. Chimerization present also a great interest to promote antimicrobial activities of the myeloperoxidase towards chloride anions.Reference: 1. Céré, C et al. BioTech, 2023. 12, DOI: 10.3390/biotech12020033. *The authors marked with an asterisk equally contributed to the work.

P-20-016
An unusual hyperthermophile glycoside hydrolase that converts starch into small malto-oligosaccharides through an integration of exo-amylase and glucanotransferase activity

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PfuAmyGT (PF0272) is a homodimeric, three-domain, GH57 glycoside hydrolase from *Pyrococcus furiosus*, and has previously been proposed to be either an α-amylase, or a 4-α-glucanotransferase. Our work demonstrates that PfuAmyGT integrates both the functionalities, enabling it to (i) efficiently convert starch into

a pool of small malto-oligosaccharides (ii) less efficiently disproportionate an individual malto-oligosaccharide into the same pool of malto-oligosaccharides. The structure of the recombinant enzymes was analysed through different biophysical techniques like circular dichroism and size exclusion chromatography and the activity through different biochemical techniques like thin layer chromatography and high-performance anion exchange chromatography with pulsed amperometric detection. The results suggest that PfuAmyGT uses (i) an exo-amylase function in domain 1, to processively excise the terminal glucose from the starch chain; (ii) a loop motion in domain 2, to relocate the excised glucose to a groove [surface binding site (SBS)] situated between domain 2 and 3; AND either (iii) a glucanotransferase function, to transfer the relocated glucose to an acceptor saccharide bound to the SBS; or (iv) a glucose-releasing function, to release the excised glucose to water which can further act as an acceptor. In-depth examinations of PfuAmyGT, including analyses of alanine-substituted mutants of PfuAmyGT and chimeras created through domain-swapping with TonAmyGT (a homolog from *Thermococcus onnurineus*), reveal that PfuAmyGT hosts more than one site, four catalytic residues (E131, D222, E224, D362), and distinct functions in all three domains. The coupled exo-amylase and glucanotransferase activities are demonstrated to be accelerated by long donors and short acceptors, indicating distinct turnover rates at separate sub-sites. Thus, this study highlights the intricate molecular architecture and versatile functionality of PfuAmyGT in starch processing.

P-20-017
Towards CATCHFIRE-based chemogenetic tools to image endogenous proteins

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A central challenge in biology is to comprehensively characterize the cellular role of the proteins encoded in the human genome. To systematically study protein function in a native cellular background, it would be highly valuable to tag proteins at their endogenous loci with a functional sequence that can give access to their localization and to ways of controlling their functions. In this project, we will take advantage of CATCHFIRE (CHemically Assisted Tethering of CHimera by Fluorogenic Induced REcognition) a fluorogenic chemically induced dimerizing technology that relies on the rapid and reversible dimerization of two small genetically encoded domains, ^{FIRE}tag and ^{FIRE}mate, upon addition of a fluorogenic chemical inducer of proximity called match[1]. The use of a small tag such as ^{FIRE}tag (only eleven amino acid residues) will facilitate genome editing in addition to reduce the genetic footprints and the dysfunctional fusions reported when using larger tags while incorporating unprecedented fluorescence imaging capabilities. Our experimental approach will be based on the functional tagging of endogenous loci with the minimal tagging sequence ^{FIRE}tag. We showed that the ability of ^{FIRE}tag to complement with ^{FIRE}mate in presence of match and form a fluorescent assembly allowed the efficient fluorescent labelling of various ^{FIRE}tagged proteins in different cellular localizations in mammalian cells (e.g. the nucleus, the endoplasmic reticulum, the Golgi apparatus, the nuclear membrane, the cytoskeleton, the mitochondria, the microtubules). Genome-edited libraries of ^{FIRE}tagged loci will next be generated via genetic knock-in by homology-directed DNA repair. This scalable method for the robust and specific tagging of endogenous proteins will be extended to a large number of mammalian proteins for imaging and functional studies. [1]Previously published in: Bottone et al. (2023) Nat Methods 20, 1553–1562.

P-20-018
Two ω-transaminases from the Ectoine biosynthesis and degradation pathways

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Omega (ω)-transaminases are biocatalysts highly sought after in the pharmaceutical industry as a green alternative for the production of valuable chiral amines. The DABA ω-transaminases EctB and DoeD catalyze the forward and reverse transaminase reactions in the bacterial ectoine biosynthesis and degradation pathways, respectively. Ectoine is a highly valuable compound used in both the cosmetics and pharmaceutical industries due to its many novel properties, such as acting as a protein stabilizer, DNA protector, and membrane, cell, and skin protectant. It is primarily produced by a method called bacterial milking, but more recent research has focused on heterologous production using non-halophilic bacteria such as *Escherichia coli*. Despite the high market demand for ectoine, research on characterizing the enzymes in the ectoine biosynthesis and degradation pathways has been limited. We hereby present our recent, unpublished work where we have solved the first crystal structure of DoeD at a resolution of 1.5 Å and completed a biochemical and biophysical characterization of the transaminase, including exploring the substrate scope for other potential uses of the enzyme as a pharmaceutical biocatalyst. Previous work by the Ectoine Research Group includes solving the crystal structure of EctB from the model organism for ectoine production, *Chromohalobacter salexigens* DSM 3043[1]. The group aims to characterize all the core enzymes involved in ectoine synthesis, including EctB, EctA, and EctC, as well as DoeD from the ectoine degradation pathway. Future work will also prioritize rational design to improve the operational stability and efficiency of the transaminases, especially EctB and DoeD. 1. Previously published in: Hillier HT et al. (2020) FEBS J 287, 4641–4658.

P-20-019

Abstract withdrawn.

P-20-020
Effect of glycine on the heterotrophic growth and [NiFe]-hydrogenase activity of *Cupriavidus necator* H16

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The chemolithoautotrophic H_2-oxidizing β-proteobacterium *Cupriavidus necator* demonstrates a versatile metabolism. It is known for its capacity to synthesize four different O_2-tolerant

[NiFe]-hydrogenases (Hyds) [1], which present promising anodic biocatalysts in microbial fuel cells. It has previously been shown that enrichment of the heterotrophic growth medium with certain amino acids significantly increase yield and activity of O_2-tolerant Hyds of *C. necator* under aerobic conditions [2]. Here, a *C. necator* variant overproducing the soluble NAD^+-reducing hydrogenase (SH) was grown microaerobically in glycerol-fructose-nitrogen medium supplemented with 7 µmol/ml glycine at 30°C and 130 rpm for 168 h and the SH was purified by Strep-Tactin-based affinity chromatography. Glycine supplementation led to a decrease of the bacterial biomass formation (OD_{436nm} = 7.8) compared to glycine-free mineral medium (OD_{436nm} = 13). However, the specific H_2-driven NAD^+ reduction activity purified from the glycine-supplemented medium, measured spectrophotometrically at 365 nm was threefold higher (70 U/mg/min) than that of SH isolated from the glycine-free culture. Glycine supplementation also increased the yield of SH enzyme, as demonstrated by SDS-PAGE and western blots using antibodies against the SH subunits HoxF, HoxH, HoxU, and HoxY. The results underline the positive effect of amino acid supplementation for the isolation of highly active hydrogenases, thus paving the way for biotechnologically relevant applications. References: 1. Lenz O et al. (2018) *Methods Enzymol.* 613: 117–51. 2. Iskandaryan M et al. (2023) *AMB Express*, 13(1), 1–12. *The authors marked with an asterisk equally contributed to the work.

P-20-021
Exploiting different strategies for the recombinant production of antimicrobial peptides

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Antimicrobial peptides (AMPs) are broad-spectrum host defense molecules, considered as promising candidates to face the crisis of antimicrobial resistance. The human cathelicidin LL37 and its shorter fragments are of great interest for their antibacterial and anti-biofilm properties, also against multi-drug resistant pathogens. Since huge amounts of AMPs are required for clinical applications, the optimization of their production is needed. In this field, recombinant expression represents the most cost-effective option [previously published in: Pennone V et al. (2024) Front Microbiol, submitted]. *E. coli* is the most used prokaryotic expression system, despite two challenges have to be addressed: potential toxicity and proteolytic degradation of AMPs. We applied a fusion strategy to express LL37 peptide in *E. coli* cells linked to the small metal-binding protein (SmbP) carrier to reduce the AMP toxicity and improve solubility. The SmbP-LL37 protein was purified from the crude extract by affinity chromatography with a volumetric yield of 2.4 mg/l and digestion with enterokinase was performed to isolate the LL37 peptide. Since obtaining the peptide without its fusion partner is challenging, an alternative production strategy was established. The FK16 peptide, a promising shorter version of LL37, was expressed in *E. coli* in the form of inclusion bodies. The peptide was solubilized upon treatment of the cell lysate with Triton X-100 and purified by affinity chromatography, obtaining ≈5 mg of peptide per liter of cell culture. The latter expression strategy seems well suited for producing additional LL37 fragments and thus to evaluate their antibacterial properties, assess their conformational changes and investigate bacterial membrane degradation using fluorescent dyes. This work has been supported by Fondazione Regionale per la Ricerca Biomedica (Regione Lombardia), project ID 3414083 AMPROject.

P-20-022
Robust hemicellulolytic enzymes secreted by thermophilic microbial consortia isolated from a biogas plant

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Lignocellulose biomass (LCB) is an abundant feedstock to produce value-added chemicals. Yet, LCB valorisation is challenging because of its complexity and recalcitrance to enzymatic hydrolysis. Thermophilic enzymes represent valuable tools to setup effective biomass deconstruction protocols; thus, aiding circular economy-inspired industrial applications. In this study, we report the characterisation of enzymes secreted by two microbiomes, namely CMC-50 and XYL-50, which were previously enriched at 50°C first on spent mushroom substrate (SMS) and then on two pure carbon sources (CMC and xylan)[1]. Microbiomes were proliferated on SMS as the sole carbon source to induce enzymes secretion. Xylanases in cell-free supernatants (CFS) were active over a wide pH range, with an optimum at pH 7. Enzymes activity was highest at 60°C for CMC-50, although they were more stable at 50°C; retaining over 80% of their initial activity for three days. On the other hand, enzymes secreted by XYL-50 displayed a higher temperature optimum (65°C). Noteworthy, both CFS fully retained their maximum active over two months when stored at 4°C. Besides xylanases, hydrolysis tests of wheat flour arabinoxylans and ivory nut mannans, highlighted the presence of α-L-arabinofuranosidases in both CFS, and of endo-1,4-β-mannanases in the CFS from CMC-50. To uncover further differences in degradation, a comprehensive phylogenetic analysis was conducted, and putative glycoside hydrolases (GHs) enzymes were predicted to be involved in CMC and xylan degradation. This analysis showed that very similar set of GHs from closely related *Bacillus licheniformis* strains played a pivotal function in both microbiomes, underlaying a crucial role of gene regulation and of synergic catalytic activities that can be exploited to formulate more effective enzymatic cocktails for biotechnological applications. Reference: 1. Bombardi, L. et al. (2024) Int. J. Mol. Sci., 25(2), 1090; https://doi.org/10.3390/ijms25021090.

P-20-023
Self-labelling-protein-tags from extreme sources: pushing on the boundaries for the development of new biotechnological tools

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SLP-tags such as the SNAP-*tag*® and the *HaloTag*®[1,2], by their nature[1] or suitably engineered[2], have the ability to specifically react with their respective substrates but covalently retaining a part of them in the catalytic site after the reaction. However, these mesophilic tags have the disadvantage to be employed only in mild reaction conditions[3]. Recently, we focused attention on the identification, characterization and engineering of AGTs from thermophilic microorganisms[4], aiming to develop new thermostable SNAP-*tags* to be applied as biotechnological tools in *in vitro* harsh reaction conditions[3-4], as well as in *in vivo* heterologous expressions in thermophilic model organisms[3,5]. Starting from the engineered variant of the *S. solfataricus* OGT used as thermostable SNAP-*tag*[3], we furtherly engineered this enzyme achieving, for the first time, a thermostable version of the CLIP-*tag*®[6], showing a modified substrate specificity, and thus allowing a simultaneous multi-protein labelling in extreme reaction conditions. Knowledge of the amino acid residues involved in substrate specificity[6] has led to the identification of AGTs from the archaebacteria *Pyrococcus furiosus* and the Antarctic fish *Trematomus bernacchii*. In the former, new engineered versions of SNAP- and CLIP-*tag* with a further increase in stability were achieved (with activities close to the water boiling point), while the AGT wild type of the latter was confirmed to be extremely active at the water freezing point. The validity of these new proteins as biotechnological tools in order to expand the SNAP-*tag* technology is discussed. References: 1. Keppler A, *et al*. (2003). *Nat. Biotechnol*., 21, 86-89. 2. England CG, *et al*. (2015). *Bioconjug. Chem*., 26, 975–86. 3. Vettone A, *et al*. (2016). *Extremophiles*, 1, 1–13. 4. Mattossovich R, *et al*. (2020). *Extremophiles*, 24, 81–91. 5. Visone V, *et al*. (2017). *PLoS One*, 12, e0185791. 6. Merlo R, *et al*. (2022). *Comput Struct Biotechnol J*, 20, 5275–5286.

P-20-024
The role of terbium ions on paraoxonase 1 activity

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Paraoxonase 1 (PON1) is a metallohydrolase involved in various biological processes, including lipid metabolism and detoxification of organophosphates. PON1 is primarily associated with high-density lipoprotein (HDL) in the blood and contributes to the antioxidant and anti-atherosclerotic capabilities of HDL. It hydrolyzes and thereby inactivates the toxic organophosphorus insecticides, as well as nerve agents. PON1's activity varies significantly among individuals due to genetic polymorphisms affecting its expression and functionality. PON1 contains two calcium ions: a structural Ca^{2+} ion that binds with high affinity and is crucial for protein stability, and a catalytic Ca^{2+} ion within the active site that can be displaced without affecting the enzyme's structure. However, the PON1's enzymatic capabilities disappear in the absence of calcium ions. Exploring the effects of lanthanide ions, which share similar ionic radii and coordination properties with calcium ions, on PON1 could enhance our understanding of calcium's structural and functional roles in the enzyme. We conducted a detailed kinetic analysis, comparing the enzyme's activity with and without terbium ions. Additionally, we investigated the binding affinities and thermodynamic interactions between PON1, calcium, and terbium ions by carrying out isothermal titration calorimetry (ITC). Our kinetic study, examining the impact of terbium ions on PON1's substrate turnover using dihydrocoumarin and thiophenyl acetate, uncovered intricate reaction mechanisms. Our findings indicate that the terbium ions can replace the catalytic calcium ions in PON1, driving the process spontaneously while leading to a reversible loss of lactonase and arylesterase activities. Kinetic and ITC analyses demonstrated that terbium ions bind over 100-times more strongly to PON1's calcium-binding sites than calcium ions themselves. *The authors marked with an asterisk equally contributed to the work.

P-20-025
Discovery and characterization of antiphage defense enzymes from cold-adapted bacteria

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Bacteria have multiple lines of defense against bacteriophages and research on the topic is rapidly expanding our knowledge regarding the complexity, prevalence, and distribution of these systems. Despite this progress, little is known about the antiphage defense systems of cold-adapted bacteria. Cold-adapted bacteria are thriving in Earth's vast cold habitats and have evolved unique mechanisms for survival, facing challenges such as reduced enzyme activity and protein cold denaturation. Using the bioinformatics tools, Prokaryotic Antiviral Defense LOCator (PADLOC) and CRISPRCasTyper we have mapped the presence and diversity of antiphage defense systems in over 900 available high-quality genomes of cold-adapted bacteria. Our findings reveal that RM, dXTPases, and Abi systems have high prevalence among the analyzed genomes, whereas CRISPR systems appear less common. Some antiphage defense systems can be modified and applied as biotechnological genome editing tools. We have identified enzymes, such as prokaryotic Argonautes, retron reverse transcriptases and CRISPR-associated (Cas) endonucleases, with potential for further characterization and development as genome editing tools. A limitation of the genome editing platforms available is that most tools are developed from and optimized for mesophilic organisms, which may restrict their utility in cold living organisms. To address this gap, we are currently recombinantly expressing and characterizing five Cas endonucleases to assess their potential as cold-active genome editing tools.

P-20-026
Immobilization study of β-mannosidase from *Cellulomonas fimi* and its application in the synthesis of glycoconjugate vaccine precursors

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The β-D-mannopyranoside linkage is found in a number of biological structures, in particular, in the core pentasaccharide of *N*-linked glycoproteins, as well as within the antigenic lipopolysaccharides of *Salmonella* sp., *Klebsiella* sp. and *Candida* sp. [1] The construction of this glycosydic bond by chemical approach is very challenging and requires cumbersome protection and activation steps prior to glycosylation. [2] In this context, β-mannosidase from *Cellulomonas fimi* (Cf-β-Man) was immobilized for the first time, and it was screened in the synthesis of a panel of different β-mannosides. In the enzymatic transglycosylation reaction, different free monosaccharides (D-mannose, D-mannose-SCH_2CN, *N*-acetyl-D-glucosamine, and *N*-acetyl-D-glucosamine-SCH_2CN) were used as acceptors and *para*-nitrophenyl-β-mannoside as a donor. Cf-β-Man immobilized on IDA-Co^{2+}-agarose allows the synthesis of the disaccharide, cyanomethyl β-D-mannopyranosyl-(1→6)-2-acetamido-2-deoxy-1-thio-β-D-glucopyranoside, with a higher conversion compared to the soluble enzyme (20% vs. 5%) after 6 h under best conditions. [3] This explorative work opens new scenarios concerning the design of engineered Cf-β-Man mutants and their immobilization in order to obtain a robust and recyclable biocatalyst for applications in the chemoenzymatic synthesis of glycans as glycoconjugate vaccine precursors. This work was supported by the Italian Ministry of Health (Project Immunoterapia: cura e prevenzione di malattie infettive e tumorali (Immuno-HUB), project number T4-CN-02). References: 1. Liu J et al. (2021) ACS Catal 11, 2763–2768. 2. El Ashry ESH et al. (2005) Curr Org Synth 2, 175–213. 3. Previously published in: Robescu MS et al. (2023) Catalysts 13, 1399.

P-20-027
Capturing oligomeric states of EcLon involved in its regulation

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LonAs are highly regulated AAA+ Ser-Lys proteases that play a crucial role in protein quality control in both prokaryotes and eukaryotes. However, many details on how LonAs operate are still not clear, including the relevance of the hexameric vs dodecameric structures. The structure of Lon N-terminal domains (NTDs) is not well defined due to flexibility required for LonA oligomers to function. To get more accurate structures of the highly dynamic NTDs of LonA we raised a number of nanobodies specific to that part of *E. coli* LonA molecule. Properties of the complexes with one of them, E9, were evaluated by both biochemistry and electron microscopy. We found that E9 is able to completely inhibit the ATPase activity of Lon and prevents both the peptidase and proteolytic activities. Negative stain and cryo-EM studies of the complex showed the enrichment of the quaternary structure in which only 10 protomers are visible, interacting through nanobody-mediated contacts between NTDs. We also observed the formation of higher-order oligomers in solution in both the presence and the absence of nanobodies and further identified different states of the oligomers using cryo-EM. We observed structures of 12-, 11-, and 10mer oligomers. In all cases the interface was formed by the intricate interaction of the NTDs of Lon. Interestingly, the arrangement of the interface NTDs is distinctly different in the three configurations. The 12mer state forms a symmetrical arrangement of the two hexamers with a contiguous channel connecting the two. The 11mer and 10mer states have the two oligomers arranged at an angle with the NTDs blocking the formation of a contiguous channel between them. We hypothesize that the different states achieved by Lon may regulate its activity and the number of active proteins in the system. However, further experiments are underway to assign functional relevance to the different quaternary states of Lon. Supported by intramural NIH and by RSF project 21-74-20154.

P-20-028
Playing lego with a chitinase structure

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Chitinase (EC 3.2.1.96) from *Moritella marina* (*Mm*Chi60) is a 60 kDa enzyme that belongs to the GH18 family and consists of 4 domains linked in a chain-like structure (from N-end): the catalytic TIM-barrel domain (CAT), 2 consecutive Ig-like domains (Ig1 and Ig2) and a chitin-binding module (CBM). The highly elongated structure of the protein was revealed by X-ray crystallography (Malecki PH et al. (2013) Acta Cryst D69, 821–829). The temperature melting profile was done using DSC and shows several distinct peaks indicating large differences in stabilities of the domains [Stavros P et al. (2015) Biochem Biophys Rep 3, 108-116]. In order to explore how each domain of the protein influences its structure, activity and stability several different mutants have been designed, each containing a different set of domains from the original *Mm*Chi60. However, this molecular lego system turned out to be difficult to research, especially in the case of mutant C (domains: CAT & CBM linked). Three mutant C versions were produced, with different links (consisting of 2 (C-2), 5 (C-5) or 8 (C-8) amino acid residues) and all were prone to degradation, occurred by an unknown mechanism, despite the fact that all were probably well folded, as the DSC data showed. Finally, we succeeded in obtaining a crystal structure for mutant C-5 with 2 crystallographically independent molecules in the asymmetric unit and the CBM domain assuming 2 alternative positions relative to the CAT domain. The flexing between the domains starts at Asp347 and results in a shift of up to 4.6Å at the other end, i.e. the "foot" of the CBM domain. The 3 Trp residues that were observed to be co-planar in the native structure and proposed to be part of the surface interacting with chitin, are observed in the C-5 mutant structure to interact with the neighboring protein molecules and are not co-planar. The research was co-funded by the National Science Centre, Poland (UMO-2017/27/B/NZ1/02201).

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P-20-029
Structural and functional characterization of phage tailspikes

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Antimicrobial resistance (AMR) is nowadays a pressing public health concern, notably exemplified by the 'ESKAPE' pathogens, resistant to traditional clinically used antibiotics [previously published in: De Oliveira DMP et al. (2020) Clin Microbiol Rev 33 (3)]. Structural insight of molecular factors which play a key role in AMR is fundamental to understand their mechanisms on and develop effective new drugs. *Klebsiella pneumoniae*, one of the most harmful bacteria belonging to ESKAPE family, exploits various instruments to evade the immune system response and antibiotic attacks. Among these is the cell envelope, consisting of two main barriers: capsular polysaccharides (CPS) and lipopolysaccharides (LPS). Bacteriophages evolved to overcome these bacterial defenses: thanks to highly specific depolymerases – enzymes incorporated into virions' tail spike – they can hydrolyze CPS structures and make them susceptible to the antibiotic action. In collaboration with the Wroclaw University of Poland, we have performed functional studies focused on CPS degradation by depolymerases action through microbiological assays and mass-spectrometry analysis and structural studies both on depolymerases and their complexes with CPS-derived sugars through X-ray crystallography. These studies have allowed us to shed light on the mechanism of action of these proteins [previously published in: Squeglia F et al. (2020) Structure 28(6):613–624]. It is known that, generally, wild type depolymerases adopt a homotrimeric structure. Surprisingly, setting up experiments of protein engineering, we also identified a smaller version of this enzyme, active as monomer [previously published in: Maciejewska B et al. (2023) mBio 14(5)]. Deep knowledge of their structures is a crucial step to engineer recombinant enzymes with a smaller size and a broader spectrum of action against infectious pathogens.

P-20-030
Regulation of lignin-modifying enzymes gene expression in *Trametes versicolor* in the presence of cytostatic drugs

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Anticancer drugs that exhibit resistance to wastewater treatment pose a potential environmental threat. Therefore, to eliminate cytostatics from waters, technologies based on white-rot fungi (WRF) are worth particular attention. These organisms possess the capability to remove a broad spectrum of xenobiotics, thanks to their substrate non-specific oxidative enzymatic system. Our prior studies have demonstrated that the effective elimination of specific cytostatic drugs is achievable through WRF technology, with the removal process primarily associated with the activity of laccases. Hence, we endeavoured to investigate whether the presence of pharmaceuticals induces biochemical changes in mycelia, specifically the regulation of oxidative enzymes. This study aimed to examine the gene expression of laccase (*Lacc*), manganese peroxidase (*MnP*), and lignin peroxidase (*LiP*) in *Trametes versicolor* (strain CB8) during 9-days biodegradation tests of bleomycin and vincristine at an initial concentration of 10 mg/l. Assessment of the studied transcripts level was performed with the use of RT-qPCR, following the SsoAdvanced Universal SYBR Green Supermix (Bio-Rad, USA) reaction protocol using a CFX96 system (Bio-Rad, USA). The results indicate that, at the end of observation, *Lacc* and *MnP* genes are poorly expressed in bleomycin-treated *T. versicolor* (CB8) cells compared to control samples without the drug. On the contrary, the induction of *LiP* expression was observed as a result of bleomycin activity. Vincristine did not alter the expression of both peroxidases, but it significantly up-regulated *Lacc* expression, correlating with the more effective removal of this drug. These findings lay the groundwork for comprehending the stimulation of metabolite production and the intensification of the cytostatic drugs' biodegradation process using filamentous fungal culture. The study was financed by the National Science Centre, Poland, project number: UMO-2020/37/N/ST8/01077.

P-20-031
Construction of a drug candidate screening system based on HIV-1 Rev/RRE functional activity

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The human immunodeficiency virus (HIV) remains a significant health problem. HIV inhibitors in clinical practice have a number of limitations due to their lifelong use and toxicity. Therefore, the development of new drugs with anti-HIV activity is essential. For this purpose, small HIV regulatory proteins (vpr, tat, rev) are being actively studied and can serve as potential drug targets for new anti-HIV compounds screening. Rev plays a pivotal role in the life cycle of the virus to translate unspliced viral RNA from nucleus to cytoplasm for subsequent translation of structural proteins. The arginine-rich motif in the Rev structure acts as a nuclear localization signal (NLS) and a binding site for the Rev response element (RRE) on the virus mRNA. Eliminating the formation of the Rev-RNA complex could be an effective antiviral strategy, which have been previously shown for a number of compounds. When screening compounds, it is rational to obtain a system to check the inhibitory activity of compounds *in vitro*. In order to create such system, it is necessary to obtain a high purity protein and choose a method for detection of the presence or absence of inhibitory activity of compounds. We have created the HIV-1 rev construct capable of protein expression in *E.coli* with the C-terminal 6His-tag. Rev isolation and purification has been optimized, allowing to obtain the protein in a good yield (up to 19 mg/l), high purity (up to 99%) and free of bacterial RNA contamination. The functional activity of recombinant Rev was confirmed *via* electrophoretic mobility shift assay (EMSA). Based on the recombinant Rev and the RNA matrix containing the RRE element, we have developed and tested a system for screening inhibitors that disrupt the formation of the rev-RRE complex. Previously unpublished compounds synthesized in our laboratory have been tested as inhibitors of the Rev-

RRE complex formation. The research was supported by the Russian Science Foundation (grant №20-74-10121-P). *The authors marked with an asterisk equally contributed to the work.

P-20-032
Development of targeted protein degradation system based on RING domain of E3 ligase Ark2C

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Ubiquitination is an essential post-translational modification of proteins in eukaryotic cells, facilitated by the sequential actions of ubiquitin-activating enzyme (E1), ubiquitin-conjugating enzyme (E2), and E3 ubiquitin ligase. The conjugation pattern of ubiquitins dictates the subsequent fate of substrate proteins, particularly their proteasomal degradation. Recently, numerous efforts to exploit the ubiquitin-proteasome system for the selective degradation of target proteins have proven successful. Proteolysis-targeting chimeras (PROTACs) are heterobifunctional molecules that recruit E3 ligase and target proteins, ultimately promoting ubiquitination and subsequent degradation. However, PROTACs have intrinsic disadvantages, such as low solubility, cellular permeability issues, and challenges in synthesis and activity regulation. Here, we developed an endogenous Bcl-xL & MCL1 degrader by engineering the E3 ligase Ark2C. Ark2C possesses a RING domain responsible for target ubiquitination, capable of mediating ubiquitination of both the target and itself, only with the assistance of regulatory ubiquitin molecules and no other partner proteins. Bcl-xL and MCL1, anti-apoptotic BCL2 family proteins, are often overexpressed as part of the survival strategy of cancer cells. Previous work by Kim et al. reported the computationally designed cobinder protein, 4H_αBM3, against Bcl-xL and MCL1, which simultaneously inhibited their function and induced apoptotic cell death. By fusing 4H_αBM3 to the RING domain of Ark2C, we successfully degraded endogenous BclxL and MCL1. Notably, the 4H_αBM3-Ark2C complex exhibited self-degradation, potentially mitigating off-target effects. In conclusion, we have developed a simple, self-destructive degrader system based on the RING domain of the E3 ligase Ark2C, which can be efficient and safe therapeutic degradation strategy applicable to various target proteins. 4H_αBM3 was previously published in: Kim et al. (2022) Comput Struct Biotechnol J 20, 3019-3029.

P-20-033
Engineering thermostable LCC for enhanced PET hydrolysis at moderate temperatures

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The breakdown of polyethylene terephthalate (PET) is essential for prompting the goals of a circular bioeconomy aimed at reducing the environmental footprint of plastic materials. In this context, the enzymatic hydrolysis of PET becomes a pivotal procedure, allowing its bioconversion into monomers which could then serve as starting materials to yield valuable chemicals. The most promising PET hydrolase, in terms of depolymerization efficiency, is the thermostable leaf-branch compost cutinase (LCC). Evolved variants of the enzyme possessing a higher activity on PET have been produced by a semi-rational design approach, such as the S101N/F243T ΔLCC variant which is able to fully depolymerize 1.3 g of untreated postconsumer PET waste in less than 3 days at 55°C [as previously published in Pirillo et al. (2023) FEBS J, 290: 3185–3202]. Although this optimal temperature is lower than that for the wild-type one, and suitable for *in vitro* polymer biodegradation, the integration of LCC into one-pot cellular systems designed for the upcycling of PET (engineered whole cell biocatalysts) is hampered. To address this issue, we focused on evolving LCC variants with significant activity at moderate temperatures while retaining a significant thermal stability. To reach this goal a high-throughput screening protocol has been set up and protein engineering studies, supported by bioinformatic analysis, are carried out by various approaches, including site-directed mutagenesis, error-prone PCR, and DNA shuffling. Interesting clones are further evaluated for their activity on PET nanoparticles under different bioconversion conditions. Within the framework of a circular bioeconomy, this research marks the beginning for the set-up of bioconversion systems with the objective of extracting value from post-consumer environmental pollutants. This work is part of the ProPla project (funded by Fondazione Cariplo).

P-20-034
Screening for new enzymes acting on modified heterocyclic bases

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Ribonucleic acids are essential to many processes of life and, to fulfil their functions, they have numerous chemical modifications. Most of such modifications are found in tRNA, where they affect metabolism, structure, stability, localisation and transport. Despite the huge progress in discovery of enzymes that introduce chemical modifications into tRNA and the numerous investigations of their role in cell physiology and disease, much less known about the catabolism of the modified nucleotides and corresponding heterocyclic bases. In this study, using the organic synthesis methods, several *N, O, S*-methylated pyrimidine (such as 2-methylthiocytosine, 2-methylthiouracil) and purine (such as 6-methylthioguanine, 6-methoxyguanine, *N6*-methyladenine) heterocyclic bases were synthesized, purified and characterized. These and other commercially obtained modified heterocyclic bases were used for screening of genes involved in their catabolism. The uracil or purine auxotrophy-based selection screening systems, similar to previously published in: Aučynaitė A et al. (2018) J Front Microbial 9, 2375; Urbelienė N et al. (2023) Sci Adv 9, 4361, allows the selection of enzymes converting the modified pyrimidines and purines into the unmodified counterparts. Also, new metagenomic libraries, created by isolating DNA from the environmental samples, sharing and cloning into the multicopy cloning vectors, were used for selection of genes involved in metabolism of modified heterocyclic bases. Several candidate DNA fragments were obtained during the screening procedure and are currently under the investigation. This project has received funding from the Research Council of Lithuania (LMTLT), agreement No [S-MIP-22-71].

P-20-035
Characterization of a novel thermophilic protein with β-xylosidase activity expressed in *Saccharomyces*

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Thermophilic enzymes have garnered significant interest due to their potential applications in various industrial processes. In this study, we employed a sequence-based metagenomics approach to explore the genetic diversity of a hot spring metagenome, with the aim of discovering new thermophilic xylanases. Xylanases, specifically endo-1,4-β-xylanases, along with β-xylosidase enzymes, play a vital role in the complete hydrolysis of xylan to xylose, a major component of plant cell walls, making them valuable in the food industry such as the production of prebiotics (Saavedra-Bouza et al., 2023). We identified a gene encoding a putative β-xylosidase from the genome of a microorganism isolated from an enriched culture. The culture was performed with water from the geothermal spring of Rio Caldo (Ourense, Spain), enriched with wheat straw (Molifibra-Molienda y Granulación, S. L., Burgos, Spain) as the sole carbon source. The gene was expressed in *Saccharomyces cerevisiae* BJ3505 to evaluate its activity and assess its potential for biotechnological applications. The purified protein obtained was subjected to extensive biochemical characterization, including determination of optimum pH and temperature and thermostability. The highest activity was measured at 70°C and pH 7. Moreover, this enzyme maintains a high relative activity at 60°C for 24 h and the activity was decreased by only Cu^{2+} and Zn^{2+} cations. The characteristics of the enzyme make it potentially interesting for xylan hydrolysis and obtaining prebiotics in the food industry. Acknowledgement to: RTI2018-099249-B-100. Ministerio de Ciencia, Innovación y Universidades. ED431C2020-08. Conselleria de Cultura, Educación e Ordenación Universitaria. Xunta de Galicia, co-financed by ERDF Reference: Saavedra-Bouza, A., Escuder-Rodríguez, J.-J., deCastro, M.-E., Becerra, M., & González-Siso, M.-I. Xylanases from thermophilic archaea: A hidden treasure. Current Research in Biotechnology, 5, 100116 (2023).

P-20-036
Application of *in silico* prediction tools for enhanced production of soluble DEK1 fragments

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The effective use of available *in silico* tools has shown to be of immense value when applied to protein engineering, whether it is only for increased solubility or creating novel functions of the target molecule. In this work, we explore the multi tool approach in engineering increased solubility of DEK1 fragments (CysPc, CysPc-C2L), notorious for their insolubility and low levels when produced in *Escherichia coli*. Since no structural determinations of these proteins are available, this work involves *in silico* structure modelling to allow solubility improvements by tools relying on these measurements and calculations. To minimize the error rate, we have opted for multiple rounds of model refinement and various tool application. The results suggest that as few as two point mutations can contribute to significant increase of particular fragment solubility and combination of five mutations stabilizes the protein for further purification steps and subsequent protein crystallography procedures. Acknowledgements: This work was supported by Slovak research and development agency grants APVV-17-0333, APVV-21-0215, APVV-22-0161 and APVV-21-0227.

P-20-037
Bioprospecting of polar environments: enzymatic activities of extremophilic bacteria studied through *in vitro* assays and genomic analyses

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Extreme regions such as the Arctic and Antarctica are being increasingly studied. Bioprospecting of these areas can lead to the discovery of novel microorganisms and biomolecules adapted for these ecological niches. Their peculiar features make them excellent candidates for potential applications in various industrial sectors, including the biodegradation of plastic pollutants. Experiments have been performed to identify and to characterize extremophilic enzymes, also called extremozymes, of the classes of the carboxylic ester hydrolases (EC 3.1.1) and of the glycoside hydrolases (EC 3.2.1). Starting from environmental samples collected in the North and the South Poles, extremophilic bacteria have been isolated through screenings with agar plate-based assays on different media. Qualitative plate analysis allowed the

detection of catalytic activities such as amylases, xylanases, and non-specific polyesterases. Spectrophotometric assays with synthetic chromogenic substrates have been used to further test the positivity *in vitro*, and extracellular enzymatic activities have been highlighted in the cell-free supernatants after bacterial growth in standard conditions until the stationary phase. This have allowed to define the specificity of the enzymes onto classes of substrates under the assay conditions. In parallel, *in silico* screenings have been implemented with the RAST Annotation Server to process the genome sequences of selected bacterial strains. The biodegradation potential is currently being validated with thermogravimetric analysis on the depolymerization of plastic pellets after static and low temperature exposure, showing cold-adapted lipolytic activities of interest. This research was partially supported by PNRA19_00073 (TENORE), PNRA18_00232 (AMICI), the PNRR European Commission – NextGenerationEU, Project "SUS-MIRRI" n. IR0000005, and by FOE-CNR FutuRaw Project.

P-20-038
Inside carbohydrate-active enzymes: a novel enzyme from Pisciarelli hot spring discovered by a metagenomic approach

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The discovery of novel enzymes by (meta)genomic approaches advanced our understanding of carbohydrate-active enzymes (CAZymes www.cazy.org), catalysts that find applications in a variety of fields encompassing industrial and biomedical biotechnological applications. Exploring the biodiversity of extreme environments has recently allowed us to increase our knowledge of CAZymes and offer the opportunity to test them in second-generation biorefineries [1,2]. In the GH122 family, currently showing 84 entries exclusively from Archaea, only the α-glucosidase from *Pyrococcus furiosus* (Pf-GH122) has been characterised [3,4], but the catalytic mechanism and the residues involved in the reaction remain unknown. Recently, a metagenomic study of the microbial community inhabiting the thermal springs of Pisciarelli (Naples, Italy) unveiled the entire repertoire of CAZymes in this site [1]. Among these sequences, an ORF encoding a potential GH122 that showed only 23% identity to Pf-GH122 was annotated and named Met-GH122. The gene expressed in recombinant form produced a functional hyperthermophilic enzyme that revealed an α-glucuronidase activity that is novel in family GH122 and distinct from its unique characterised counterpart. In addition, the comparison of the 3D-structures of Pf-GH122 and Met-GH122 predicted by using AlphaFold, allowed us to propose the possible molecular determinants of the different substrate specificities of these enzymes. Our data allow a new functional and phylogenetic annotation of family GH122. References: 1. Strazzulli A et al. (2019). FEBS J 287 (6): 1116–1137. 2. Iacono R et al. (2022) Int. J. Mol. Sci. 23(18):10505. 3. Chang Stet al. (2001) Methods Enzymol. 330:260-9. 4. Comfort DA et al. (2008) Appl Environ Microbiol, 74(4):1281–3.

P-20-039
TED: a structural charachterization pipeline in support of white biotech at EMBL

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The European Molecular Biology Laboratory (EMBL) Hamburg unit is developing a structural characterization pipeline dubbed TED (Trim, Express & Diffract) to support enzymology in white biotech. TED is designed as a service to provide external researchers, both from academia and industry, a qualitative crystal-based system. This crystal system should be robust enough to be manipulated and allow for the determination of high-quality experimental structures of the enzyme of interest in different states allowing for better and targeted follow-up research, development and engineering. In offering this service, we hope to lower the threshold enzymologists and biotechnologists face to undertake the structural characterization of their system under study. TED is designed to leverage the wealth of sequence information coming from metagenomic initiatives in combination with putative structural models which have recently become available thanks to the revolution in machine learning. Together, these clear the path to arrive at the structure function relationship of the enzyme of interest supported by experimental evidence. The TED service is well located at EMBL Hamburg with access to state-of-the-art macromollecular crystallography beamlines at the PETRA III storage ring at DESY and the EMBL Sample Preparation and Characterisation Facility. Here we showcase proof-of-principle results with in-house targets including both soluble enzymes and enzymes residing in the membrane. We encourage researchers involved in white biotech, interested in obtaining an experimentally derived structure as an experimental basis for further research and development, to contact us.

P-20-040
Engineering prenyltransferase NphB for enhancing regioselectivity and catalytic activity toward olivetolic acid

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Cannabinoids are a class of natural products originally isolated from the plant *Cannabis sativa*. The legalization trend of the compounds from *C. sativa* has made a global surge in demand for cannabinoid-derived medications. Nevertheless, their utilization is hindered by the lack of cost-effective cannabinoid production methods. To address the issue, research employing a synthetic biology approach to produce various cannabinoids enzymatically is currently underway. Cannabigerolic acid (CBGA) is a pivotal precursor which can be converted into valuable cannabinoids such as tetrahydrocannabinolic acid (THCA), cannabidiolic acid (CBDA), and cannabichromenic acid (CBCA). NphB is a soluble aromatic prenyltransferase from *Streptomyces* sp. which has been shown to be able to prenylate diverse aromatic substrates including olivetolic acid (OA) to form CBGA. However, during CBGA production by NphB, unwanted side product has been also produced, consequently reducing yield of CBGA. In this presentation, we engineered NphB to enhance

regioselectivity and catalytic efficiency toward OA via computational rational design. Based on the structural analysis and information from previously reported mutants, nine residues have been selected for analysis using the online available tool named FuncLib. Through the analysis, critical residues influencing regioselectivity and activity were identified. The variants including the residues produced CBGA over 15-fold increase than wild-type without side product. Our NphB variants hold promise for producing valuable cannabinoids with improved regioselectivity and catalytic activity, thereby advancing cannabinoid-based therapeutics.

P-20-041
Bacterial encapsulins as a novel tool for biotechnological applications

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Encapsulins are high-molecular weight, capsid-like protein structures, consisting of a shell and a cargo protein encapsulated within. They have proven to be a useful alternative to conventional nanoplatforms due to their outstanding stability, non-toxicity and biodegradability. We have previously shown that stable expression of encapsulins can be achieved in mammalian cells [Gabashvili AN et al. (2020) Nanomaterials 12, 1657–1671], and various cargo proteins can be encapsulated into the nanocompartment shell. Here we present a stable hTERT immortalized MSCs cell line expressing the encapsulin system from the bacterium *Quasibacillus thermotolerans* (ASC52telo-Qt cells) comprising the encapsulin shell, a ferroxidase cargo protein, and a divalent iron transporter protein. The ferroxidase ensures the biomineralization of iron ions within the shell, while the transporter protein provides a high intracellular concentration of iron. As a result, iron-containing nanoparticles are formed inside the encapsulin nanocompartments, which have T2*-contrasting properties and serve as genetically encoded labels for MRI. To allow ASC52telo-Qt cells visualization via optical methods the cells were also transduced using RFP encoding lentiviral vector. The use of an immortalized line allowed us to overcome the limitations associated with obtaining cells from a donor, as well as the limit on the number of passages inherent in primary MSCs cultures. ASC52telo-Qt cells acquire a characteristic blue coloration when stained with Prussian blue, which will make it possible (along with the presence of RFP) to identify them on histological sections during in vivo studies. The presence of new sequences in the MSCs genome does not have a negative effect on cell viability, proliferation and differentiation. The obtained cell line can be used in the field of regenerative medicine and developmental biology for multimodal studies of the mechanisms of MSCs migration and homing. *The authors marked with an asterisk equally contributed to the work.

P-20-042
Unraveling conformational changes and alternative catalytic pathways in the crystal structures of *Lactobacillus plantarum* tannase

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Tannins are widespread polyphenols in plants. Tannase (E.C.3.1.1.20) is an esterase breaking down galloyl ester bonds in hydrolyzable tannins to release gallic acid. *Lp*TanB, a subtype B tannase from *Lactobacillus plantarum*, hydrolyzes tannic acid into glucose and gallic acid. Here, we present eight high-resolution crystal structures of *Lp*TanB ranging from 1.16 Å to 1.41 Å. These structures include the wild-type enzyme, three single active-site mutants (S163A, D419A, and H451F), and a triple mutant (S163A/D419A/H451F), either in the apo-form or in complex with gallic acid. The wild-type *Lp*TanB crystallized in two different forms I and II, both containing a glycerol (a cryoprotectant component) in the active site. In the presence of tannic acid, each single mutant also crystallized in form I, but the active site contained a gallic acid. The binding site was adjacent to the Ser163 side chain, and the gallic acid was probably a hydrolysis product of tannic acid. The proteins in all six crystals of wild-type or single mutant LpTanB exhibited similar conformations, as did the ligand-free S163A in form II crystal, which exhibited an empty active site. In contrast, the triple mutant, crystallized in both forms I and III with tannic acid, displayed significant conformational changes in the His451 loop, presenting an open configuration that rendered the formation of the catalytic triad impossible. However, adjacent to the active site, a gallic acid was covalently attached to the side chain of Cys204. These observations suggest alternative binding modes capable of accommodating the large tannic acid substrate in various orientations. This could potentially facilitate the formation of an alternative catalytic triad, enhancing catalytic efficiency. Furthermore, our findings underscore the significant role of Cys204 in the catalytic mechanism of tannic acid hydrolysis by *Lp*TanB.

P-20-043
Innovative utilization of *Escherichia coli* for PET valorization: a pathway to amino acids production

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One of the major concerns in today's world is the microplastic pollution caused by unsustainable usage and disposal. Microplastics (mainly polyethylene terephthalate, PET) with their potential risks to organisms, are dramatically impacting the marine

environment and having extreme repercussions for human beings. In the modern era, the exponential growth of knowledge and increased awareness presents a significant opportunity within the field of applied science. In this scenario, the ProPla project (Proteins from Plastic) proposes the recovery of PET microplastics from wastewater and their conversion into valuable amino acids, such as L-Ala, using a combination of protein engineering and systems biology approaches. A novel biosynthetic pathway (made by 9 enzymes also containing the LCC PET-degrading enzyme) has been developed for the bioconversion of PET into pyruvate, and has been divided in two major modules, then integrated into the *E. coli* K12MG1655 strain using CRISPR/Cas9 technology. With the first module we exploit optimized enzymes to convert PET into protocatechuic acid (PCA). The subsequent step focuses on the transformation of PCA into pyruvate, a key intermediate for amino acids production. Through our bottom-up approach to gene integration, we have obtained preliminary results in achieving stable integration of the enzymatic pathway required for the bioconversion of PCA into pyruvate. Using Flux Balance Analysis and related constraint-based techniques, we will determine the optimal combinations of metabolic fluxes leading to the maximum production of L-Ala [previously published in: Cazzaniga, P. et al. (2014) Metabolites 4,1034]. Upon establishing a stable cell factory, a large amount of pyruvate will be generated and will be then effectively converted into valuable amino acids utilizing either a single or multiple additional enzymatic steps. The ProPla project is funded by Fondazione Cariplo.

P-20-044
Computational study of the structure and catalytic mechanism of xylene monooxygenase from *Pseudomonas putida*

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Xylene monooxygenase (XMO) from *Pseudomonas putida* stands out for its ability to oxidize inert substrates with a structure like toluene and xylene. Nevertheless, its catalytic efficacy is curtailed by a mysterious inhibition mechanism, the details of which remain elusive. Presently, our research endeavors focus on employing wet and computational methodologies to unravel the structural intricacies and catalytic dynamics of XMO atoms. This enzyme exhibits high sequence homology with alkane monooxygenase (alkB), with its distinctive catalytic site where two iron atoms are situated at an unusual distance of 6 Å within a histidine-rich environment. This peculiar catalytic site underlines a catalytic mechanism unknown to this day. While our investigation is ongoing, our preliminary molecular modeling and simulation efforts aim to shed light on the fundamental aspects of its function and inhibition. Through this ongoing work, we anticipate laying the groundwork for potential strategies to enhance the stability and efficiency of XMO. Our endeavors hold significant promise for advancing both the fundamental understanding and practical applications of XMO in biotechnological contexts such as industrial processes.

P-20-045
Development and optimization of a novel "SEAP-GFP chimeric reporter" screening assay to profile TGFß signaling *in vitro*

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TGFß (Transforming growth factor-ß) is a major driving factor for wound healing, and it regulates context-dependent processes including apoptosis, proliferation, migration, and differentiation. Over-active TGFß signaling is associated with irreversible fibrosis and is pivotal in cancer, autoimmune diseases, and atherosclerosis. Thus, assessment of TGFß signaling is key in the development of any therapeutic means. We aimed to develop an innovative cell-based SMAD (suppressor of mothers against decapentaplegic) sensitive dual reporter vector system that allows us to profile the TGFß signal *in vitro*. Thus, we created two chimeric proteins to fuse secreted alkaline phosphatase (SEAP) and green fluorescent protein (GFP) using "Overlap Extension PCR" method and create an open reading frame to express the dual reporter. One construct contains a rigid chimeric protein structure without a linker region between two proteins, and the other one is a flexible structure harboring a linker region. Both vectors were transfected to C2C12 cells and SEAP enzyme activity was assayed in the supernatant. Variant conditions were tested and GFP was quantitated using fluorometric measurements. Following the transfection limited GFP was observed in cells, but SEAP activity was not detectable (following 24, 48, and 72 h). It's likely that SEAP cannot fold correctly in this chimeric structure, even with a linker. While optimization strategies are pursued, this model is a key to screening and optimization of anti-TGFß compounds and supports drug development efforts towards augmentation of TGF-ß signaling. The ultimate aim of this approach is to obtain a stable cell line expressing the reporter construct to be used as an in vitro screening assay to monitor TGFß signaling towards high-throughput screening.

P-20-046
Harnessing diversity-generating retroelements for directed evolution of proteins in *E. coli*

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The field of directed evolution mimics and quickens the natural process of evolution to drive genes of interest toward a desired function. An *in vivo* directed evolution system is the longstanding goal of directed evolution experiments. It would allow us to perform continuous evolution and overcome the limits of manually staged *in vitro* steps. Diversity-generating retroelements (DGRs) are genetic mutagenesis systems first identified in the *Bordetella* bacteriophage BPP-1 and that generates dense targeted mutagenesis with high error rates on adenines. To adapt the DGR natural system to *E. coli*, we combined DGR proteins with recombineering proteins and termed the strategy DGRec. Thanks to the DGRec system, we successfully mutagenized some targets (eg. regions of sacB and gpJ genes). However, early results have shown that not all targeted sequences were producing the same mutagenesis efficiency. It was therefore crucial to be able to predict if a template region would result in DGRec mutagenesis or not. In order to unravel the determinants of DGRec mutagenesis efficiency, we built libraries of random targeted regions, cloned

them into DGRec plasmids, and induced mutagenesis. We performed amplicon sequencing to determine the mutagenesis efficiency of each template region of the cloned libraries. This allowed us to train a machine learning models to be able to predict the mutagenesis efficiency based on features such as primary DNA sequences and RNA secondary structure predictions. With stringent constraints, the models can predict good DGRec targets with an accuracy above 95%.

P-20-047
Novel lactobacilli enzymes against pathogenic microorganisms

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Lactobacilli are well known important for human health bacteria. It was revealed that some enzymes are secreted by lactobacilli in response to pathogenic infection. Proteomic analysis showed that two strains of lactobacilli, *Limosilactobacillus reuteri* LR1 and *Lacticaseibacillus rhamnosus* F secrete several groups of enzymes: hydrolases of nucleic acids, metabolic enzymes and hydrolases of bacterial cell walls [Previously published in Savinova OS et al. (2021) Int. J. Mol. Sci. 2021, 22, 10999]. We selected the enzymes from each group which are secreted in the greatest quantities. Among them we can highlight ribonucleoside hydrolase C (RihC), L-lactate dehydrogenase (LLDH), and cysteine synthase A (CysK). Genes encoded these three enzymes were cloned and expressed in *E. coli* cells. The enzymes were obtained in active and soluble forms, purified using affinity chromatography. Kinetic analyses of RihC [Previously published in Shaposhnikov et al. (2024) Int. J. Mol. Sci. 2024, 25, 538] and LLDH revealed that they have higher catalytic constants compared to same enzymes from other sources. Maybe it can be connected with its particular role in response of lactobacilli to pathogens. Structural analysis of these two enzymes showed, that they are tetramers which was confirmed by gel-filtration. Also, the main features of their active sites were studied by structure analysis. The antibacterial properties of the enzymes were studied by several methods. It was revealed that they have synergetic effect against pathogens with some antibiotics and also negatively influences the biofilm production. The work was supported by a grant from the Russian Science Foundation № 23-64-10029, https://rscf.ru/project/23-64-10029/.

P-20-048
Graphene oxide decorated with gelatin: a nanocarrier for delivering pH-responsive therapeutics to improve its efficacy towards atherosclerotic plaque

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The progressive inflammatory disease atherosclerosis promotes myocardial infection, stroke, and heart attack. Anti-inflammatory drugs treat severe atherosclerosis. They are inadequate bioavailability and cause adverse effects at higher doses. A new nanomaterial coupled pH-apperceptive drug delivery system for atherosclerotic plaque is outlined here. We have synthesized a graphene oxide-gelatin-atorvastatin (GO-Gel-ATR) nano drug characterized by spectroscopic and imaging techniques. The encapsulation efficiency of GO-Gel-ATR (79.2%) in the loading process is observed to be better than GO-ATR (66.8%). The internal milieu of the plaque cells has a pH of 6.8. The GO-Gel-ATR displays a sustained and cumulative release profile at pH 6.8 compared to ATR and GO-ATR. Our proposed nanocomposite demonstrated high cytocompatibility up to 100 μg/ml in foam cells induced by oxidized-low density lipoprotein (Ox-LDL) and lipopolysaccharides (LPS) compared to normal macrophages for 24 and 48 h. The uptake efficacy of the nanodrugs is shown to be enhanced in foam cells compared to normal macrophage. Oil red O staining of foam cells with and without drugs confirmed therapeutic efficacy. Foam cells treated with nanodrugs had more lipids and efflux than ATR cells. In an $ApoE^{-/-}$ mice model of atherosclerosis, treated GO-Gel-ATR nano-drugs for 6 weeks showed significant decreases in lipids, aortic lesion region, and area relative to free ATR. The finding of the *in-vitro* and *in-vivo* study reveals that the GO-Gel-ATR nanocomposite carriers have the potential to deliver anti-atherosclerotic drugs effectively and inhibit atherosclerotic plaque progression.

P-20-049
Sub-crystalline protein assemblies through crystal contact engineering

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Crystal lattice contact modulation between consensus tetratricopeptide repeat (CTPR) protein chains allowed to synthesise crystalline two-dimensional planar and one-dimensional tubular assemblies. Supramolecular protein assemblies are highly desirable for nanoscale applications as a means to confer order on the molecular level, with considerable effort spent on the development of self-assembling proteins. The predominantly employed approach relies on artificial design of interacting protein surfaces to sustain the protein assemblies, grafted on protein scaffolds of suitable topology to support desired architectures. Targeted disruption of the crystal lattice-supporting contacts restricts lattice growth in selected directions, resulting in lower-dimension assemblies with crystalline order. CTPR proteins, made of small repeating modules, are particularly apt for supramolecular designs, as individual repeats can be modified without affecting the functions of the neighbouring modules. Folding into rigid right-handed superhelices, CTPR proteins create a regular helical

surface that can be used to impose molecular order on the nanoscale for functional nanomaterial construction. The large library of deposited crystal structures, however, provides an abundance of targets for sub-crystalline assembly design. Thus, crystal contact engineering presents a promising rapid and attractive approach for the design of ordered protein assemblies.

P-20-050
Biochemical characterization of the binding mode of apo-inhibitors towards the human enzyme indoleamine 2,3-dioxygenase 1

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Human indoleamine 2,3-dioxygenase 1 (hIDO1) is a cytosolic heme-containing oxygenase expressed at low levels in various tissues and cells, including the lungs, epididymis, placenta, central nervous system cells, macrophages, and dendritic cells. In its heme-containing form (holo-form), it catalyzes the first step of the kynurenines pathway, which is the oxidative cleavage of the indole ring of tryptophan to form N-formyl-L-kynurenine. This enzyme plays a crucial role in tumor immune escape and is therefore an attractive target for cancer immunotherapy (1, 2). Classical catalytic inhibitors were unsuccessful, so attention was focused on a new class of molecules defined as apo-inhibitors. These agents can effectively compete with the heme group by binding to the apo form of the enzyme. We expressed and purified the holo-IDO1 protein at a high yield in *E. coli* in order to analyze a library of compounds selected in a virtual screening aimed at identifying apo-inhibitors. We characterized the molecular mechanism by which this class of molecules efficiently and selectively inhibits IDO1 by targeting its apo-form. We used an innovative assay based on the decrease of Soret peak to define the dissociation constants of specific candidate apo-inhibitors. We observe cooperative mechanism of tested compounds despite IDO1 is traditionally described as monomeric in solution. To validate the possible correlation between the specific cooperative binding and the quaternary assembly, we set up an experimental framework based on SEC-SAXS and western blot under non-reducing conditions, which confirmed the protein dimerization. Additional experiments, such as in cellulo fluorescence resonance energy transfer (FRET) and blue native-polyacrylamide gel electrophoresis (BN-PAGE), will be conducted to further validate this hypothesis. Previously published in: 1. Molinier-Frenkel, V. et al. (2017) FEBS Lett. 591, 3135–3157. 2. Adamo, A. et al. (2021) Front. Immunol. 11, 613069.

P-20-051
Rational engineering of oxalate decarboxylase improves activity and stability under physiological conditions

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Oxalate decarboxylase from *Bacillus subtilis* (OxDC) is a Mn-dependent hexameric enzyme which converts oxalate to carbon dioxide and formate. Recently, OxDC has attracted the interest of the scientific community, mainly due to its biotechnological and medical applications for the treatment of hyperoxalurias, a group of pathologic conditions caused by oxalate accumulation. Indeed, studies performed in healthy subjects indicate that the oral administration of OxDC could degrade intestinal oxalate, thus preventing absorption. The enzyme has an optimum pH in the acidic range, where it shows the highest stability, but most applications involve processes occurring at neutral pH. Under physiological conditions the activity and stability of OxDC are remarkably reduced as compared with acidic pH. Here, guided by a bioinformatic approach, we designed engineered forms of the protein, and characterized the functional and stability properties of the corresponding variants in the purified form. Through combinatory mutagenesis, we identified which mutations increase catalytic efficiency and thermal stability under physiological conditions. We selected a double mutant showing the best functional and structural properties. The data obtained hold promise to exploit engineered OxDC as improved tool for efficient intestinal oxalate degradation in hyperoxaluria patients.

Enzyme and Cell Therapies (Medicinal Biochemistry)

P-21-001
New endocannabinoid system modulators: design, synthesis, characterization and selectivity

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The endocannabinoid system (ECS) is an endogenous signalling system, composed by receptors, lipid mediators, endocannabinoids (ECs) and N-acylethanolamides (NAEs), and their biosynthetic and metabolic enzymes. ECs are liberated in some enzymatic steps from membrane precursors and released on demand. The signal is turned off by the three main degradative enzymes: monoacylglycerol lipase (MAGL), fatty acid amide hydrolase (FAAH), and N-acylethanolamine-hydrolysing acid amidase (NAAA). The ubiquitous role of ECS, involved in a wide range of physiological processes and deregulated in different pathological conditions, promotes its components as

pharmacological targets. Here we report the design, synthesis and characterization of new compounds based either on the structures of well-known modulators, like oleoyl ethyl amide or the ureas and carbamates URB602, BIA-2474 and ARN726 and on EC structures. For the design of new compounds carbamate or urea functional groups, the palmitic or oleic chains, as mimic of the endogenous substrates, and aromatic cyclic or linear groups as second substituent were chosen. A library of more than 60 new compounds were synthesized, completely characterized, and assessed on human recombinant MAGL, FAAH or NAAA by using fluorescent high-throughput screening technique. Commercially available probes 7-HRO for MAGL, AAMCA for FAAH and PAMCA for NAAA, allowed to investigate novel compounds modulatory activity and IC50 were calculated for the most active ones and were lower than known inhibitors in our conditions. The best performers specifically designed for each hydrolytic enzyme have also been assessed on the others to verify specificity. The preliminary data allow to formulate hypothesis on structure activity relationship suggesting the carbamate functional group as primary actor in the selectivity toward FAAH and NAA hydrolytic enzymes.

P-21-002
Investigation of the relationship between diabet and epoxieicosatrienoic acid metabolism

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Epoxyeicosatrienoic acids (EETs), derivatives of arachidonic acid, exhibit vasodilatory, anti-inflammatory, and profibrinolytic properties. These metabolites are implicated in various physiological processes, and their potential impact on pancreatic beta cell mass in type II diabetes has attracted attention. To explore the relationship between diabetes and EET metabolism, we conducted a study involving 100 healthy individuals without diabetes and 100 patients diagnosed with type 2 diabetes for a duration of 10 years. Blood samples were collected from both groups, and the expression levels of the soluble epoxide hydrolase enzyme (sEH), a key player in EET metabolism, were determined at the mRNA level using quantitative polymerase chain reaction (qPCR) with cDNAs extracted from whole blood samples. For this purpose, blood samples were taken from 100 healthy individuals without diabetes, from one hundred patients who had type 2 diabetes for 10 years. In the study groups, the expressions of enzyme involved in EET (sEH) were determined at the mRNA level by qPCR using cDNAs obtained from whole blood samples. Furthermore, in the DNA samples obtained from the groups, EPHX2 rs62504268 and rs4149232 polymorphisms were determined by Rt-PCR device using hydrolysis probes. It was observed that EPHX2 expression at mRNA and protein levels in T2DM patients was similar to each other compared to healthy controls. In addition, a significant association was found between the rs62504268 polymorphism, which causes an increase in sEH activity, and T2DM. Conversely, no discernible association was observed between type 2 diabetes and rs4149232 polymorphism. In conclusion, our findings suggest a potential link between EET metabolism, particularly the rs62504268 polymorphism in the EPHX2 gene, and type 2 diabetes. Further research is warranted to elucidate the intricate mechanisms underlying these associations and explore potential therapeutic implications.

P-21-003
Variations on the theme of L-asparagine hydrolysis

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The simple hydrolysis of the amide group at the side chain of L-asparagine, leading to L-aspartate and ammonia, has at least three enzymatic solutions in the living organisms, catalyzed by enzymes classified in three structural Classes and five types. Class 1 L-asparaginases, also known as bacterial-type (a misnomer, as representatives are found in all domains of life), are the most thoroughly studied, as high-potency type II enzymes of Class 1 are used as antileukemic drugs. Their catalytic center, originally interpreted as a T-K-D version of the serine protease triad, has been recently re-interpreted as a system of two T residues and a proton relay with an activated Y residue and a chain of water molecules, that works according to a double-displacement mechanism. The origin of the Y residue in these homotetrameric enzymes differentiates types I and II. Class 2 L-asparaginases are Ntn-hydrolases, i.e. are expressed as single-chain precursors that undergo autoproteolytic activation, liberating a T residue at the N terminus of subunit β, which is also responsible for the maturation. The enzymatic activity (which can also hydrolyze L-Asn modifications, e.g. β-peptides or glycopeptides) is based on a pair of T residues, which also work in a ping-pong mode. Some Class 2 L-asparaginases are activated by K^+ cations bound in an activation loop. The prototypes IV and V of Class 3 L-asparaginases (so far found in bacteria and fungi) are from *Rhizobium etli*. By structural homology to such enzymes as serine β-lactamases or glutaminases, the active site in this Class was identified as consisting of two S-K tandems and of an odd Zn^{2+} binding site that plays a role in substrate docking, but not in catalysis. One of the S residues is the primary nucleophile but its activation is a mystery. It is also a mystery why, and by what mechanism, some of these proteins have the S nucleophile phosphorylated. Supported by NCN grants 2020/38/E/NZ1/00035 and 2020/37/B/NZ1/03250.

P-21-004
Development of optimized IgG fractionation technology from small plasma-pools as a groundwork for future antibody-based therapies against infections caused antibiotic resistance bacteria

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Antibiotic resistance is a global health problem that is becoming increasingly serious. As antibiotics often fail to work, alternative treatment approaches are needed to address this issue. One of promising strategies is the development of antibody-based passive immunotherapies, which provide immediate, high-titer antitoxic antibodies to control infections. The main goal of our project is to develop a hyperimmunoglobulin product for treating infectious diseases caused by methicillin-resistant *S. aureus* (MRSA) bacteria through evaluation of various available immunization schemes. To achieve this goal, it was crucial to develop proper techniques for the separation of the IgG fraction from plasma. For this purpose, the classical Kohn 6 cold ethanol fractionation method was adapted during the study, with additional purification steps to meet the quality criteria of the product according to European Pharmacopoeia. Small plasma pools were used for the experimental production of intravenous Immunoglobulin (IVIG) preparations in three independent replicates. Based on the obtained data, the yield of IVIG was an excellent 4.5 ± 0.2 g/l, comparable to the yields achieved by the largest plasma fractionation centers using both the Cohn and/or chromatography methods. The quality and safety parameters of the obtained IVIG samples were tested according to the requirements and methods of the European Pharmacopoeia. Based on the obtained data, all parameters met the standards, including protein composition, molecular parameters, stability, etc. In the next stage, animal immunization schemes will be implemented to obtain immunized serum. The isolation of hyperimmunoglobulin preparations from the animal's serum and the assessment of their quality indicators will be carried out using the methods developed in this study, with some adjustments for animal sera. Upon completion of the project, we expect to successfully develop hyperimmunoglobulin products specifically targeting MRSA toxins.

P-21-005
In vitro and *in vivo* studies of lactonases for the control of virulence factors in *Pseudomonas aeruginosa*

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The emergence of multidrug-resistant (MDR) bacteria stands as a paramount threat to current public health. Traditionally, MDR bacteria are linked to nosocomial infections, but there is a notable shift as certain MDR bacteria increasingly contribute to widespread community-wide MDR, through horizontal gene transfer processes. Biofilm communities may serve as a reservoir for MDR bacteria, and one strategy to combat MDR pathogen infections involves disrupting quorum sensing (QS) signals using lactonases. Our focus is on phosphotriesterase-like lactonases (PLLs), initially studied as phosphotriesterases, but later found to primarily target lactones. Specifically, we are investigating AhlA, a PLL from the mesophilic bacterium *Rhodococcus erythropolis*. This enzyme, derived from a synthetic His-tagged gene, has been expressed, purified, and thoroughly characterized. AhlA exhibits high thermophilicity, thermostability, a prolonged shelf life at 4°C, and stability under oxidizing conditions[1]. His-AhlA proves to be an effective quorum quenching enzyme, hydrolyzing acyl-homoserine lactones, 3oxo-C12-HSL, and C4-HSL, inhibiting the formation of *Pseudomonas aeruginosa* (PAO1) biofilm. To enhance the impact on PAO1 biofilm formation, we developed an enzymatic formulation comprising three enzymes with high lactonase activity: recombinant form of human paraoxonase 2 (rPON2)[2], microbial his-AhlA, and SsoPox 4Mut[3] from thermostable archaeal *Saccharolobus solfataricus*. This formulation underwent testing in wound healing assays on immortalized HeLa cells *in vitro*. We also, tested the formulation on infected wounds skin in an *in vivo* model of CD1 mouse. Results demonstrated improved wound closure and significant reduction in biofilm formation and bacterial cell number inside the wound. References: 1. Marone M, et al. (2023) Chem Biol Interact. 25:383, 110657. 2. Mandrich L, et al. (2015) PLoS One 10(12), e0144579. 3. Suzumoto Y, et al. (2020) Int J Mol Sci 21(5), 1683. *The authors marked with an asterisk equally contributed to the work.

P-21-006
Biological organic peroxides as substrates/inhibitors of bacterial AhpC

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Multi-drug resistant (MDR) is a global threat and the search for novel biological targets/compounds is of considerable importance to public health. Antibacterial drugs and immune cells produce reactive oxygen species in order to annihilate pathogens. In the course of evolution, bacteria have developed highly efficient enzymes that mitigate these defenses and strategies aimed to inactivate these enzymes may represent an important alternative for controlling MDR strains. Bacterial peroxiredoxins, so called AhpC, are among the most abundant peroxidases in cells and have been shown to act as a virulence factor in some species. Curiously, these enzymes can be inactivated by overoxidation at very high H_2O_2 concentrations (mM). In previous work, we have shown that a natural compound (prenylated benzoic acid) from *Piper crassinervium* is able to efficiently inhibit AhpC from *Pseudomonas aeruginosa* (IC_{50} = 20.3 μM). Among its chemical functions, the compound has a carboxylic acid and a hydrophobic tail that resembles long-chain fatty acids. The aims of this work were to assess whether oleic and linoleic peroxides and a peroxide extracted from the plant *Drimys brasiliensis* (DBEGH 3.5) could be AhpC substrates/inhibitors by means of computer assisted simulations, enzymatic assays and toxicity tests on *P. aeruginosa* cells. The molecular docking results revealed that the compounds are stabilized in the microenvironment of the active site by various hydrophobic and polar interactions (ΔG: 4.90 to −6.80 kcal/mol). Biochemical assays revealed that AhpC has a high affinity for lipid peroxides and *D. brasiliensis* peroxide (K_m:

4.08 – 200 μM). By fluorescence/immunoblotting approaches we showed that lipid peroxides are capable of inactivating the enzyme at very small amounts (μM) and high rates ($10^{5\text{-}6}M^{-1}s^{-1}$). Finally, tests on cells revealed that the association with antibiotics (gentamicin or ciprofloxacin) is able to increase the bacterial death promoted by the antibiotics.

P-21-007
Development of a novel enzyme replacement therapy against phenylketonuria based on a stabilized version of AvPAL using the IC-Tagging system

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Phenylketonuria is the most common hereditary defect in amino acid metabolism and is characterized by the presence of excessive levels of phenylalanine in blood and tissues. Currently, the only available treatment of this pathology is based on a strict diet without phenylalanine. In this context, our work proposes the use of the IC-Tagging system patented in our laboratory to develop an enzyme replacement therapy. Based on the production of a stabilized version of the enzyme phenylalanine ammonium lyase from *Anabaena variabilis* (AvPAL), which can reduce the phenylalanine levels of patients. The IC-Tagging methodology is a protein labeling platform based on the use of the avian reovirus-derived muNS-Mi protein [Brandariz-Nuñez A et al. (2010) PLoS One 5.11, e13961]. This system is able to produce protein micro- or nanospheres (MS or NS) that can be loaded with proteins of interest in any expression system through their tagging with a tag called IC. Our results show that it is posible to use our system to produce NS in bacteria loaded with the stabilized AvPAL enzyme maintaining its catalytic activity, correct folding and without altering its kinetic parameters in an inexpensive and simple way. The encapsulation of the AvPAL enzyme in these NS significantly improves its thermoresistance, has a stabilizing effect over a wide range of pH values and provides great resistance to incubations with low pH values. This nanoencapsulation also provides formidable protection against denaturing agents and proteolytic digestion and increases its long-term storage stability. Moreover, it is possible to coat the NS with different polymers without losing specific activity to avoid degradation and increase the oral bioavailability as we demonstrated in *in vivo* biodistribution assays. In conclusion, our work presents a new and disruptive approach to develop an orally administered enzyme replacement therapy against phenylketonuria.

P-21-008
Improvisation of existing treatment strategies of Glioblastoma through PAMAM dendrimer: an *in vitro* approach

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The aggressive brain cancer, glioblastoma (type IV glioma), occupying 16% of all central nervous system malignancies, has a survival rate of <5% post-prognosis. Current treatment approaches, such as surgical excision of tumors, radiotherapy, and chemotherapy, face various challenges. These encompass challenges in precisely delineating the boundaries of the tumor, limited drug delivery across the blood–brain barrier, and the occurrence of side effects such as nausea, vomiting, fatigue, headache, and myelosuppression. To address this situation, drug delivery through specifically designed nanoformulation has been developed. In this study, G4 PAMAM (polyamidoamine) nanoparticle (NP) is surface modified with folic acid (FA) and NSCD (N and S doped carbon dot) for better recognition of the tumor cells with enriched folate receptors and to endow multicolor imaging capabilities, respectively. In addition, platinum-based anticancer drug carboplatin (CP), which is responsible for blocking DNA replication, is loaded. The synthesized G4 PAMAM_FA_CP was analyzed through UV-Vis, FTIR, XPS, TEM, and MALDI-TOF MS, which confirms successful surface conjugation of FA and CP, uniform size distribution and indicates probable molecular weight. The average size and molecular weight were determined to be ~30 nm and ~20 500–22 000 g/mol, respectively. The zeta potential of it is ~−25 mV, establishing it to be a stable system. It has a slow, sustained acidic pH specific drug release profile with a ~70% release rate. Compared to the free drug (CP), *in vitro* experimentations on LN229 and LN18 cells exhibited higher antiproliferative effects and apoptosis by G4 PAMAM_FA_CP. Moreover, normal cells are unaffected by this nanoformulation. In conclusion, G4 PAMAM_FA_CP can be a potential therapeutic strategy for treating glioblastoma.

P-21-009
Effective reconstitution of the human retinal guanylate cyclase-activator complex in a cell line

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Guanylate cyclase-activating proteins (GCAPs) are neuronal calcium sensors that tune the light sensitivity of rods and cones by regulating membrane-bound guanylate cyclases (GCs) through a Ca^{2+}-mediated feedback mechanism. Three isoforms of GCAPs have been described in the human retina (GCAP1, 2 and 3) with a different distribution in the photoreceptor cells [1,2]; however, the physiological meaning of this apparent redundancy is not well understood. Indeed, it is confirmed that the GCAP1-GC1 protein complex is the main regulator of the subtle interplay between cGMP and Ca^{2+} in human photoreceptors, but the physiological role of human GCAP2 and 3 has not yet been fully clarified. A powerful tool for obtaining information on protein-protein interaction and protein trafficking within cells is the delivery of functional recombinant proteins into the cytosol. In

order to effectively reconstruct the human retinal guanylate cyclase- activator complex in HEK293 cells, in this work we tested three different delivery systems: (i) free protein, (ii) protein encapsulated into liposomes [3], (iii) protein with HEPES as transfection reagent [4]. The efficiency of protein delivery was evaluated by immunofluorescence, western blot and live cell imaging. Our data suggest that the free protein was not internalized into the cytosol in 24 h time frame, while the protein encapsulated into liposomes was internalized and released after 48 h. Interestingly, the presence of intracellular protein was complexed with HEPES, it was detected intracellularly already after 4 h. Previously published in: 1. Avesani A, et al. (2021) J Biol Chem. 296,100619. 2. Avesani A, et al. (2021) J Biol Chem. 296,100619. 3. Asteriti S et al. (2023) Cell Mol Life Sci. 80(12), 371. 4. Chen SH et al (2018) Mol Ther Methods Clin Dev.13, 99–111.

P-21-010
Mycobacterium tuberculosis tryptophan synthase as a target for novel antitubercular drugs

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Mycobacterium tuberculosis tryptophan synthase (TrpAB) is an essential enzyme for bacterial growth absent in human, thus considered an interesting target for drug development. TrpAB is a heterotetramer of two alpha and two beta subunits; alpha-subunits hydrolyse indole-3-glycerol phosphate into glyceraldehyde-3-phosphate and indole, while beta-subunits condensate indole and L-serine into L-tryptophan. GSK3778839 (GSK839) is a potent antitubercular compound active against Mtb clinical isolates, without cross-resistance with other antitubercular drugs and low frequency or resistance. Sequencing of resistant mutants suggested the involvement of TrpAB in GSK839 mechanism of action. To confirm this hypothesis, the effects of GSK839 have been evaluated against the recombinant enzyme, showing a specific inhibition of the beta-subunits without affecting the alpha-reaction. An in-depth characterization revealed a mixed uncompetitive inhibitor behaviour, with a Ki value of 0.25 ± 0.01 μM and residence time of 1.5 min. To confirm the suitability of TrpAB as druggable target, different chemical class inhibitors have been evaluated against both reactions. Some compounds showed a moderate inhibition of both enzyme activities without specificity against one of the two reactions, possibly affecting the allosteric regulation rather than a specific active site. This study confirmed the TrpAB enzyme as feasible drug target opening the possibility to the development of different chemotypes. This project has received funding from the Innovative Medicines Initiative 2 Joint Undertaking (JU) under grant agreement No 853989. The JU receives support from the European Union's Horizon 2020 Research and Innovation Programme and EFPIA and Global Alliance for TB Drug Development Non-Profit Organisation, Bill & Melinda Gates Foundation, University of Dundee. This work reflects only the author views, and the JU is not responsible for any use that may be made of the information it contains.

P-21-011
Exploring the biochemical relationship between alpha 1-antitrypsin and human neutrophil elastase in BALf samples of covid-19 patients

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Sars-CoV-2 is the virus responsible for COVID-19 disease. The major damage at the lung level observed in BALf samples is given by a large release of proteases and ROS that destroy lung parenchyma. The most important protease is HNE that, in healthy individuals, is balanced by its natural inhibitor (AAT) which blocks the protease activity by forming a stable complex HNE-AAT. COVID-19 lack this complex for two different reasons: first, both proteins undertake different fates by complexing with other proteins (as far as HNE is concerned) and with NETs (this is the case of AAT) [1]. Second, the AAT aliquot that remains free can be oxidized by the strong hypoxic environment in the lung resulting from ROS release. This latter hypothesis was confirmed by LC-MS/MS analysis of BALf samples [2]. Molecular dynamics analysis showed that the overall small deviations observed in the protein backbone are not significant enough to cause destabilization of the protein structure. This suggests that the structural integrity of both proteins is maintained, and the presence of oxidized methionines in the AAT-OX is tolerated. The effects of oxidation on the secondary structure of the antiprotease were explored by circular dichroism analysis [2]. Because AAT is ineffective in inhibiting HNE, alternative inhibition processes were investigated. The effects of two synthetic protein inhibitors (Sivelestat and Alvelestat) and a mutagenized protein form of AAT were evaluated. The results look promising. Thus, the ultimate goal of this work is to analyse the whole protein profiles in samples (BALf and serum) from patients with different lung diseases (asthma, bronchitis, emphysema, fibrosis, pneumonia, and broncho-pneumopathy) find a viable pharmacological endpoint of these severe disorders.

P-21-012
In vitro investigating the cytotoxic effects of biogenic AgNPs on the ZR-75 breast carcinoma cell line

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Silver nanoparticles (AgNPs), with unique properties, are increasingly popular in various industries, raising concerns about their environmental impact. [1]. Thus, it is crucial to accurately assess the biocompatibility of AgNPs. The purpose of this study was to study *in vitro* the effects of biogenic AgNPs stabilized in a 50% *Ocimum araratum* extract. The object of the AgNPs cytotoxicity study was the breast carcinoma cell line ZR-75 (ATCC) grown on RPMI 1640 medium nutrient medium (EuroClone, Italy) under standard conditions. The load of AgNPs per well was 4–0.125 μg, and the antitumor drug used in the treatment of breast carcinoma – Fluorouracil (Accord, England) – 100–1.5625 μg. Cytotoxicity was determined by IC_{50} equivalent viable cell count curves using standard MTT test protocols [2]. Biogenic synthesis

of AgNPs was carried out after the congregation of salt and 50% *O. araratum* extract. HPLC analysis of the extract under study revealed rosmarinic acid (RA) at a concentration of 5.43 µg/ml, which is a major component. The synthesized AgNPs were not genotoxic [3]. The results of the study revealed a dose-dependent effect of exposure to AgNPs. The highest concentration of AgNPs was absolutely cytotoxic and resulted in 98.8% ± 0.2 mortality and an IC_{50} of 2.6 µg/well. It should be noted that neither the 50% ethanol extract of *O. araratum* nor its major component PA had cytotoxicity at the concentrations studied. AgNPs had more pronounced cytotoxicity against the ZR-75 breast carcinoma cell line compared to Fluorouracil, whose IC_{50} was 1.56 µg/well and their complex effect has absolute cytotoxicity. Consequently, AgNPs double the effectiveness of Fluorouracil. This research was funded by MESCS RA SC, grant number 10-2/23-I/RAU-BIOL, 21T-1F243. References: 1. Nie P., et al., 2023, doi.org/10.1016/j.ecoenv.2023.114636. 2. MTT Cell Proliferation Assay Instructions for use ATCC® 30-1010K. 3. Kazaryan Sh. et al., 2022, doi: 10.1080/21691401.2022.2149931.

P-21-013
Selective targeting of NAD synthesis in *Plasmodium falciparum* red blood infected cells

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Nicotinamide adenine dinucleotide (NAD) and its phosphorylated form (NADP) are essential and ubiquitous coenzymes, which play a fundamental role in cellular metabolism. Thus, NAD biosynthesis is a vital metabolic pathway in all living organisms and can be considered as a generous source of enzymatic targets for drug development. NAD(P) is synthesised through two major pathways: (i) *de novo* synthesis and (ii) salvage routes, where NAD is generated from nicotinamide (Nam) and nicotinic acid (NA). The unicellular parasite *Plasmodium falciparum* is the cause of the most severe form of malaria and is responsible for 300 million infection and approximately 2 million deaths a year. Infected erythrocytes clump and block capillaries in the peripheral circulation, brain and placenta, and are a major contributor to the pathology of malaria. In *P. falciparum* infected human red blood cells (PRBCs), a remarkable increment in NAD content was demonstrated by Zerez, C R et al. Blood vol. 75,8 (1990): 1705–10, possibly supporting the major request of the cofactor caused by a burst in the activity of glycolytic enzymes, and its appears to be mediated by increases of NAD synthesis from NA. In particular *Pf*RBCs have an important increase in nicotinic acid phosphoribosil transferase (NaPRTase) activity, and in addition, of NMN/NaMN adenylyltransferase (NMNAT) and NAD synthetase (NADS) activity in contrast with uninfected RBCs. We purified all three principal plasmodium enzymes to perform a complete biochemical and structural characterisation of the *Pf*NAD(P) synthetic pathway as a source of robust and innovative drug-target. By applying a multidisciplinary approach that integrates enzymological and structural investigations with drug-design and chemical synthesis, we first characterise the assembly of *P. falciparum* NAD synthesis proteins complex, and we start to identify a few lead compounds showing high inhibiting potency against all *Pf*NAD biosynthetic enzymes.

P-21-014
Acid ceramidase inhibition as a mechanism to treat lysosomal storage disorders

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Lysosomal storage disorders (LSDs) are complex neurodegenerative disorders characterised by an abnormal build-up of toxic materials within lysosomes as a result of lysosomal enzyme defects. Due to the complex nature of these disorders, currently, there are no available treatments for the majority of LSDs. Acid ceramidase (ACase), a lipid hydrolase that cleaves ceramide, contributes to the pathology of LSDs by deacylating accumulating glycosphingolipids into lyso-glycosphingolipids, which are potent signalling lipids and are toxic to cells. Therefore, ACase makes a promising therapeutic target as a potential treatment for LSDs. The chemotherapeutic drug Carmofur is a potent, covalent inhibitor of ACase, however it is unsuitable as a treatment for LSDs as over-inhibition of ACase causes the development of Farber disease – an aggressive LSD. Therefore, our aim is to inhibit ACase to an extent that can alleviate the lysosphingolipid-induced pathology, but not induce Farber disease. We aim to do this by developing a non-covalent inhibitor of ACase. After establishing a fluorescent ACase activity assay in-house, we have tested numerous in-house compounds for ACase inhibition. Currently, our most promising compound (compound A), inhibits ACase with an IC_{50} of 45 nM. Preliminary biophysical characterisation has also shown that compound A is a non-covalent inhibitor of ACase. Compound A provides a promising starting point to find a compound that will inhibit ACase via a mechanism suitable for treating LSDs. Further work, including crystallisation of ACase and compound A, will help discover more potent and specific inhibitors of ACase for onward development towards the first therapy for several life-shortening and life-limiting LSDs.

P-21-015
Immobilization of enzymes on nanomaterials as a targeted method of restoring the balance in extracellular signaling

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Under physiological conditions, biological systems are maintained in homeostasis, understood as the appropriate concentration of ligands to the number of receptors/transporters, or the amount of substrates required for the metabolic needs of cells. All these processes are fine-tuned by enzymatic activity of specific enzymes. Inhibiting or activating enzymatic activity may be a way to regulate the metabolic and signaling functions of cells in their specific niches. Moreover, expanding the catalytic capabilities by immobilization on biocompatible materials further increases the potential for the use of enzymes. The research

aimed to test how immobilization on carbon nanomaterials affects the kinetic parameters of selected enzymes and how such constructs influence cell biology in *in vitro* cultures. Immobilization of enzymes, adenylate kinase, β-galactosidase, and catalase, was carried out for 24 h by adsorption on carbon quantum dots (CQD) or graphene oxide (GO). Kinetic parameters were determined under optimal conditions for each enzyme. The effect of the nanobiocatalysts on cell viability *in vitro* was determined using the MTT assay. Hemocompatibility of carbonaceous nanomaterials was also confirmed by evaluating platelet aggregation and hemolysis. We observed that enzyme adsorption modifies its kinetic parameters and stability. Additionally, enzymes immobilized on nanomaterials induce changes in the viability of treated cells. The obtained results indicate that immobilization is a way to expand the catalytic and regulatory capabilities of the enzyme and open up wider possibilities of using enzymes in therapies. However, the impact of nanobiocatalysts and the direction of changes are not universal and are closely related to the cell line used for research. They deserve further scientific attention as beneficial tools for developing personalized therapies. Reference: Hetmann et al. 2023, J Biomed Mater Res A. 111(10):1565–1576.

P-21-016
Effects of molecular hydrogen in mammalian cells

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Until recently, molecular hydrogen was considered an inert substance in the context of mammalian cells, which does not interfere with their physiology and metabolism. However, studies in recent years have revealed specific antioxidant properties and subsequently signalling, anti-inflammatory and anti-apoptotic effects have been described. Although it has been the subject of intensive research and its positive effects have been demonstrated in the treatment of many diseases, its exact mechanism of action has not yet been fully elucidated. Here, we focused on the study of the effects of molecular hydrogen in cell cultures and also in isolated cell organelles. Using the Oroboros O2k oxygraph, cellular respiration was measured in the affected cell lines RAW 264.7 and AC16 and their respective controls, and reactive oxygen species (ROS) production was determined using fluorescence assays. ROS production was also measured in mitochondria and microsomes isolated from mouse liver tissue. The measured data suggest that acute administration of molecular hydrogen leads to an increase in maximal cellular respiration, whereas normal respiration is not significantly affected. Paradoxically, it also increases ROS production. Our results suggest that a single administration of molecular hydrogen has a significant effect on cellular processes and could have a number of signalling effects through ROS production that could lead to, among other things, better adaptation to stress conditions and stimulation of antioxidant mechanisms. This work was supported by a university specific research grant – grant no. A2_FPBT_2024_040.

P-21-017
Unraveling the interplay of human pericyte-like adipose-derived mesenchymal stem cells in a blood-retinal barrier *in vitro* model of diabetic retinopathy

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The blood-retinal barrier (BRB) disruption is a hallmark of diabetic retinopathy (DR), characterized by pericyte detachment from retinal microvessels, leading to increased permeability and impaired BRB integrity. Our previous results demonstrated that, under high glucose (HG) conditions, human adipose stem cells (ASCs) and their differentiated pericyte-like phenotype (P-ASCs), exhibited high rate of proliferation and remarkable migratory capabilities[1]. Moreover, when co-cultured with human retinal endothelial cells (HRECs), P-ASCs were able to maintain BRB integrity under HG conditions by restoring the expression of adherens and tight junction proteins (VE-cadherin and ZO-1), downregulating the expression of pro-inflammatory cytokines and pro-angiogenic factors, and preventing HG-induced activation of the pro-inflammatory phospho-ERK1/2/phospho-cPLA$_2$/COX-2 pathway. Furthermore, our latest findings shed light on key molecular alterations, particularly highlighting the role of the PDGF-B/PDGFR-β axis. Specifically, we observed a significant simultaneous increase in PDGF-B expression and enhanced PDGFR-β activation in P-ASC/HREC co-cultures under HG conditions. Additionally, our investigations revealed: (a) an increase in HO-1 protein content and nuclear translocation of the transcription factor NRF2; (b) a significant reduction in cPLA$_2$ and PGE2 release, both attributed to P-ASCs/HRECs co-cultures under HG; and (c) a decrease in VEGF-A levels in media in response to HG in co-cultures of P-ASCs/HRECs compared to ASCs/HRECs. These findings collectively underscore the potential therapeutic efficacy of differentiated autologous ASCs in mitigating DR-induced damage. The observed molecular alterations within the BRB *in vitro* model suggest the promising potential of P-ASCs in future clinical applications focused on cell therapy to restore BRB function and counteract the detrimental effects of DR. Previously published in: [1]Mannino G et al. (2021) Int. J. Mol. Sci. 22, 4604.

P-21-018
In vitro selection of the single chain antibody fragment against the respiratory virus antigen

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Respiratory diseases (RDs) are reported to be one of the leading causes of death worldwide. The World Health Organization has identified RDs as one of the four major chronic diseases affecting humans. RDs are a significant economic burden and a major global health issue in terms of morbidity and mortality. Recombinant single-chain antibody fragment variables (scFv) are promising alternatives to full-length immunoglobulins. Antibody fragments offer significant advantages over traditional monoclonal antibodies. These characteristics include enhanced affinity and specificity, cost-effective large-scale production, higher stability, decreased immunogenicity and the capacity to refold after

heat denaturation. Ribosome display technology is commonly utilized for functional protein or peptide screening and directed evolution of protein molecules *in vitro*. In this study, ribosome display method was used to select scFv antibody fragments against the human respiratory virus antigen. The developed scFv fragments were recombinantly produced in *E. coli* expression system, and then purified with metal-chelate affinity chromatography. The equilibrium dissociation constant (Kd) of the selected antibody fragment was determined by using isothermal titration calorimetry. This study was supported by ERAS Innovation and Advanced Engineering Technologies Inc.

P-21-019
Characterization of a new elastase and proteinase 3 inhibitor for a potential treatment of neutrophil-mediated inflammatory lung diseases

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Neutrophils have a critical role in the innate immune response to infection and in the regulation of inflammatory processes. A key component of the neutrophilic activity is the secretion of proteolytically active neutrophil elastase-related serine proteases (elastase, proteinase 3 and cathepsin G), which are known to play a role in immune modulation and tissue repair following injury (1). In physiological conditions, neutrophil serine proteases (NSPs) activity is controlled by endogenous antiproteases. However, disruption of the protease-antiprotease balance can cause diseases in which neutrophilic inflammation is central to the pathology including non-cystic fibrosis bronchiectasis (NCFB) and cystic fibrosis (CF). In sputum samples from patients with NCFB, elastase and proteinase 3 have been shown to be a marker of pathology severity (2). In this study, we first quantified proteolytically active NSPs in sputa from patients with NCFB (non-infected patients n = 45, infected patients n = 45) and cystic fibrosis (n = 23). We then assessed the inhibitory efficacy of a novel compound against elastase and proteinase 3 in sputum samples from patients. Our results show the potential of this new inhibitor to restore the protease-antiprotease balance in chronic inflammatory diseases characterized by a neutrophilic inflammation. References: 1. Korkmaz B et al. (2010). Pharmacol Rev. 62(4), 726–59. 2. Chalmers JD et al. (2017) Am J Respir Crit Care Med. 195(10), 1384–1393.

P-21-020
Effect of multitargeted kinase inhibitor midostaurin (PKC412) and its complex with the drugs delivery system of supramolecular ribbon-like structures on bladder cancer cells

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As previous studies show, c-KIT receptor inhibitors can induce apoptosis in bladder cancer cells by inhibiting the activity of p-Akt kinase, the excessive activity of which may contribute to tumour progression, e.g., by inhibiting apoptosis, programming various changes in cell metabolism and proliferation, and by affecting the regulation of their ability to migrate and invade. Studies show that the invasion and progression of this cancer may be associated with overactivation of the phosphatidylinositol 3-kinase signaling pathway and the expression of micro-RNA 145 and micro-RNA 146b. Interestingly, overexpression of these micro-RNAs can inhibit the PI3K/AKT pathway. Unfortunately, it is very difficult to administer the drug without avoiding toxicity to healthy cells. Therefore, great hopes are attached to drug carriers that could deliver the drug directly to cancer cells, without reducing the therapeutic dose, and then safely remove it from the body. The purpose of our research was to evaluate the *in vitro* impact of the CR-PKC412 compound on selected properties of bladder cancer cells. The obtained results showed the cytostatic and cytotoxic effect of CR-PKC412 on bladder cancer cells. The resulting PKC-412 complex not only inhibited growth and mainly caused apoptosis of cancer cells, but also influenced the organisation of the cytoskeleton of the tested cell lines. It also inhibited the migration and invasion of cancer cells. The evaluation of the impact of the tested compounds on selected signaling proteins allowed us to determine which of them reduced the level of Akt and Erk1/2 phosphorylation in the cell lines. The cytostatic and cytotoxic effects of CR-PKC412 on bladder cancer cells highlighted its potential as a targeted therapy while indicating avenues for future research in this promising field. This research was funded by the National Science Centre in Poland (grant number K/MNT/000232).

P-21-021
Hypophospho-modified ATP derivatives as substrates for NPP1

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Nucleotide pyrophosphatase/phosphodiesterase 1 (NPP1) is one of the most important enzymes involved in the regulation of purinergic signaling *via* the degradation of extracellular nucleotides. Signaling pathways activated by extracellular nucleotides have been shown to regulate cancer progression and metastasis, as well as tissue regeneration. Extracellular nucleotides regulate proper cell function through interaction with nucleotide receptors (P2X and P2Y), membrane enzymes such as NPP1, and other cell surface proteins like glucose-regulated protein 78 (GRP78) [1]. Therefore, stable, biologically active analogues of nucleotides for therapeutic purposes are still being sought. The newly synthesized beta, gamma-hypophospho-modified ATP derivatives seem to be good candidates for potential therapeutic applications. Obtained compounds exhibit increased hydrolytic stability in biological conditions compared to ATP. These ATP analogues have been shown to act as substrates [2] and cofactors [3] for T7 RNA polymerase and T4 DNA ligase, respectively. The current research presents in-depth analysis of a series of hypophospho-modified ATP derivatives as substrates for nucleotide-degrading enzymes. Both, theoretical and experimental analysis were performed to verify their potential activity as substrates for NPP1, possible binding to the enzyme and the rate of hydrolysis. Experimental investigation confirmed conversion of beta, gamma-hypophospho-modified ATP derivatives in the presence of NPP1. Our studies provided new data for the studies of NPP1 substrate preferences, as well as allowed for the assessment of possible therapeutic use of hypophospho-modified nucleotides. References: 1. Suwara J et al. (2023) Curr Med Chem 30, 1232–1255. 2. Pawlowska R et al. (2024) ACS Omega 9, 9348–9356. 3. Pawlowska R et al. (2016) Bioorg Chem 67, 110–5. This research was supported by the grants 2017/26/D/ST5/01046 and 2023/49/B/ST4/03288 from National Science Centre in Poland.

P-21-022
Cytostatic and cytotoxic effect of nilotinib (AMN107) and its complex with supramolecular carriers on bladder cancer cells

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The progress that has been made in recent years in the field knowledge about the biology and genomics of bladder cancer has led to intensive research on new therapeutic possibilities. Changes in tyrosine kinase receptors, phosphoinositide 3-kinases, protein kinase B, mammalian target of rapamycin and important stages of the cell cycle are potential therapeutic goals in cancer patients with a high degree of malignancy. The aim of the project was to analyse the emerging complex between nilotinib (AMN107) and Congo red (CR) and to evaluate *in vitro* the effect of free drug and drug in a complex with a supramolecular carrier on normal human urinary tract cells and bladder cancer cells. The analysis of the formation of carrier-drug complexes allowed the determination of optimal molar proportions of the carrier-drug and the detection of changes in the size of the hydrodynamic diameter of the formed complexes using the dynamic light scattering method. The stability of the obtained carrier-drug systems was also analyzed using ultracentrifugation. Measurements made using this technique allowed not only the assessment of hydrodynamic parameters, but also allowed for the determination of changes occurring in the tested systems under the influence of the interaction of individual inhibitors and the supramolecular carrier. The experiments revealed that investigated compounds inhibited the proliferation of bladder cancer cells, reduces their survival and causes changes in the cell cycle. Furthermore, CR-AMN107 complex has a much smaller cytotoxic effect on normal cells of the urinary bladder. The results obtained and the conclusions drawn from them shed new light on anticancer therapy but also emphasize the importance of research on supramolecular structures, opening new possibilities for more effective and safe anticancer therapies. This research was funded by grant from the Ministry of Science and Higher Education through Jagiellonian University Medical College N41/DBS/001132.

P-21-023
New insights in GH116 family of carbohydrate-active enzymes: two novel hyperstable archaeal GH116 from the metagenome of Solfatara Pisciarelli

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The glycoside hydrolase family 116 (GH116) encompasses 1,186 protein sequences belonging to Eukarya, Archaea and Bacteria, including the human glucosylceramidase 2 (GBA2) implicated in Gaucher disease. The family has been organized, based on phylogenetic analysis, into three different subfamilies, which showed also different substrate specificity and inhibitor sensitivity. However, the functional study of this family is currently limited by few characterized members and, in particular, subfamily II [MM1][1] is currently represented only by a N-acetyl-beta-glucosaminidase from the Archaeon Saccharolobus solfataricus, as we reported previously.[1,2,3,4] This study focuses on the characterization of two novel GH116 enzymes from subfamily II. These enzymes were discovered in metagenomic datasets obtained from

two distinct mud/water pools within Solfatara Pisciarelli, Agnano (Naples, Italy), which greatly differ in both temperature and pH (T = 85°C and pH 5.5; T = 92°C and pH 1.5, for Pool1 and Pool2, respectively).[5] The characterization of these two novel enzymes revealed a substrate specificity for 4Np-β-glucopyranoside (4Np-Glc), as common substrates for all GH116 enzymes, but also a substrate specificity for 4/2Np-Galactopyranoside for one of them and inhibitor sensitivity among the two members of the same subfamily. The results increase our understanding of the functional aspects of GH116 family, emphasizing that members of the same subfamily can exhibit distinct characteristics. References: [1]Ferrara, M.C. et al. (2014) Biochim Biophys Acta 1840(1): 367-377. [2]Charoenwattanasatien, R., et al. (2016) ACS Chem Biol 11(7): 1891-1900. [3]Strazzulli, A., et al. (2020) FEBS J 287(6): 1116-1137. [4]Meelua, W., et al. (2023) Chem Biol Interact 384: 110717. [5]De Lise, F., et al. (2021) Molecules 26(7).

D-amino Acids and Pathological States

P-22-001
The human metabolic assembly for L-serine biosynthesis

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L-Serine (L-Ser) is the precursor of the neuroactive signaling molecules D-serine (D-Ser) and glycine, which modulate the activity of N-methyl-D-aspartate receptors. L-Ser biosynthesis in the mammalian brain proceeds through the phosphorylated pathway (PP) involving 3-phosphoglycerate dehydrogenase (PHGDH), phosphoserine aminotransferase (PSAT) and phosphoserine phosphatase (PSP), expressed in astrocytes. L-Ser plays a major role in the development and function of the human CNS: various severe, infantile, neurological disorders have been linked to its deficiency. Using the proximity ligation assay and confocal microscopy, we demonstrated that in iPSC-derived differentiated human astrocytes the three enzymes of the PP co-localise in cytoplasmic clusters, which size is similar to the one reported for other metabolons (i.e. the purinosome). Kinetic studies of the in vitro reconstituted pathway generated using the recombinant human PHGDH, PSAT and PSP (at physiological enzymes and substrates concentrations) supported the production of an enzymatic agglomerate: PHGDH catalyzes the rate-limiting step and PSP reaction is the driving force for the whole pathway. We propose that human PHGDH, PSAT and PSP can cluster in a transient metabolic assembly, the putative "serinosome" [previously published in: Rabattoni V. et al. (2023) FEBS J. 290(15), 3977–3895], providing a channeling solution for the pathway intermediates and delivering a relevant level of sophistication to the control of L-Ser biosynthesis. Its modulation by known pathological SNPs of the three proteins, by metabolites and interacting partners in physiological and pathological conditions is under investigation. This project was founded by "PRIN-2017 – Dissecting serine metabolism in the brain".

P-22-002
Discovering new promising inhibitors of human D-aspartate oxidase for D-aspartate modulation in schizophrenia

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D-aspartate (D-asp) contributes, with D-serine, to the neurotransmission of N-methyl-D-aspartate receptors (NMDAR) in mammalian brain. Neurotransmission impairment is typical of some psychiatric and neurodevelopmental pathologies like schizophrenia, where depletion of D-asp is caused by overexpression of its catabolic enzyme human D-aspartate oxidase (hDASPO). In this context, hDASPO is considered an interesting target for the development of new inhibitors in attempt to modulate excessive D-asp depletion and improve neurotransmission in patients. Olanzapine is a weak inhibitor of hDASPO and was chosen as scaffold to rationally synthetize new derivatives with better inhibitory activity. The first intermediate of olanzapine synthesis, 2-amino-5-methylthiophene-3-carbonitrile (AC-51), represents new promising inhibitor (IC_{50} = 5 μM). Understanding the molecular interactions between hDASPO and this new inhibitor is crucial to guide further rational design and achieve better potency. To this aim, the wild type hDASPO was co-crystallized with AC-51 and molecular replacement was applied to diffraction data exploiting the available crystallographic structure (PDB: 6RKF) as a search model [previously published in Molla G et al. (2020) The FASEB Journal. 2020; 34: 1182–1197]. The best crystal diffracted at 2.5Å resolution, showing a C2221 symmetry, with six molecules in the asymmetric unit. However, only four out of six protein molecules display electron density in the active site and further evaluations are needed to understand the nature of the bound ligands. Another issue is the poor reproducibility of the crystals. In the hDASPO crystals, protein molecules form dimers thanks to the presence of a phosphate molecule at the protein-protein interface. Thus, we propose to insert site-specific mutations that could stabilize the proteins towards a dimeric form with the aim of favouring crystallization and improving the order and stability of the crystal lattice. *The authors marked with an asterisk equally contributed to the work.

P-22-003
D-aspartate serum levels are altered in patients with treatment and non-treatment-resistant schizophrenia but not with autism spectrum disorder

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Schizophrenia (SCZ) and autism spectrum disorder (ASD) are neurodevelopmental diseases characterized by different psychopathological manifestations and divergent clinical trajectories. Various alterations at glutamatergic synapses have been reported in both disorders, including abnormal NMDA and metabotropic receptor signaling. We conducted a retrospective bicentric study to assess the levels of neuroactive D- and L-amino acids and their precursors in the serum of ASD, SCZ patients and their respective healthy controls. Twenty patients diagnosed with ASD and twenty-four control subjects were recruited from Istituto Giannina Gaslini (Genoa, Italy), while additional thirty-three ASD patients and six controls were recruited from the Federico II Hospital (Naples, Italy). Moreover, twenty-six SCZ patients and thirteen non-psychiatric control subjects were recruited from the same Neapolitan Hospital. Specifically, the SCZ patients were subdivided into treatment-resistant and non-treatment-resistant SCZ patients, based on their responsivity to conventional antipsychotics. We quantified serum concentrations of L-glutamate, L-aspartate, glycine, L-glutamine, L-asparagine, D-aspartate, D-serine, L-serine through High-Performance Liquid Chromatography. No significant differences between cases and controls were found in amino acid concentrations in the two independent ASD cohorts analyzed. Conversely, D-serine and D-aspartate serum reductions were found in SCZ patients compared to controls. This result further encourages future research to evaluate the predictive role of selected D-amino acids as peripheral markers for SCZ pathophysiology and diagnosis. *The authors marked with an asterisk equally contributed to the work.

P-22-004
D-amino acid metabolism is altered in the brain of autism spectrum disorder animal models

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Glutamatergic synaptic dysfunction contributes to the pathophysiological alterations observed in neurodevelopmental psychiatric diseases, including schizophrenia and autism spectrum disorder (ASD). In support of this notion, altered metabolism of two NMDA receptor (NMDAR) modulators, D-serine (D-Ser) and D-aspartate (D-Asp), has been reported in schizophrenia brains. Besides schizophrenia, recent studies also evidenced altered cerebral D-Asp levels in the idiopathic ASD mouse model, BTBR. Consistent with preclinical observations, a clinical investigation also identified a duplication of the D-aspartate oxidase gene, which encodes the enzyme responsible for endogenous D-Asp catabolism, in a young patient with ASD symptomatology and intellectual disability. Based on these preliminary findings, we performed a comprehensive HPLC analysis of the endogenous ligands of NMDARs, including D-Ser, D-Asp, L-glutamate, L-aspartate, glycine and their precursors, in the serum of two different cohorts of ASD patients. Additionally, we measured the same neuroactive amino acids in the plasma and different brain regions of adolescent and adult ASD rat models, exposed during pregnancy to lipopolysaccharide (maternal infection) or valproate administration. Despite unaltered amino acid blood levels in both ASD patients and rat models, compared to their respective controls, our results highlighted a remarkable deregulation of D-Asp and D-Ser levels in the striatum of ASD rats, accompanied by altered cerebral expression levels of serine racemase, an enzyme intimately involved in D-Ser and D-Asp biosynthesis. Altogether,

our clinical and preclinical findings suggest that neuroactive NMDAR-related amino acids may not be regarded as reliable peripheral markers of ASD despite their altered cerebral levels may be a key factor contributing to the glutamatergic dysfunctions observed in ASD.

P-22-005
pLG72 and the modulation of D-aspartate catabolism in human

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D-aspartate (D-Asp) plays important roles in growth, reproduction, and endocrine mediatory functions, but has been mainly investigated as an alternative agonist of N-methyl-D-aspartate receptors (NMDAr). Albeit D-Asp biosynthesis is still obscure, the occurrence of this D-amino acid in the brain is strictly controlled: it is highest during the embryonic phase and drops after the first days of life. Notably, despite its low levels in adult tissues, disruption of D-Asp metabolism was proposed to cause dysfunctions in NMDAr-mediated neurotransmission during the onset of various disorders, among which schizophrenia [as previously published in Nuzzo T et al. NPJ Schizophr. 2017. 3:16]. Here, we focus on the only known enzyme crucially involved in controlling D-Asp brain levels, the peroxisomal FAD-containing flavooxidase D-aspartate oxidase (DASPO) responsible for the selective catabolism of this D-amino acid, and on factors acting in its regulation. By combining *in vitro* and cellular studies, we show that human DASPO (hDASPO) interacts with the primate-specific protein pLG72, previously identified as a negative chaperone of the homologous human flavoenzyme D-amino acid oxidase (hDAAO), deputed to the degradation of the NMDAr co-agonist D-Ser. Worthy of note, pLG72 and hDASPO generate a cytosolic complex impairing the enzyme cellular stability: the expression of pLG72 negatively affects hDASPO level by reducing its half-life. We propose that pLG72 binding may represent a protective mechanism, common to hDAAO and hDASPO, aimed at preventing the excessive degradation of their substrates (i.e. the depletion of D-Ser and D-Asp cellular pools). In the case of the highly active hDASPO, this would also allow to avoid cytotoxicity associated to H_2O_2 production, before the final targeting of the enzyme to peroxisomes. This project was founded by "PRIN-2020 – Biochemical modulation of D-aspartate metabolism in brain functions".

P-22-006
Study of the regenerative effect of opioid peptides on a canine trichophytosis model

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The study is aimed at studying the regenerative effect of opioid peptides in a canine model of trichophytosis, assessing the possible enhancement of the therapeutic effect when combined with antifungal drugs due to the revealed resistance of the causative agent of trichophytosis (Mikrosporum and Trichophyton fungi) to griseofulvin therapy. The study was conducted on 24 dogs with a confirmed diagnosis of trichophytosis. Two groups of animals were formed: group 1 – standard therapy terbinafine (25 mg/kg); Group 2 – terbinafine (25 mg/kg) + sedatin buccal films 0.2 mg 2 times a week for a 4-week course of therapy. The effectiveness of therapy was assessed visually by measuring the area of the lesion, reducing the inflammatory process in the lesion, and epithelization of skin defects. The indicators were assessed on days 7, 14, 21 and 28. Itching was less pronounced in animals receiving combination therapy (16%), versus 29% in animals from the 1st experimental group. 76% of healing was established in the 2nd group of animals, versus 53% in the first group of experimental animals ($p = 0.891$). Combined therapy "terbinafine + sedatine", a defect in the epidermis and dermis filled with purulent-necrotic masses was detected in the skin of dogs. By the 21st day, healing of the skin defect was observed by 86% in the 2nd group and by 69% in 1st group; by the 28th day, in animals of the 2nd group, healing of the area of the skin recorded by 93%, versus 81% in experimental animals of the 1st group ($p = 0.931$). The use of opioid peptides makes it possible to shorten the healing time of a skin defect with the mycotic lesion under study and reduce the manifestation of clinical signs such as itching, exudation, and purulent lesions.

P-22-007
On the modulatory role of phosphorylation and acetylation of the human 3-phosphoglycerate dehydrogenase

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Post-translational modifications (PTMs) are well-known to reversibly activate/inactivate proteins. In the case of 3-phosphoglycerate dehydrogenase (PHGDH), the first enzyme of L-serine (L-Ser) synthesis through the phosphorylated pathway (PP), PTMs have been reported to affect activity, stability and compartmentalization [previously published in: Ma et al. (2021) Nat Metab. 3, 1357–1371]. Based on that and the sex-dependent modulation of the serine metabolism during healthy and Alzheimer's disease (AD)-marked aging [previously published in: Maffioli et al. (2022) Cell Rep. 40, 111271], we hypothesized that PTMs of the PP's rate-limiting enzyme could represent a regulatory strategy of the anabolic route, possibly contributing to the neurodegenerative pathophysiology. To this purpose, the endogenous PHGDH was isolated from human hippocampal tissues of both healthy and AD-affected males and females with an ad-hoc optimized immunoprecipitation procedure; the search of the modified residues was conducted via nLC-MS/MS analyses. While the targeted MS/MS approach highlighted the same phosphorylation pattern at five positions among the four categories of subjects, conversely, the selective deacetylation of PHGDH K289 in AD-affected men suggests a distinctive adaptation of males to the decreased L-Ser levels observed in AD. Although not sex-specific or correlated to the pathogenesis, *in silico* analyses of the modified residues allowed us to propose phosphorylation as a constitutive regulatory mechanism of the enzyme. Accordingly, biochemical studies on PHGDH variants mimicking the

phosphorylation state are ongoing. Overall, these studies are paving the way to the elucidation of the processes modulating the PP and L-Ser synthesis in fields other than oncogenesis, the one explored so far. This project was founded by "PRIN-2017 – Dissecting serine metabolism in the brain".

P-22-008
Structural properties of human D-3-phosphoglycerate dehydrogenase

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L-Serine (L-Ser) plays a pivotal role in the central nervous system and alterations of its level have been linked to severe neurological disorders [previously reported in: Tabatabaie L et al. (2011) J Inherit Metab Dis., 34(1):181–184]. D-3-phosphoglycerate dehydrogenase (PHGDH) catalyses the rate-limiting step in the synthesis of L-Ser via the phosphorylated pathway. Structurally, the human PHGDH is an homotetramer with an N-terminal region involved in the dimerization and formation of the substrate and nucleotide binding sites, and a C-terminal region including two regulatory domains: the ACT (aspartate kinase-chorismate mutase-tyrA prephenate dehydrogenase) and ASB (allosteric substrate binding) domains. The crystal structure of human PHGDH was solved only for a truncated dimeric form consisting of the substrate and cofactor binding domains. In this work, *in silico* analysis using AlphaFold and focusing on the inter-subunit interactions at the tetrameric interface, allowed to produce a model of the tetrameric PHGDH and the identification of essential residues involved in the oligomerization, which were eventually analysed via alanine-scanning mutagenesis. Interestingly, R454A, L478A, P479A, and Y495A variants within the ACT domain appear crucial for preserving a stable tetrameric assembly. These substitutions lead to significant structural alterations, resulting in hampered activity, decreased stability and misfolding. Conversely, the F418A variant in the putative ASB domain, halved the activity, slightly modified the tetrameric structure and the protein stability. This latter position appears to be mainly involved in the recognition of dimers leading to the formation of the tetramer. In conclusion, the predicted residues appear to be crucial for the tetramer formation and the proper folding of human PHGDH. This research was funded by a grant from the Ministero Università e Ricerca Scientifica PRIN 2017.

Gene Editing Technologies to Treat Diseases and Disorders

P-24-001
Generation of rescued Japanese encephalitis virus genotype 1 from infectious full-size clone using reverse genetics

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Japanese encephalitis virus (JEV) is a pathogen responsible for high mortality and morbidity rates among children with encephalitis. The JEV genotype 1 (GI) strain has recently become dominant in South Korea. Hence, corresponding research and vaccine development are urgently required. Molecular genetic studies on JEV vaccines can be boosted by obtaining genetically stable full-length infectious JEV complementary DNA (cDNA) clones. Furthermore, the significance of the reverse genetics system in facilitating molecular biological analyses of JEV properties has been demonstrated. The system may serve as a valuable tool for heterologous gene expression and developing new strains for JEV vaccines. Therefore, in this study, we describe the development of such clones. A recombinant JEV-GI strain was constructed using a reverse genetics system based on a Korean wild-type GI isolate (K05GS). cDNA was synthesized using RNA extracted from JEV-GI, and a recombinant full-length JEV clone, pTRE-JEVGI, was generated from the DNA fragments. Rescued JEV-GI viruses were generated by transfecting BHK-21 cells with pTRE-JEVGI. We analyzed the rescued JEV-GI viruses by performing *in vitro* experiments, including examination of cytopathic effects, plaque morphology, protein expression, and growth kinetics and *in vivo* experiments in mice to compare pathogenicity. The rescued JEV-GI exhibited similar characteristics to wild-type JEV. These results suggest that our reverse genetics system can generate full-length infectious clones that can be used to analyze molecular biological factors that influence viral properties and immunogenicity. Additionally, it may be useful as a heterologous gene expression vector and help develop new strains for JEV vaccines. *The authors marked with an asterisk equally contributed to the work.

P-24-002
Modelling ciliopathies in zebrafish: a morpholino approach

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Ciliopathies are a group of autosomal (or X-linked) recessive disorders caused by mutations in proteins of the primary cilium. Central nervous system, retina, kidney, liver and skeletal system are the organs mostly affected in these diseases. An extensive clinical heterogeneity, with many patients presenting overlapping phenotypes has been previously published in Mitchison and Valente (2017) J Pathol 241(2):294. Furthermore, variants of unknown significance (VUS) in the same gene can be responsible

for a wide spectrum of ciliopathy outcomes, making difficult a definitive genetic diagnosis. Three morpholinos that specifically bind and block the expression of the ciliopathy related genes, *ahi1, tmem67* and *rpgripl1*, known to be associated with Joubert Syndrome, were injected in 2-cell stage zebrafish embryos and their development was investigated. Three days post fertilization, ahi1, tmem67 and rpgrip1l morphants showed hydrocephalic head, abnormal body curvature and heart bilateral symmetry. Whole mount immunofluorescence using anti acetylated tubulin antibody proved an impairment in cilia development at the level of olfactory placode ranging from few misshaped cilia in *tmem67* morphants to complete loss of cilia in *ahi1* and *rpgrip1l* morphants. To demonstrate the correlation of morphants phenotype to ciliopathies, a rescue experiment was carried out by simultaneously injecting in the embryos the morpholino and the human wild -ype mRNA. Morphological analyses confirmed a successful rescue of the phenotype. This approach will be exploited to investigate the effect of VUS on the penetrance and on the expressivity of the canonical recessive ciliopathy mutations. The possibility to easily generate zebrafish morphants and dissect the effect of specific mutations could help in solving diagnostic dilemmas also in other similarly "complex mendelian" disorders.

P-24-003
Application of type IV-A3 CRISPR-Cas system from *Klebsiella pneumoniae* to modulate gene expression in mammalian cells

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Identification of CRISPR-Cas tools as precise genome editors opened new avenues for the treatment of various human diseases. However, conventional CRISPR techniques rely on the generation of site-specific DNA double-strand breaks (DSB), which inevitably come with risks, from unspecific mutations at the site of the break to large-scale chromosome rearrangement; therefore, alternative approaches to DNA cutting and editing are required. Recently described type IV-A3 CRISPR-Cas system from *Klebsiella pneumoniae* do not require DSB to target specific gene expression, as this system, composed of a multi-subunit effector complex and a CasDinG helicase, uses a nuclease-independent transcriptional interference pathway to suppress gene expression. While type IV-A3 CRISPR-Cas system has gene repression activity in bacterial cells, its application in mammalian cells has not yet been established. In this study, we expressed the type IV-A3 system along with non-targeting RNA in human embryonic kidney HEK293T cells. To optimize the expression, we used immunofluorescent labeling to quantify the proportion of cells expressing type IV-A3 complex proteins and evaluated DNA interference at single-cell level. To apply type IV-A3 CRISPR system for transcription interference in HEK293T cells we targeted highly conserved PPIB gene, which is used for the benchmarking of the modulation of gene expression. For PPIB expression interference, we used ten RNA guides for both coding and non-coding regions of PPIB gene. We evaluated transcription interference by quantitative real-time PCR. In parallel, we have evaluated transfection efficiency by immunofluorescence microscopy. Our study revealed that type IV-A3 CRISPR system can be used for targeted genome modulation in HEK293T cells. Application of this CRISPR system in mammalian cells offers the opportunity to expand the CRISPR toolbox with potentially safer techniques that elicit targeted changes to the genome without the introduction of a DSBs.

P-24-004
CRISPR/Cas9-mediated UBC knockout in gastric cancer cell lines

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Gastric cancer (GC) is one of the most common and lethal cancers. Alterations in the ubiquitin proteasome system (UPS) play key roles in the carcinogenetic process. Hence, the ubiquitin pathway is considered a promising target for therapeutic interventions. Upregulation of polyubiquitin genes *UBB* and *UBC* is observed in various cancers, with studies demonstrating that silencing *UBB* reduces proliferation rates in multiple cancer types, while knockdown of *UBB* and *UBC* inhibits lung cancer cell growth both *in vitro* and *in vivo*[1]. Our investigations in GC cell lines show that siRNA-mediated knockdown of *UBB* and *UBC* induces apoptosis predominantly in primary cells[2]. The CRISPR/Cas9 system has recently used to deplete ubiquitin pools by knockout of *UBB* and *UBC*[3] . Our aim was to knockout the unique coding exon of the *UBC* gene using the CRISPR/Cas9 system in primary and metastatic GC cell lines. Dual guide RNAs (gRNAs) targeting *UBC* were cloned into the Cas9 expression vector PX459, facilitated precise excision of the *UBC* coding exon, confirmed through PCR amplification of genomic DNA. Initial trials in HEK293T demonstrated successful *UBC* knockout. Western blot analysis confirmed a decrease in total ubiquitin levels in *UBC*-KO cells. Further, we conducted CRISPR/Cas9-driven *UBC* KO in GC cell lines, observing varying responses in ubiquitin pool depletion between the two GC cell lines, resulting in reduced *UBC* mRNA expression and diminished total ubiquitin levels. Clonal expansion of transfected GC cells yielded *UBC*-knockout cell lines for further analyses, focusing on elucidating effects on other ubiquitin gene expression, proliferation rates, cell cycle distribution, and migration capabilities. Targeting the ubiquitin pathway via gene knockout shows promise as a novel therapeutic strategy in GC, with further understanding of its role in cancer biology potentially leading to new therapeutic avenues. References: [1]Tang, Y. et al. (2015) Sci Rep **5**, 9476 (2015). https://doi.org/10.1038/srep09476 [2]Scarpa, E.S. et al (2020) Int J Mol Sci 21(15), 5435 https://doi.org/10.3390/ijms21155435 [3]Park C.W. et al. (2020) Cell Biochem Biophys 78(3):321-329 doi: 10.1007/s12013-020-00933-2

P-24-005
Utilizing MPC1 microexon islands for splicing-mediated gene correction

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The MPC1 microexon island can facilitate splicing-mediated gene correction. Alternative splicing of microexons, ranging from 3 to 30 bp, plays a crucial role in brain development and the progression of human cancers. Our research reveals that inserting the MPC1 microexon island into a target exon or at a splicing site can induce a splicing pattern alteration, leading to the replacement of the exon with the microexon. Initially, we successfully substituted the 14th exon of ACLY, an alternatively spliced microexon (30 nt), with the MPC1 microexon island, achieving the constitutive splicing-in of the 30 nt sequence. Furthermore, by inserting the MPC1 microexon island containing a 5 nt exon into DMD exon 20 (242 bp), we observed the splicing-in of the 5 nt MPC1 microexon instead of the original 242 nt, effectively preserving the reading frame. Overall, our findings demonstrate that the MPC1 microexon island can significantly alter genuine splicing patterns, offering new avenues for exploring splicing-mediated gene correction strategies.

Epigenome and Transcriptome

P-25-001
The role of epigenetic factors in the development of ovarian cancer

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Ovarian cancer (OC) is a complex disease with diverse tumor types and abnormal genetic and epigenetic changes, leading to uncontrolled cellular growth and malignant tissue formation. Long non-coding RNAs (lncRNAs) are key players in cancer biology, influencing processes in carcinogenesis and metastasis. Therefore, a promising objective is to identify potential molecular markers that can be valuable for the diagnosis and prognosis of this type of cancer. Our study aims to evaluate the impact of methylation on the changes in expression level of the lncRNA genes SNHG6 and SEMA3B-AS1 in OC. Using qMS-PCR and qRT-PCR (Bio-Rad), nonparametric Mann–Whitney U test and Spearman criteria (RStudio) we revealed statistically significant decrease in the expression level ($p \leq 0.001$, FDR = 0.1) for the SNHG6 and SEMA3B-AS1 lncRNA genes in a set of 27 paired (tumor/normal) samples of the OC. The suppressor potential of the same lncRNA genes was also confirmed by identification of a statistically significant increase in the level of methylation ($p \leq 0.001$, FDR = 0.1) that was found in the general set of samples. A negative correlation between changes in methylation and expression levels (Rs = (−0.77)-(−0.62), $p \leq 0.0006$) was revealed. Moreover, it was shown that there is a positive correlation between SNHG6 and SEMA3B-AS1 in terms of methylation level (Rs = 0.36, $p < 0.0001$) and expression level (Rs = 0.56, $p < 0.003$). Based on the results obtained and additional validation on a larger set of samples, it is possible to use these lncRNAs as a potential prognostic marker of OC. This research was funded by the Russian Science Foundation, grant number 20-15-00368-P.

P-25-002
Transcriptome profiling of pancreatic neuroendocrine tumour tissue reveals potential non-invasive biomarkers

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Pancreatic neuroendocrine tumours (PanNETs) are a heterogeneous group of neoplasms that arise from the neuroendocrine cells of the pancreas and demonstrate various clinical manifestations and malignancy potential. Even though the prognosis for patients with PanNETs is usually encouraging, the majority of PanNETs are diagnosed at an advanced or metastatic stage, decreasing the chance of survival and treatment. The molecular pathogenesis and alterations of PanNETs are only partially understood despite being extensively studied in the past decade. Investigation of their transcriptomic landscape can identify novel genes involved in tumour development and uncover missing puzzle pieces in understanding PanNETs' progression. Considering the rarity of PanNETs, 72 FFPE PanNET samples, including normal adjacent and metastatic tumour tissues, were collected. RNA was then extracted, and libraries were prepared, continuing with transcriptome sequencing, which was carried out on the DNBSEQ-G400 platform (MGI). Finally, ten differential gene expression (DGE) analyses for seven sample groups were performed to identify differentially expressed genes (DEGs). Among the identified DEGs, we highlighted several, including *CTRB2*, *LAMA4*, *CPA1*, *CPB1*, and *REG1A*. Subsequently, we showed that the progression of the disease and risk of metastasis can be detected based on differential gene expression, primarily linked to extracellular matrix remodeling, angiogenesis, cell migration, and motility pathways. We also demonstrated that genes *CPA1* and *CPB1*, involved in a carboxypeptidase activity pathway and commonly found in healthy pancreatic tissues, were markedly downregulated in PanNET tissues, indicating their potential as biomarkers. Overall, this study underscores the importance of PanNET transcriptome profiling for predictive biomarker investigations, which can be performed in FFPE tissues to enable the non-invasive detection of these tumours in liquid biopsy.

P-25-003
Role of PHF10 isoforms in spreading and migration of melanoma A375 cells

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A specific subunit of PBAF chromatin remodeling complex, PHF10, plays a crucial role in transcriptional regulation. There are four isoforms of PHF10 in mammalian cells that are incorporated in the PBAF complex alternatively. Isoforms are expressed from one gene and proteins differ by N- and C-ends. PHF10-P isoforms contain DPF domains at C-end and bind histone modification; PHF10-S isoforms contain PDSM motif for SUMO1 conjugation. Domain organization of PHF10 isoforms determines different cellular localization, stability and modification patterns. According to transcriptome analysis, knockdown (KD) of PHF10 isoforms leads to changes in the expression of genes associated with cell adhesion and motility. We analyzed the role of PHF10 isoforms in spreading and migration of A375 melanoma cells with downregulated expression. We performed KD of PHF10-P and PHF10-S isoforms in A375 cells using specific siRNAs and analyzed cell morphology and spreading rate from the beginning of cell attachment on the fibronectin-coated plate during 60 min. The spreading rate of cells with PHF10-S and PHF10-P KD was 1.5 times higher compared to control. A375 cells with KD PHF10-P also polarized faster than control cells and cells with KD PHF10-S. Next, we analyzed the A375 cell migration parameters: migration speed, track length and total displacement. The total displacement was 40.11 ± 3.12 μm for control A375 cells, 42.01 ± 4.83 μm for cells with KD PHF10-P and 94.14 ± 12.89 μm for cells with KD PHF10-S. Thus, downregulation of the PHF10-P expression leads to increase in cell spreading rate and cell polarization, while downregulation of PHF10-S expression enables more directional movement. We assume that PBAF complexes containing PHF10-P and PHF10-S isoforms regulate adhesion and cell motility genes in different ways. This study was supported by the Russian Science Foundation [grant number 21-14-00258 to N.S.]. *The authors marked with an asterisk equally contributed to the work.

P-25-004
Phospho-DJ1: Disclosing a novel link between epigenetic dysregulation and breast cancer

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Recently a plethora of histone non-enzymatic covalent modifications, correlating epigenome landscape and metabolic rewiring, has been described. These modifications are tightly related to cells metabolic fitness and are able to impair chromatin architecture. Our group demonstrated how in breast cancer cells, the high glycolytic flux induces carbonyl stress, a damaging condition that increases reactive carbonyl species levels making histones more susceptible to glycation. This particular non-enzymatic modification leads to the formation of advanced glycation endproducts (AGEs) and induces a fatal deconstruction of the histone code. In this scenario, DJ-1, a deglycase enzyme found to be dysregulated in several human tumours, appears to be crucial in preserving the proliferative potential of cancer cells counteracting AGE formation. Using omics strategy, we identified a novel isoform of the protein establishing how DJ-1 pro-tumorigenic abilities are dependent on Akt-pathway. In breast cancer cells, the over-activation of Akt-signaling prevents, through a functional tuning of DJ-1 proteoforms, glycation induced histones dysregulation. To corroborate the role of DJ-1 in preserving the malignant proliferative potential of cancer cells, we used the novel DJ1-proteoform as a molecular template for a docking simulation and we identified a parterre of DJ-1 inhibitors able to selectively target DJ-1 glyoxalase activities. Virtual Screening studies were done using the Food and Drug Administration (FDA)-approved drugs database. Histone glycation profiling in response to treatment with DJ-1 inhibitors showed a parterre of histone marks critical for chromatin homeostasis, an Achilles' heel that might improve targeted therapy in breast cancer. Therefore, our results reinforce the notion that targeting the novel DJ-1 proteoform might be a promising therapeutic strategy.

P-25-005
The common and opposite roles of MCPIP family members in keratinocyte biology

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Monocyte chemoattractant protein-induced protein-1/-3 proteins (MCPIP1/3), encoded by the *ZC3H12A/C* genes, possess a PIN domain that has RNase properties and a CCCH-type zinc finger motif, which is required for RNA binding. The interaction is based on the recognition of specific stem-loop structures in the 3'UTR. MCPIP1 regulates the stability of transcripts coding for inflammatory cytokines (e.g., IL-6, IL-12p40) and transcription factors (e.g., c-Rel, C/EBPβ), thereby functioning as an essential regulator of inflammation, cell differentiation, and proliferation. Based on current knowledge, MCPIP3 similarly binds to the 3' UTR sequences and degrades transcripts, e.g., *IL6* and *ZC3H12A* (thus, like MCPIP1), but also *TNFA*. Furthermore, both proteins are highly expressed in the human psoriatic epidermis at the mRNA level. Within this study, we developed cancerous keratinocyte cell lines with stable silencing of MCPIP1, MCPIP3, or both proteins simultaneously. Functional analyses showed that silencing MCPIP1/3 affected cell viability. Next, global transcriptome analyses of RNA isolated from MCPIP1-, MCPIP3-, MCPIP1/3-silenced, and control cells were performed. Bioinformatic analysis revealed lists of differentially expressed genes in each comparison, which were mostly assigned to biological processes such as immune response and the development of the epidermis. We further identified potential new common and specific targets of MCPIP1 and MCPIP3 RNases by analyzing the Venn diagrams. Finally, we confirmed some direct regulation of selected mRNAs by MCPIP1/3 using luciferase reporter assays. Our results showed that *IL6* and *IL33* mRNAs are common targets for both proteins, whereas *IL36G* and *MMP9* are preferentially targeted by MCPIP1 and *TNIK* by MCPIP3. In

conclusion, this study showed that both MCPIP1 and MCPIP3 proteins play relevant roles in modulating keratinocyte biology. This study was supported by the National Science Centre grant no. 2022/47/D/NZ3/01654 (to ALC).

P-25-006
Unlocking the screening potential of transcriptomic-based salivary biomarkers in gastric cancer as a proxy of malignant transformation

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Saliva, due to its ready accessibility, emerges as an appealing candidate for liquid biopsy applications in gastric cancer (GC) detection. However, there is a paucity of research on this field. This study aims to assess the potential of saliva as a proxy of gastric malignant transformation, as well as its diagnostic and prognostic value. Leveraging available data from Gene Expression Omnibus (GEO) and The Cancer Genome Atlas (TCGA), and using R software, a comprehensive analysis of saliva and tissue transcriptomic profiles was conducted, including differential gene expression, receiver operating characteristic (ROC) curve analysis, hazard ratio (HR) computation, and multivariable Cox regression. From the pool of 1,881 differentially expressed genes (DEGs) identified in gastric tissue, only a subset of five – *DSP, MMP12, SDR16C5, TUFT1*, and *ZFAND2A* – preserved a dysregulated pattern in saliva, unveiling a distinctive molecular landscape. A robust salivary gene expression model was defined that effectively discriminated between cancer and normal samples (AUC = 0.807, 95% CI: 0.602–0.966), including the *DSP, MMP12*, and *ZFAND2A* genes. Furthermore, a worse overall survival was associated with a high expression of *TUFT1* (HR = 1.64, p = 0.026) and *ZFAND2A* (HR = 1.55, p = 0.041), and a worse progression-free survival was reported for patients underexpressing *SDR16C5* (HR = 0.69, p = 0.030). Our study underscores the potential of tumor-derived salivary biomarkers as a non-invasive tool for screening and managing GC, despite the scarcity of publicly available data on saliva. Further research of liquid biopsy applications in personalized medicine is imperative to enhance diagnostic and prognostic methodologies.

P-25-007
The significance of the malfunction of the chromatin remodeling machinery in clear cell renal cell carcinoma

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Renal cell carcinoma (RCC) is a prevalent urological cancer, commonly diagnosed in individuals aged between 60 and 70 years. Nevertheless, it can also affect younger people. The predominant subtype of RCC, accounting for over 80% of cases, is clear cell renal cell carcinoma (ccRCC). ccRCC is distinguished by alterations in cellular metabolism leading to the accumulation of glycogen and lipids in tumor cells, resulting in their pale appearance. Typically, the disease is identified at an advanced metastatic stage and exhibits resistance to conventional chemotherapy and radiotherapy, posing challenges in treatment. Analysis of the TCGA database has revealed that genes encoding BAF60 subunits of SWI/SNF CRC are partially inactivated in primary ccRCC tumors, but their expression increases in metastases. Additionally, NGS analysis of patient samples has shown that 50% of ccRCC tumors harbor a mutation in the SMAD4 coding sequence, specifically a Gln to Leu change at position 256, potentially impacting the functions of this transcription factor and its interactions with the SWI/SNF complex. The aim of this study is to enhance our understanding of the disruption of regulatory processes specific to ccRCC, with a focus on transcriptional control mechanisms. The coding sequences of BAF60s and mutSMAD4 were cloned and the proteins were overexpressed. Subsequently, we successfully identified VHHs that bind to the BAF60A protein, while the search for anti-mutSMAD4 and anti-BAF60B VHHs is ongoing. Furthermore, our investigation revealed long-range chromatin interactions with the promoter regions of PBRM1, ARID1A, ARID1B, SMARCA4, and SMARCD2 genes. Overall, our findings suggest that the regulation of chromatin remodeling machinery function may be compromised at different levels in ccRCC, with potential variations depending on the disease stage. Sources of financing: National Science Center (Poland) grant No. 2018/30/M/NZ1/00180 for TJS and IBB minigrant No. FBW SD 6/2024 for JS.

P-25-008
Chromatin remodeling dynamics in HPV infection: evaluating gene expression profile

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Cervical cancer (CC) is by far the most common human papillomavirus (HPV) related disease. Cervical carcinogenesis involves multiple epigenetic alterations (changes in viral and host genomes), including chromatin remodelling. The aim of this study was to identify host genes with modified expression pattern by NuRD complex deposition related to E6 and E7 HPV16 viral proteins. Chromatin immunoprecipitation sequencing for MBD2, MBD3 (NuRD components) genome wide DNA binding pattern

was evaluated in *in vitro* model (CaSki cell line). Four genes were selected: SF3B1B, EIF4G3, DCP2 and FAT1 and their mRNA level expression was evaluated in patients. Further, 55 patients with different cytological status were considered: LGSIL Low-Grade Squamous Intraepithelial Lesion (n = 12), HGSIL High-Grade Squamous Intraepithelial Lesion (n = 12), SCC Squamous Cell Carcinoma (n = 13) and control group: NILM – Negative for Intraepithelial Lesion or Malignancy negative (−) (n = 10) and HPV positive (+) (n = 10). Total RNA was extracted from investigated samples and then was used for cDNA synthesis. In order to quantify expression levels of selected genes, qRT-PCR was performed. Statistical analysis was performed using GraphPad Prism 5.0. Regarding investigated genes expression levels, we noted that SF3B1B, EIF4G3 and DCP2 display an increased expression pattern in LGSIL lesions versus NILM (−) group ($p = 0.141$; $p = 0.0047$ and $p = 0.0400$). An interesting result was displayed by SF3B1B and EIF4G3 genes, with an increased expression level in NILM (+) versus NILM (−) ($p = 0.0413$, $p = 0.0283$). EIF4G3 gene was also significantly increased in SCC group ($p = 0.0485$). The expression levels of FAT1 did not display any difference between studied groups. The results showed that epigenetic gene control was mediated by HPV infection both in in vitro and patients, suggesting that the expression of identified genes could serve as potential biomarkers. Acknowledgment: Romanian Academy

P-25-009
Unraveling colorectal cancer dynamics: insights from transcript isoforms

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Colorectal cancer (CRC) progression is intricately linked to aberrant splicing events, notably impacting cell division, invasion, apoptosis, angiogenesis, and drug resistance. Understanding transcript isoforms at a cohort level is imperative for comprehensive analysis. receptor tyrosine kinases (RTKs) are pivotal in cancer, driving cellular growth and influencing signaling pathways, including those implicated in drug resistance. Despite their significance, RTK-related alterations and their interaction within the tumor microenvironment (TME) remain incompletely understood. Leveraging nanopore technologies, we mapped transcript isoforms in CRC, elucidating associations between RTK transcripts and TME subgroups. Analysis of our colorectal cancer cohort revealed disease-exclusive RTK isoforms, correlating significantly with prognosis. Specifically, a distinct RTK isoform associated with poorer prognosis and an immune-depleted TME. Our findings propose this isoform as a potential prognostic biomarker and underscore its relevance in isoform-targeted therapies, fostering precision oncology.

P-25-010
Histone H3K4ac, as a marker of active transcription start sites and enhancers, plays roles in histone eviction and RNA transcription

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The lysine 4 of histone H3 (H3K4) can be methylated or acetylated into four states: H3K4me1, H3K4me2, H3K4me3, or H3K4ac. Unlike H3K4 methylation, the genome-wide distribution and functional roles of H3K4ac remain unclear. To understand the relationship of acetylation with methylation at H3K4 and to explore the roles of H3K4ac in the context of chromatin, we analyzed H3K4ac across the human genome and compared it with H3K4 methylation in K562 cells. H3K4ac was positively correlated with H3K4me1/2/3 in reciprocal analysis. A decrease in H3K4ac through the mutation of the histone acetyltransferase p300 reduced H3K4me1 and H3K4me3 at the H3K4ac peaks. H3K4ac was also impaired by H3K4me depletion in the histone methyltransferase MLL3/4-mutated cells. H3K4ac peaks were enriched at enhancers in addition to the transcription start sites (TSSs) of genes. H3K4ac of TSSs and enhancers was positively correlated with mRNA and eRNA transcription. A decrease in H3K4ac reduced H3K4me3 and H3K4me1 in TSSs and enhancers, respectively, and inhibited the eviction of histone H3 from them. The mRNA transcription of highly transcribed genes was affected by the reduced H3K4ac. Interestingly, H3K4ac played a redundant role with regard to H3K27ac in eRNA transcription. These results indicate that H3K4ac serves as a marker of both active TSSs and enhancers and plays a role in histone eviction and RNA transcription by leading to H3K4me1/3. *The authors marked with an asterisk equally contributed to the work.

P-25-011
Changes in gene expression and RNA processing induced by thymidylate synthase inhibitory drugs

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Thymidylate synthase (TS) inhibitory chemotherapeutic drugs perturb the cellular dUTP/dTTP ratio by preventing the efficient formation of the DNA precursor dTTP, thus dUTP is used during DNA synthesis, and great amounts of uracil appears in the genome [1]. Uracils are errors in the DNA and can be efficiently processed by the base excision repair (BER) initiated by the uracil-DNA-glycosylases (UDGs) [2]. The drug-induced elevated genomic uracil and dUTP levels lead to hyperactive futile cycles of the BER, which finally leads to thymine-less cell death. Interestingly, when the main UDG is inhibited the TS inhibitory drug treatments still lead to cell death, suggesting that these drugs might have some additional mechanisms of action. To better understand the underlying processes during TS inhibitory drug treatments we have analysed the total RNA set of HCT116 cells with different DNA-repair capacities after 48 h treatment with 5-fluoror-2'-deoxyuridine (5FdUR) a covalent inhibitor of TS, or with raltitrexed (RTX) a competitive inhibitor of the same enzyme. We have found many differentially expressed genes in the protein coding group, for example p53 pathway is more affected in case of 5FdUR treatment than during RTX treatment, while in RTX treated cells the upregulation of components involved in RNA processing is stronger than in case of 5FdUR treatment. We have observed enlarged and round nuclear

speckles after 48 h drug treatment with immunocytochemistry which appeared more evenly in case of RTX treatment than in case of 5FdUR treatment. Nuclear speckles are phase separated nuclear organelles which contain many proteins involved in RNA transcription, splicing and RNA maturation [3], and it is known that inhibition of RNA polymerase or splicing leads to enlarged and round speckles [4], which eventually suggests altered RNA processing in our case as well, serving additional information of these drugs. References: 1. PMID:9579853. 2. PMID:12483510. 3. PMID:28977640. 4. PMID:12923522.

P-25-012
Genomic uracil in single-stranded DNA regions

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In addition to the widely prevalent double-stranded B-DNA, more unconventional DNA structures are also found within living cells, even under normal conditions. These structures are often accompanied by tracks of single-stranded DNA (ssDNA), which, despite the protection provided by ssDNA binding proteins, remain more susceptible to degradation or base edition. Recently, we discovered that two thymidylate synthase inhibitory drugs, RTX and 5FdUR, similarly induced the cellular expression of the base editor APOBEC DNA cytosine deaminases in DNA repair deficient colon cancer cell lines (submitted paper). Interestingly, the expected DNA editing activity was observed within the genome only following 5FdUR treatment, suggesting either additional targeting factors and/or altered accessibility of the genomic DNA upon the two drug treatments. Here, we present new results on the differences in ssDNA content of these drug-treated cells that are actually arrested in S-phase and whose transcription might also be perturbed by elevated thymine-replacing uracil in the template DNA strand. Hence, we investigated the prevalence of RPA coated ssDNA in arrested replication forks and also quantified the transcription-associated R-loops in the treated cells. In addition, we found that conventional methods of genomic DNA isolation and short-read sequencing library preparation fail to capture genomic ssDNA fragments. Therefore, establishing an appropriate experimental pipeline, we measured the genomic uracil content for dsDNA and ssDNA, respectively. Moreover, we refined our previously published U-DNA-Seq approach [Palinkas HL et al. (2020) eLife 9, e60498] to preserve ssDNA fragments, enabling the measurement of genomic uracil patterns in these regions as well. The observed differences showed functional coherence, suggesting that the applied methods could enhance detection of genome editing activities in the chromatin context. Supporting grants: ÚNKP-23-5-BME-467, BO/726/22/8, FK-137867.

P-25-013
Construction of innovative platform based on Arabidopsis, human cell lines, mice and VHH for identification of drugs targeting metabolome-related human diseases

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Tumours exhibit impaired regulatory processes, including the control of chromatin structure and gene expression by SWI/SNF-type chromatin remodeling complexes (CRCs) and mRNA splicing. These perturbations lead to changes at the transcriptomic and proteomic levels, affecting key cellular regulatory functions such as metabolism. TOR hyperactivation and metabolic changes are typical of clear cell renal cell carcinoma (ccRCC) treated with TOR kinase inhibitors. We used the model plant *Arabidopsis thaliana* and the human cell lines to investigate evolutionary conserved processes between eukaryotes and the VHH-based platform for protein targeting. We show that the nuclear fraction of mTOR kinase interacts with the Nineteen Complex (NTC) subunit involved in mRNA splicing. TOR pathway controls NTC and SWI/SNF CRCs subunit phosphorylation. We found the TOR-SWI/SNF interaction and show that inactivation of the SWI/SNF complex causes TOR hyperactivation. The SWI/SNF CRCs and TOR kinase bind directly to the promoter region of the fructose-1,6-bisphosphatase (FBP1) gene and regulate its expression. Also, inhibitor everolimus, a drug used in oncology, does not significantly affect the transcriptome of the wild plant *Arabidopsis thaliana*, whereas it causes widespread transcriptomic changes in the swi3c mutant with inactivated SWI/SNF core subunit. We're using swi3c mutants as test plants to screen for compounds such as everolimus. We are currently using the system established in our laboratory to find VHHs that target the catalytic domain of TOR kinase to inhibit its activity. Our study revealed, evolutionarily conserved, multi-level cellular control system involving SWI/SNF-dependent chromatin remodelling, RNA splicing and metabolic regulation. Our results have allowed a better understanding of the genesis of the molecular alterations observed in ccRCC, and may thus serve as a solid basis for further research. Financial support: NCN: UMO-2018/30/M/NZ1/00180 (TJS) 2021/43/D/NZ2/02461 (SK)

P-25-014
Dynamics of the LDB1 complex and the activation of hematopoietic development

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During development, hematopoiesis occurs in successive waves to fulfil specific needs of the developing embryo by generating all blood cellular components. This intricate process is regulated by a combination of transcription factors (TFs) and their associated protein complexes. Among these, the TFs Gata2/1 and Tal1 are essential for hematopoietic gene transcription and, in combination with Ldb1, constitute a multimeric protein complex (LDB1 complex). We investigate the regulatory roles of the LDB1 complex across three critical stages of mouse hematopoiesis: hemangioblast (HB), pro-erythroblasts (Pro-E) and E13.5 erythroblasts. As differentiation progresses, the analysis of TF occupancy reveals a redistribution to new genomic regions, an increased co-occupancy by the four TFs, and correlation with H3K27Ac marks, indicating higher LDB1 complex activity during later stages of differentiation. Moreover, the LDB1 complex occupancy profile shifts from genes associated with endothelial/vascular development to primitive hematopoiesis and ultimately to definitive hematopoiesis and erythroid maturation. Motif analysis on LDB1 complex occupied regions identifies the potential involvement of the transcription factor EVI1 as a cofactor of the LDB1 complex, possibly influencing hematopoietic commitment from early stages of mesoderm commitment. Preliminary transcriptome analysis of EVI1-depleted embryoid bodies indicates dysregulated expression of relevant mesendoderm, cardiovascular and hematopoietic genes during mesoderm specification. These findings underscore the importance of LDB1 complex redistribution during hematopoietic differentiation and identify EVI1 as a key regulator of lineage commitment during the early stages of mesoderm specification towards hematopoietic and cardiovascular lineages. *The authors marked with an asterisk equally contributed to the work.

P-25-015
Regulation of the transcription pre-initiation complex by nutrient-dependent *O*-GlcNacylation of TATA-box binding protein

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O-GlcNAcylation is a dynamic and finely-tuned post-translational modification that regulates the activities of molecular players involved in all cellular processes, including cell signaling, metabolism, epigenetics, and transcription. Gene expression is carried out by three RNA polymerases (RNAPs), each of which is devoted to a specific class of gene. The assembly of specific molecular complexes at gene promoters to form pre-initiation complexes (PICs) ensures the recruitment and positioning of the correct RNAP. Among the variety of factors necessary to form a functional PIC, the TATA-box binding protein (TBP) is the sole common factor required for all RNAP recruitment and transcription initiation. Recent data showed that TBP, previously considered a passive platform for PIC assembly, is a key player in the dynamic regulation of transcription initiation. TBP is dynamically modified by *O*-GlcNAcylation on its N-terminal domain (NTD) at T114 and S158. *O*-GlcNAcylation at T114 regulates TBP dynamic interaction with chromatin and alters the expression of genes involved in lipid metabolism driven by RNAP-II. Yet, the primary role of S158 *O*-GlcNAcylation remains unknown. Using CRISPR/Cas9 edited cell lines and multi-omics approaches, we endeavor to further decipher the molecular mechanism of the regulation of TBP by *O*-GlcNAcylation and unveil the site-specific effects on cell physiology. Our data show that each site plays a specific role, allowing for the targeted transcription of a subset of genes. Interatomic analysis pointed out that *O*-GlcNAcylation of TBP regulates the assembly of selective factor 1 complex involved in RNAP-I recruitment, and the formation of alternative transcription factor II D complexes involved in RNAP-II recruitment. Altogether, our data provide evidence suggesting that an "*O*-GlcNAc code" is written at TBP-NTD to regulate the assembly of various PICs and hence gene expression.

P-25-016
Inhibition of thioredoxin reductase-1 in a lung cell line as a central regulator level in NRF2 pathway

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The epithelial cells of the lungs play a crucial role in maintaining the function of this organ and are a significant component of defense against potentially harmful substances from the environment. The pulmonary epithelium serves as a protective barrier between the external environment and the internal respiratory system. The inhibition of thioredoxin reductase-1 in a lung cell line acts as a central regulator in the NRF2 pathway. The primary source of regulation for cellular growth and metabolism is reactive oxygen species (ROS), known for their potential anti-cancer antioxidant properties. Cancer cells exhibit a higher demand for ROS, which can be exploited in therapy. The NRF2 pathway is pivotal, controlling the expression of antioxidant genes and maintaining redox homeostasis following detoxification and cell death. Activation of this pathway coincides with an increase in ROS. The study involved three cell lines: Beas2B wild type (WT), positive control, and Beas2B with GPX4 knockout (KO) obtained through CRISPR/Cas9 genome editing. The cells were exposed to erastin, a ferroptosis inducer, at two doses (5 and 10 μM) for 24 h. Subsequently, the expression levels of *NRF2, TRX*, and *TXNRD1* were assessed using RT-qPCR. An elevation in *NRF2* and *TXNRD1* levels, accompanied by a reduction in *TRX* levels, was observed in GPX KO cells. In Beas2B

WT cells, *NRF2* and *TXNRD1* levels decreased, while *TRX* levels increased. The results confirm an enhanced antioxidant activity in cells lacking the glutathione shield, expressed through *NRF2* and *TXNRD1*, responsible for antioxidant regulation and *TRX* reduction, respectively. Funding: The work was carried out thanks to the co-financing of Project-Based Education-PBL (Excellence Initiative – Research University program), in accordance with the Regulations No. 54/2020 and 55/2020 of the Rector of the Silesian University of Technology of March 13, 2020.

P-25-017
Unravelling the FOXM1-dependent biochemical signals controlling self-renewal and differentiation in epidermal stem cells

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Epidermis is constantly renewed during homeostasis or wound healing. Keratinocytes stem cells orchestrate this process, through a tight regulation of self-renewal and differentiation. Leveraging proteomic and transcriptomic data, our study delves into the intricate biochemical signaling pathways and transcriptional factors governing the generation, maintenance, and differentiation of human epidermal stem cells. Previously, we identified FOXM1 as a transcription factor critical for self-renewal of epidermal stem cells, already demonstrated as key player in in controlling DNA repair, cell cycle progression, and metabolism in other cellular system. Investigating the biochemical signals modulating FOXM1 activity and downstream functions, we pinpoint CDK1 as a key kinase governing FOXM1 stability through phosphorylation at Ser251. Our single-cell transcriptomic analysis underscores CDK1 as highly expressed in stem cells compared to transient amplifying progenitors. We unveil a positive feedforward loop wherein phosphorylated FOXM1 enhances CDK1 expression, further amplifying its own activation. Moreover, we identify novel epigenetic mechanism by which FOXM1 control stemness traits: it orchestrates the transcriptional expression of histone linker H1 isotype B, predominantly found in stem cells. H1B levels correlate significantly with stem cell abundance and epigenetically silence the expression of differentiation markers, thereby maintaining stemness. Additionally, integrated transcriptomic and metabolomic analyses, along with bioinformatic predictions, hint at heightened glycolytic activity in stem cells compared to transient amplifying progenitors, a process under the control of FOXM1. Our comprehensive findings shed light on the intricate biochemical mechanisms underlying FOXM1 activity and function in human epidermal stem cells, offering insights into skin biology and paving the way for innovative regenerative therapies. *The authors marked with an asterisk equally contributed to the work.

Cancer and Metabolism

P-26-001
Disrupting protein methylation in pancreatic cancer cells alters KRAS signalling and suppresses autophagy

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Pancreatic cancer cells with KRAS mutations require significant basal autophagy for growth and viability. In this study, we observed how protein methylation mediates some of the processes that enable the maintenance of this basal autophagy in pancreatic cancer cells. Therefore, methylation of key proteins, such as PP2A and MRAS, underpins the autophagic activity of these cells. Disruption of protein methylation through hypomethylating treatment (HMT), which reduces levels of S-adenosylmethionine in cells while increasing the amount of S-adenosylhomocysteine, leads to autophagy inhibition and endoplasmic reticulum stress activation in pancreatic cancer cells. Hypomethylation conditions that decrease MRAS membrane localisation cause KRAS signalling imbalance, leading to ERK partial inactivation and overactive PI3K/AKT-mTORC1 pathway. Significantly, HMT impedes CRAF activation by interrupting the SHOC2 ternary complex (SHOC2/MRAS/PP1), which functions as a CRAF-S259 holophosphatase. PP2A inactivation by demethylation processes also prevents autophagy activation by inhibiting ULK1 activity, in addition to restoring the cytosolic localisation of MiT/TFE transcription factors. Since autophagy allows pancreatic cancer cells to adapt to various stresses, while at the same time promoting pathogenesis and resistance to KRAS pathway inhibitors, this proposed hypomethylation treatment represents a potential therapeutic opportunity for patients with pancreatic adenocarcinoma.

P-26-002
Development, characterization and *in vitro* evaluation of dual drug-loaded pH-sensitive polymer nanoconjugate for ovarian cancer

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Ovarian cancer (OC) is the 7th most common malignancy among women and ranks 8th in cancer-related mortalities. However, even after achieving complete remission in first-line treatment, 75–80% of patients experience recurrence. Therefore, more effective treatments are needed. It is well-known that the VEGF and LPA pathways undergo alterations in OC, promoting survival, migration, invasion, tumor growth, and angiogenesis. Cabozantinib (CBZ) is a multi-receptor tyrosine kinase inhibitor that effectively targets VEGFR-1, 2, 3. Ki16425 is a selective inhibitor of LPA receptors 1, 2, and 3. In OC, it is known that there is a pathological feedback loop between VEGF and LPA receptors. In this research, the aim was to develop a polymer-drug nanoconjugate targeting both VEGFR and LPAR inhibition. For this,

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polyethylene glycol (PEG), known for its advantages in cancer studies, was selected as the carrier system. A nanoconjugate containing Ki16425 and CBZ was synthesized and its treatment potential was assessed. CBZ and Ki16425 were conjugated to the PEG_{5000} through pH-sensitive hydrazone and ester bonds with EDC/NHS and DCC/DMAP coupling, respectively. After characterization, *in vitro* drug release was conducted and OC treatment potential was evaluated with cytotoxicity, apoptosis, migration and gene expression analysis on A2780, OVCAR3 and SKOV3 cell lines. It was observed that CBZ and Ki16425 were efficiently bound to PEG_{5000}, displaying controlled and sustained release profile. *In vitro* studies have demonstrated that the nanoconjugate exhibits a higher cytotoxic effect at a lower dose compared to free drugs on OC cells and triggers a greater level of apoptosis. In conclusion, it has been found that the developed nanoconjugate exhibits quite effective therapeutic potential in OC cells and it is considered to be a promising nanoconjugate. Acknowledgments: This work was supported by the Scientific and Technological Research Council of Türkiye (TUBITAK) (Project number: 121S625).

P-26-003
Glutamate accumulation in myelofibrosis microenvironment induces mesenchymal stromal cells senescence by fumarate overproduction

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Primary myelofibrosis (PMF) is a myeloproliferative neoplasm characterized by inefficient hematopoiesis. Malignant clone proliferation is regulated by its interaction with the tumor microenvironment (TME), characterized by high-grade fibrosis and inflammation. Here, mesenchymal stromal cells (MSCs) are reprogrammed to become senescent, releasing proinflammatory cytokines as part of senescence-associated secretory phenotype (SASP), therefore enhancing the PMF-TME inflammatory status. The latter is also enriched in several metabolites contributing to reeducating TME to promote malignant clone progression. We thus investigated the abundance of several metabolites on PMF peripheral blood by HPLC, detecting an accumulation of gutamate (Glu) and its byproduct fumarate, both reported to prompt cell senescence. Therefore, we assayed the impact of these metabolites on the mesenchymal compartment using an *in vitro* model of healthy MSCs. Cells treated with Glu and fumarate accumulated ROS, increasing the overall DNA damage, and fumarate-associated epigenetic modification H3K36me2. Furthermore, fumarate hampered cell proliferation, as reported by clonogenic assay and triphosphate accumulation. Most importantly, Glu and fumarate supplementation triggered cell senescence, increasing β-Galactosidase activity. This outcome was supported by phospho-p53 accumulation and increased expression of SASP-associated genes IL6, TFN, and TGFβ. Furthermore, senescent cells were also characterized by increased 5mC, also detected on primary PMF-MSCs. Finally, we also detected Col1a1 enhancement upon fumarate supplementation, unveiling the contribution of this metabolite in the establishment of the typical fibrotic PMF-TME. Therefore, our data unveil the Glu metabolism contribution to PMF onset. Its conversion in fumarate primes MSC senescent profile, promoting the typical TME fibrosis. Further studies will be needed to inhibit Glu uptake to achieve novel therapeutic strategies against PMF.

P-26-004
The effect of nutrients, hormones and signaling molecules on the expression of lysophospholipase PNPLA7 and β-actin in hepatocellular carcinoma cell line

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PNPLA7 (*Patatin-like phospholipase domain containing protein 7*) is an intracellular membrane-bound lysophospholipase highly expressed in testes, heart and insulin-targeted tissues – liver, skeletal muscles and adipose tissue. Studies have shown that it is regulated by metabolic signals such as insulin and glucose, thereby suggesting it plays a role in fast/feeding cycles and energy metabolism. While the physiological role and regulation of PNPLA7 in liver is still unknown, it was shown that mice with liver *PNPLA7* deficiency display hypoglycaemia, hypolipidemia, low energy consumption and reduced secretion of very-low-density lipoproteins, which overall suggests its importance in the whole-body energy metabolism. Furthermore, it was shown that *PNPLA7* is downregulated in the hepatocellular carcinoma (HCC) as a result of hypermethylation of its promotor. However, the role of PNPLA7 in HCC development is unclear. The purpose of our study was to investigate the regulation of PNPLA7 in HCC *in vitro*, since one of its hallmarks is dysregulation of lipid metabolism. Therefore, we exposed HepG2 cells to different concentrations of insulin, glucocorticoid dexamethasone, activators of protein kinase A pathway (i.e. forskolin, cAMP) and glucose, and examined the protein abundance of PNPLA7 and β-actin by immunoblotting after 16 or 24 h of the treatment. First, our results show that different glucose concentrations affect protein levels of β-actin, thereby implicating it might not be a good housekeeping gene under these conditions. Secondly, glucose and insulin tended to lower protein abundance of PNPLA7, which is consistent with the literature data on non-cancer cells. Conclusively, these results suggest that regulation mechanisms for PNPLA7 are preserved and therefore, the potential of PNPLA7 as a therapeutic target in HCC should be studied further.

P-26-005
STAT3 post-translational modifications (PTMs) involved in carcinogenesis and energy metabolism

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The oncoprotein STAT3 is a hub of numerous signaling pathways, its activity is modulated by a variety of PTMs. The STAT3 canonical pathway involves phosphorylation at Y705 in response to cytokine, growth factors. Additionally, the phosphorylation on S727 residue of STAT3, PTM present in a more aggressive

tumor, activates non-canonical pathway that induces its localization to mitochondria, where it can regulate oxidative phosphorylation. Our aim is to investigate how the STAT3 and its PTMs could influence energy metabolism in prostate cancer (PCa). Literature showed that the STAT3/HIF-1α/PKM2 loop is involved in the energy metabolism, in particularly in the "Warburg effect," which is characteristic of the late stages of carcinogenesis. Consequently, our purpose, is how it is possible to modulate the activity of STAT3 PTMs and the proteins involved in the loop, in order to slow down or reverse the progression of PCa. Experiments were performed on DU-145 AR-PCa cell line. Whereas some allosteric modulators of PKM2 prevent its translocation into the nucleus and the reactive oxygen species (ROS), typical of a highly proliferative tumor, activate both HIF-1a and STAT3 pathways; we chose to treat the cells with Serine as a PKM2 modulator, N-acetylcysteine (NAC) as an anti-ROS and S3I-201 as a selective STAT3 inhibitor. Immunofluorescence and western blot assays showed cytosolic PKM2 localization, reduced HIF-1α and pS727 STAT3 expression in treated samples. In all treatments, can be observed a reduction in ROS species and the Warburg effect, in fact, lactate/pyruvate dosages obtained by gas/mass revealed a decrease in lactate production and an increase in pyruvate. These results underline how STAT3 is the hub of this circuit and lead us to hypothesize that modulation of the tumor microenvironment could contribute to controlling the progression of carcinogenesis.

P-26-006
Investigation of the relationship between ARID1A and EZH synthetic lethal interaction in T-ALL

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In common human malignancies, genes encoding SWI/SNF chromatin remodeling complex proteins are commonly altered. ARID1A, an essential member of this complex, attaches to DNA and directs SWI/SNF complexes to the chromatin region that requires remodeling. ARID1A mutations have been discovered in numerous cancer types by genome-wide sequencing investigations. Furthermore, research into ARID1Amt malignancies has led to the identification of certain susceptible genes. Typically, targeting these susceptible genes in ARID1Amt tumors results in synthetic lethality. The catalytic subunit of Polycomb repressive complex 2 (PRC2) is EZH2, which suppresses histone methylation and transcription. Defects in SWI/SNF function via mtARID1A promote Polycomb activity on chromatin, which may make cells more susceptible to EZH2 inhibition. The aim of this study is to investigate the possible synthetic lethality effects of inhibition of EZH2 using Jurkat and Molt-4 cell line, which contain a mutated in ARID1A (frameshift), and ARID1Awt respectively. GSK2816126 (GSK) was used to inhibit EZH2 in Jurkat and Molt-4 T cell acute lymphoblastic leukemia (T-ALL) cell lines. Cell viability and apoptosis/necrosis were measured using WST-1 and Annexin V/7AAD, respectively. WB analysis was used to evaluate the protein levels of ARID1A, EZH2, H3K27me3, total H3, and GAPDH. Results: The suppression of EZH2 by GSK therapy was identified in Jurkat and Molt-4 cells by a decrease in H3K27me3 protein levels. EZH2 inhibition reduced cell survival and caused apotosis in Jurkat cells in a time and dose-dependent manner. However, no change in cell viability or apoptosis was observed in the Molt-4 cell line at any concentration tested. GSK inhibited EZH2 in ARID1Awt Molt-4 and ARID1Amt Jurkat cells, resulting in a synthetic lethal impact via apoptosis. These findings imply that EZH2 could be a therapeutic target in ARID1A-deficient acute lymphoblastic leukemia.

P-26-007
Loss of Sirtuin 3 in mouse embryonic fibroblasts augments sex-specific differences in redox and metabolic signaling

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Sirtuin-3 (Sirt-3) is the most important mitochondrial deacetylase and regulates numerous biological processes. It has been shown that Sirt-3 is an important anti-ageing molecule and regulator in many age-related diseases, and is also involved in various metabolic pathways and cancers. These processes are known to differ between the sexes, but there is little information on the sex-specific role of Sirt-3. Using mRNA sequencing, we investigated the differences in global gene expression patterns between Sirt-3 wild-type (WT) and knockout (KO) mouse embryonic fibroblasts (MEF). We performed differential expression analysis, gene-set enrichment analysis (GSEA) and pathway analysis between WT and KO MEF of both sexes. To confirm our findings, levels of key proteins were analysed by western blot. Our results show that the effects of Sirt-3 knockout differ significantly between the sexes. In male KO MEF, a decrease in mitochondrial function leads to pseudohypoxia and a subsequent hypoxia-inducible factor 1α (Hif-1α) response. Moreover, male KO MEF are under chronic oxidative stress and induce a sustained Atf-4-mediated integrated stress response, together with the unfolded protein response in both mitochondria and the ER, as a means to adapt to the loss of Sirt-3. On the other hand, female KO MEF can compensate for the absence of Sirt-3 by an as yet unknown mechanism and are able to keep the level of oxidative stress low enough to avoid the effect observed in male KO MEF. Since Sirt-3 plays a role in various age-related and metabolic diseases as well as cancer and is considered a potential therapeutic target, these results should prompt future research to routinely consider sex as an experimental variable.

P-26-008
Unveiling the role of C3G in glioblastoma: stemness, initiating capacity, and metabolic reprogramming

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Glioblastoma (GBM) is an astrocytoma characterized by its aggressiveness and inter- and intra-tumor heterogeneity. Glioblastoma-initiating cells (GICs) comprise a cellular subpopulation with high plasticity and self-renewal capacity, responsible for resistance to treatments, tumor initiation and recurrences. A

metabolic reprogramming is observed within GBM cells and GICs, being upregulated both glycolysis and fatty acid synthesis. C3G is a Rap guanine nucleotide exchange factor (GEF), which also acts through GEF-independent mechanisms. C3G regulates several cellular functions, playing a dual role in cancer. C3G levels are downregulated in GBM, which enhances migration and invasion promoting the acquisition of a mesenchymal phenotype [previously published in: Manzano S. et al. (2021) Cell Death & Disease 12(4):348]. We have analyzed the role of C3G on GBM stemness and initiating capacity, using U87 and non-commercial patient-derived (12Φ12) cells with permanent C3G silencing, grown under non-differentiated conditions. C3G-silencing favors the generation of more spheres, but smaller, in both cell lines. C3G downregulation also increased cell initiating capacity measured by limited dilution assays, and increased mRNA expression of stem cell markers, such as OCT4, SOX2, NANOG, PROM1 or BMI1. On the other hand, C3G-silenced GBM spheres increased PKM2 (mRNA and protein) levels, a multifunctional protein that favors glycolysis and regulate transcription of genes like OCT4 upon translocation to the nucleus. Besides, Seahorse experiments showed that C3G knock-down increases basal and maximal respiration, ATP production and extracellular acidification rate in U87 cells, which supports an enhanced mitochondrial respiration and glycolysis. Therefore, we can conclude that low C3G levels promote GBM stemness and metabolic reprogramming, favoring tumor initiating capacity and glycolysis. The underlying mechanisms should be further characterized.

P-26-009
Investigation of the role PPM1D/WIP1 phosphatase in genotoxic stress-induced autophagy in Rhabdomyosarcoma

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Autophagy can be induced as a result of DNA damage and activation of DDR by chemotherapeutic agents in tumor cells. PPM1D/WIP1 is a Ser/Thr phosphatase activated upon genotoxic stress in a p53 dependent manner. Wip1 is amplified and over expressed in common human cancers such as breast, ovarian, pancreatic, neuroendocrine, medulloblastoma, neuroblastoma, colon carcinoma, and thus exerts oncogenic functions. Deregulated and overexpressed Wip1 negatively regulates global cellular stress responses such as ATM/ATR-mediated DNA damage response, DNA repair, cell cycle checkpoints, apoptosis and cellular senescence. Rhabdoid tumors (RT) are a type of soft tissue sarcoma that is highly aggressive and resistant to chemotherapy. In our previous study, we showed that Wip1 was overexpressed and amplified in the A204 RT cell line. Here, we aimed to investigate the role of oncogenic Wip1 in regulation of chemotherapy induced autophagy in RT cells. Etoposide was used to induce autophagy in A204 RT cells, Chloroquine (CQ) and GSK2830371 were used to inhibit autophagy and Wip1, respectively. The conversion of LC3I-II and P62 degradation, as well as protein levels of total and Ulk1 p-Ser638 were analysed by WB. Wip1-Ulk1 interaction was demonstrated by coIP analysis. LC3 puncta formation and detection of Wip1-Ulk1 proteins were analyzed by immunofluorescence staining. Apoptosis, cell cycle, and senescence were measured by AnnexinV/7AAD, BrdU/PI analysis and SAβgal staining, respectively. Colony formation assay was used to assess cells ability to colonize. In A204 cells etoposide induced autophagy is confirmed by LC3 I-II conversion and P62 protein degradation. Inhibition of Wip1 increased phosphorylation of Ulk1 from Ser638 during etoposide induced autophagy. Co-inhibition of autophagy and Wip1 increased the rate of apoptosis in response to etoposide. Thus, targeting Wip1-Ulk1 has been identified as a promising therapeutic strategy to improve chemotherapy response in A204 cells.

P-26-010
Studies of the cellular response induced by highly active unsymmetrical bisacridines in pancreatic cancer cells cultured in 2D and 3D

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Pancreatic cancer is considered a treatment-resistant cancer with one of the worst prognosis. The great heterogeneity as well as altered cell metabolism and many genetic mutations (TP53, CDKN2A, SMAD4, and K-RAS) contribute to tumorigenesis and the high metastatic potential of this solid tumor. Moreover, the MYC oncogene is overexpressed in 43.5% of primary pancreatic cancers, therefore chemotherapeutics suppressing this gene are in demand. Unsymmetrical bisacridines (UAs) are patented compounds with high cytotoxic and antitumor activity against many cancers, particularly human pancreatic cancer. In this study, we examined the cellular response induced by the most active UA derivatives: C-2028, C-2045, and C-2053 in human pancreatic cancer cell lines: Panc-1, MIA PaCa-2, AsPC-1, and BxPC-3 with different mutations status of mentioned above genes, cultured in 2D and 3D condition. Western blot analysis showed that in all tested cells (2D) UAs caused a decrease in c-Myc protein levels which correlates with previously obtained results proving the induction of apoptosis by these compounds. However, C-2045 and C-2053 derivatives caused a complete loss of this protein in AsPC-1 cells after 120 h of incubation. Additionally, studies regarding the 3D culture model showed that spheroids generated from the tested cells were highly sensitive to UAs. Spheroids derived from Panc-1 and AsPC-1 cells exposed to UAs became less compact over time. In contrast, spheroids derived from MIA PaCa-2 cells after treatment with the compounds were dense and shrank during incubation. Cell viability analysis showed that tested cells cultured in both models were the most sensitive to the same derivative, reaching up to 62 and 49% of dead cells after 72 h in 2D and 3D, respectively. In conclusion, we have demonstrated that UAs exhibit high anticancer properties in both 2D and 3D culture conditions and decreased c-Myc protein levels, making them promising therapeutic candidates.

P-26-011
Can umbilical cord-derived mesenchymal stem cell exosomes suppress the SMAD3/SLUG signalling pathway in cervical cancer when loaded with paclitaxel?

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Mesenchymal stem cells (MSC), which have high exosome-releasing capacity, have the potential to be used as a drug carrier system. Exosomes also effectively demonstrate their ability to enter cells as a drug delivery system. This study aimed to identify the mechanisms that MSC-derived exosomes influence during drug delivery in cervical cancer cells (HeLa). In this study, mesenchymal stem cells were isolated from the umbilical cord (UC-MSC) taken at birth. Isolated UC-MSCs were characterized by CD34, CD90, CD105, and CD34 markers. The exosomes were examined for size and morphology using an electron microscope. The potential of using paclitaxel (Exo-PAC) in HeLa cancer treatment was investigated by loading the released exosomes with paclitaxel (Exo-PAC) by electroporation. It was determined that Exo-PAC affected HeLa cells at lower concentrations and in a shorter time. Exo-PAC suppressed SMAD3 and SLUG proteins, which are effective in cell metastasis and angiogenesis. At the same time, PAC showed its effect on proteins in the apoptotic pathways and induced the BAX/BCL-2 ratio. In this study, it was shown that SMAD3 and SLUG transcription factors, which are effective in epithelial-mesenchymal transition mechanisms, can be suppressed by exosomal drug carriers. It has been shown that UC-MSCs can be used as drug delivery systems to suppress cell invasion by blocking SMAD3 and SLUG signaling pathways in the cell. This study was supported by TUBITAK 1002 with project number 120S682.

P-26-012
Targeting metabolism to impair tumor lymphangiogenesis

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Lymphangiogenesis, the generation of new lymphatic vessel from pre-existing ones, contributes to cancer progression and metastasis. Targeting the metabolism of lymphatic endothelial cells (LEC) has emerged as a promising strategy to block lymphangiogenesis and hinder cancer progression. Through a comprehensive meta-analysis of breast cancer single cell RNA-sequencing datasets encompassing both normal and tumor-associated LECs, upregulated genes were identified in the latter. This finding was validated through *in vitro* experiments involving direct and indirect coculture of LECs with different cancer cell lines. We further silenced different genes in LECs and studied their functional effects. Cell viability, proliferation, migration, invasion, spheroid sprouting, and tube formation were affected by the absence of these genes. Moreover, analysis of the redox balance revealed higher levels of oxidative stress and lower antioxidant power upon gene silencing. Besides, mitochondrial reactive oxygen species were elevated, accompanied by increased mitochondrial fragmentation. In this context, analysis of oxygen consumption rates revealed that silenced LECs had lower glycolytic rates but increased ATP production. To substantiate the relevance of these metabolic genes *in vivo*, the murine fat pad model was performed, and tumor growth and metastasis were evaluated. Collectively, our findings show that LEC metabolism is as a promising target to be harnessed for preventing cancer progression and metastasis.

P-26-013
Rivaroxaban – an anticoagulant agent – appears to have a role in reversing the hypoxia-induced metabolic adaptation observed in colorectal cancer cell lines

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The pro-coagulant activity of cancer is known for its ability to induce FXa and thrombin. FXa promotes cancer growth and metastasis via PAR, according to recent literature reports. This study aimed to investigate the effect of the inhibition of FXa, which is known to play a crucial role in cancer metabolism in hypoxia, on the behaviour of colon cancer cell lines HCT-116 and HT-29. We performed studies under both normoxic and hypoxic conditions. We used immunofluorescence to assess the expression of key elements in tumor metabolism, including HIF-1 alpha, LDH-A and GLUT-1, following administration of rivaroxaban, an FXa inhibitor known to not affect cell viability. The expression of HIF-1 and LDH-A increased under hypoxia. However, it was observed that the expression decreased in the rivaroxaban-treated group. Strikingly, no statistically significant difference was found for GLUT-1 expression. Furthermore, in the analysis of E-cadherin and N-cadherin expression levels, the effect of rivaroxaban on migration under hypoxia was statistically significant in comparison to the control group. These findings were further supported by the statistical results of the wound patency in the wound healing experiment. Based on the results of this study, we demonstrate that the inhibition of FXa with rivaroxaban may represent a novel target for the treatment of tumor hypoxia. O.B. supported by the CoHE 100/2000 project. The study is funded by Dokuz Eylul University Scientific Research Projects Coordination Unit with project number TSG-2022-2576.

P-26-014
Circadian-based metabolomics and lipidomics of bone marrow adipose tissue and implications in myeloid leukemia pathogenesis: the crucial role of cell synchronization

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Circadian rhythms are crucial in regulating metabolic processes. Based on literature demonstrating the close association between leukemia and abnormal lipid metabolism, we hypothesized that dysregulation of the circadian clock of bone marrow adipose tissue (BMAT) may contribute to the pathogenesis of myeloid leukemia. It has been shown that acute myeloid leukemia (AML) blasts often exhibit altered metabolism, and circadian disruptions could be both contributors to and outcomes of these metabolic changes. Thus, we conducted a study involving human BMAT-mesenchymal stem cells (MSCs) from healthy donors (HD), AML, and Fanconi Anemia (FA) patients, which is an inherited disease characterized by AML predisposition. Our primary goal in circadian metabolomics experiments includes identifying rhythmic metabolic cues of circadian clocks to help understand the consequences of clock disruption or misalignment in BMAT MSCs in leukemia pathogenesis. Depending on the specific question, we collected cell culture supernatants under asynchronized or synchronized conditions (dexamethasone exposure for one hour at 100 nM) at zeitgeber times 6 and 18. The metabolomic analysis involved using two orthogonal metabolomics platforms: gas chromatography-mass spectrometer and liquid chromatography quadrupole time-of-flight mass spectrometry. Metabolomics datasets were analyzed for pathway enrichment and function. Hierarchical clustering analyses showed 50 metabolites at two different time points which were highly distinct after synchronization. Healthy donor samples showed significant enrichment in metabolites related to the pentose phosphate pathway upon synchronization, whereas catecholamine biosynthesis pathways were enriched in FA and AML groups. Our results suggest incorporation of circadian considerations into metabolomics studies can contribute to a more comprehensive understanding of disease mechanisms. This study was supported by TUBİTAK with grant number 220S759.

P-26-015
Simvastatin triggers a metabolic shift towards lipid metabolism in B16.F10 murine melanoma cells

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Simvastatin (SIM), a competitive inhibitor of 3-hydroxy-3-methylglutaryl-Coenzyme A reductase (HMG-CR), has proved anti-tumoral effects at elevated doses. This research explores the impact of SIM on B16.F10 murine melanoma cells, specifically focusing on energy metabolism. In close connection with SIM's primary pharmacological role in inhibiting the cholesterol biosynthesis pathway, we also investigated statin's effect on the transcriptional and post-synthetic regulation of this metabolic pathway. Moreover, our previous study has proved that under normoxic conditions, the expression of hypoxia inducible factor 1 (HIF-1) – a central regulator of cancer cell metabolism, was inhibited by SIM. To gain insight into the molecular mechanisms of the SIM-induced inhibition of HIF expression, we also explored the metabolic effects of SIM on melanoma cells. RNA-seq analysis was conducted for mRNA expression, while western blot analysis assessed levels of HIF-1, glucose transporter 1, fatty acid synthase. Enzymatic assays measured catalytic activities of key enzymes involved in main metabolic pathways affected by SIM administration. SIM treatment significantly reduced glycolysis by decreasing the catalytic activities of PFK1 and LDH, but led to a shift toward fatty acid metabolism. Additionally, SIM significantly reduced gene expression of sterol regulatory element binding transcription factors (SREBPs) and forkhead box M1 (FOXM1), linked to mevalonate pathway regulation. Several mevalonate pathway enzymes were upregulated following SIM administration, excluding HMG-CR. In summary, SIM efficiently inhibited glycolysis in melanoma cells, leading to compensatory responses in de novo fatty acid synthesis. Also, SIM affected regulatory factors for cholesterol biosynthesis, associated with cancer processes like cell proliferation and metastasis. L'Oréal – UNESCO "For Women in Science" (no. 914/26.11.2020), UEFISCDI PN-III-P2-2_1-PED-2021-0411 (No. 659PED/2022) (Alina Sesarman).

P-26-016
SLC6A14-dependent glutamine metabolic reprogramming mediates the cancer stemness and early onset of breast cancer

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The age at diagnosis for breast cancer patients is progressively decreasing, and the prognosis and survival rates for those with early-onset breast cancer (EOBC) are notably poor. Understanding the carcinogenic mechanisms behind EOBC is crucial for developing new therapeutic strategies. Using exposure to plasticizers, a well-known risk factor for EOBC, as our research

model, our data reveals that DEHP, a commonly used plasticizer, stimulates cancer stemness, leading to early onset and tumor progression. In response to DEHP exposure, transcriptomic analysis indicates a significant upregulation of gene sets associated with mitochondrial respiration-related functions. The increased expression of SLC6A14 glutamine transporter may contribute to DEHP-induced glutamine uptake and mitochondrial respiration, ultimately promoting the spheroid formation of breast cancer cells. Consequently, our findings suggest that EOBC-associated cancer stemness may involve an increase in oxidative phosphorylation, potentially by enhancing the cross-talk between glutamine and fatty acid metabolism. This study not only offers molecular insights into plasticizer-induced tumor initiation and breast cancer progression but also presents novel strategies to enhance the therapeutic outcomes for patients with EOBC.

P-26-017
SIRT2 deacetylase regulates TKI drug sensitivity in renal cell carcinoma cells and SIRT2 inhibitor helps overcome drug resistance

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Inhibiting vascular endothelial growth factor receptors-2 and -3 (VEGFR-2, VEGFR-3) and platelet-derived growth factor receptor beta (PDGFR-beta) was approved by U.S. Food and Drug Administration (FDA) for the treatment of advanced renal cell carcinoma (RCC). The chemical inhibitors for these receptor tyrosine kinase (RTK) targeted therapies include sorafenib and sunitinib. Although effective to RCC patient treatment, the main limitation of these targeted therapy is drug resistance. To uncover molecular mechanism of RTK inhibitor drug resistance acquaintance, we made sunitinib resistant RCC cell lines after treating with increasing dose of sunitinib. Comparing mRNA expressing levels between original and sunitinib resistant RCC cells, we found that several proteins were highly expressed in resistant cells. Among them, we focused on Sirt2 deacetylase enzyme. Sirt2 protein and mRNA expression was increased after sunitinib treatment in all tested RCC cells. When we repressed Sirt2 expression in original and sunitinib resistant RCC cells using shRNA expressing viral vector, significant changes in cell viability were observed after sunitinib treatment. And apoptotic signaling was induced in Sirt2 repressed RCC cells after sunitinib treatment. Finally, double treatment of sunitinib and Sirt2 chemical inhibitor increased apoptotic cell death in tested RCC cells. Those data showed us that Sirt2 protein is involved in sunitinib resistant acquaintance in RCC cells and could be the therapeutic target for overcoming RTK inhibitor drug resistant.

P-26-018
Increment of prostate tumor formation and metastasis in TRAMP mice subjected to a high-fat diet: targeting AMP-activated kinase (AMPK) as a therapeutic option

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Prostate cancer (PCa) is the second-leading cause of cancer death in the world. About 1 in 8 men will be diagnosed with prostate cancer during their lifetime. Europe is the region of the world with the highest percentage of new cases (37.5%). Nutrients, including fat, protein, carbohydrates, vitamins (vitamins A, D, and E), and polyphenols, potentially affect PCa pathogenesis and progression. Some research suggests that eating lots of saturated fat might be linked with an increased risk of prostate cancer coming back after surgery, and of developing advanced prostate cancer. It has been previously shown that dietary fat for 11 weeks enhances prostate cancer progression in the TRAMP (Transgenic Adenocarcinoma of the Mouse Prostate) mouse model. In this study, we have used the TRAMP prostate cancer model to investigate the influence of a high-fat diet (HFD) on lung and liver metastasis. 24 mice were randomly divided into 4 groups (n = 6) and fed for 24 weeks with a standard diet, a high-fat (45% Kcal fat) diet, or each diet supplemented with a compound that induces AMPK activation. We measured every three days the mice's weight, waist circumference, and diet consumed. After 24 weeks, the mice were sacrificed and prostate tumors were dissected, weighed, and frozen at −80°C until their use. Number of metastases in the lungs and liver was counted. Results showed that, as expected, the weight and waist circumference of mice fed with HFD were significantly higher than that of mice fed with the standard chow. In addition, HFD-fed animals presented more metastasis, and the prostate tumors formed were bigger and had higher weight than the controls. Interestingly, mice fed with HFD and the AMPK activator had weight and waist circumferences similar to controls. Likewise, tumors formed and metastases number were significantly lower than that of HFD-fed mice. In conclusion, Targeting AMPK may be a therapeutic option for prostate cancer. Financial support: Fundación Tatiana and ISCIII. *The authors marked with an asterisk equally contributed to the work.

P-26-019
Investigation of the cytotoxic and antimetastatic effect of quetiapine fumarate on pancreatic cancer cells

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Quetiapine fumarate is a typical antipsychotic agent from the dibenzothiazepine class and is approved by the US Food and Drug Administration for the treatment of psychotic symptoms

such as schizophrenia and mania. Besides their use in psychiatric findings, there is also research on the use of antipsychotics in cancer treatment. Moreover, cell and animal models have been used to study the anti-cancer properties of antipsychotics. Antipsychotics have been shown to have potent anti-cancer properties that play a role in suppressing tumor growth, invasion, metastasis and reversal of chemoresistance. However, no studies have been encountered in the literature regarding its effect on pancreatic cancer cells. For this reason, we aimed to investigate the cytotoxic effect and antimetastatic potential oof quetiapine on pancreatic cancer cell lines (Panc-1 and MiaPaca-2 cell lines). Firstly, pancreatic cell lines were treated five different doses of Quetiapine fumarate (150–75–39–18 and 9 μM). Then, cell viability was determined using MTT assay. In addition, the effect of Quetiapine fumarate on metastatic character of pancreatic cell lines was analyzed with scratch and colony forming assay. As a result, it has been determined that, quetiapine fumarate had the cytotoxic effect on pancreatic cells in a dose-dependent manner. Particularly, it has showed more toxic effect on Panc-1 cells at 48 h. These results are important in terms of determining the suitability of active substances that have passed the phase stages, especially for pancreatic cancer use.

P-26-020
In silico and *in vitro* determination of potential RSK inhibitors for colon cancer therapy

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The ribosomal S6 kinase (RSK) family comprises four isoforms (RSK1/2/3/4), functioning as serine/threonine kinases. RSK plays a crucial role in various physiological processes like cell growth, proliferation, and migration. RSKs play an important role due to their two catalytic regions, thus, they are proposed as cancer drug targets. To date, several small molecule inhibitors (SL0101 and BID1870) have been reported to be used in the treatment of breast and colon cancers. However, more research should be designed for novel specific inhibitors of RSKs. The aim of this study is to perform the determine of potential RSK inhibitors with *in silico* virtual screening and molecular modeling studies and then, evaluate cytotoxic effect and inhibitor potential of the selected compound in *in vitro* studies on colon cancer cell lines. For this purpose, we screened the databases and performed molecular docking study via Glide/SP method of Maestro (Schrödinger). For cytotoxicity assay, the cells were treated with five different doses of inhibitor (150, 75, 39, 18 and 9 μM). The cell viability values were obtained at 550 nm spectrophotometrically. Also, the inhibitory activity of potential inhibitor was searched with western blotting method using p-RSK antibody. As a result, the potential inhibitor demonstrated a pronounced cytotoxic effect on HT29 cells. Additionally, it led to approximately a 2-fold downregulation of the RSK protein compared to the control group (non-treated cells). The expression of apoptosis-related proteins was assessed through western blotting, revealing an upregulation of Bax and a downregulation of Bcl-2 in the presence of the potential inhibitor compared to the control group. According to our findings, substituted-benzofuro compound have a promising candidate as RSK inhibitor against colon cancer cell lines. *This study is supported by Health Institutes of Türkiye (TÜSEB) (Project Number: 27777).

P-26-021
Mutant p53 stimulates mitochondrial fragmentation in pancreatic ductal adenocarcinoma (PDAC)

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Mitochondrial dynamics play a crucial role in numerous tumors, including pancreatic ductal adenocarcinoma (PDAC), a highly lethal cancer marked by a poor prognosis and frequent mutations in the TP53 tumor suppressor gene. Given these considerations, our goal is to investigate the correlation between mutant p53 and mitochondrial dynamics in PDAC cell lines. To achieve this, PANC-1 cells, a PDAC cell line harboring the R273H gain-of-function mutation in the TP53 gene, underwent a transient transfection with sip53 and was compared to PANC-1 cells transfected with a non-targeting siRNA as a control using transmission electron microscopy (TEM) study. TEM analysis unveiled a higher aspect ratio, serving as an index of mitochondrial length, in sip53 PANC-1 cells compared to control condition. Even live cell imaging, with a confocal microscope, reaffirmed that the loss of mutp53 significantly shifts mitochondrial morphology towards an elongated phenotype, sign of an increased mitochondrial fusion. To conduct a comprehensive analysis of the 3D mitochondrial network a specific tool, called Mitochondrial Network Analysis (MiNA), was used, confirming the presence of more elongated branches and junctions when mutp53 is downregulated. To gain deeper insights into the correlation between mutp53 and mitochondrial dynamics, we analyzed mitochondrial extracts from both PANC-1 cells and PANC-1 cells with p53 knockout using mass spectrometry. Among the proteins emerged to be up regulated in PANC-1 cells we investigated the non-muscle myosin IIC (MYH14) which appears to be involved in the constriction process of mitochondrial fragmentation. To date, our findings suggest a notable association between mutant p53 and mitochondrial fragmentation; further investigations will be conducted to underscore mitochondria as a promising therapeutic target in PDAC.

P-26-022
Bi-directional crosstalk between NAD/NAMPT and IFN-γ PD-L1 axes in melanoma

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Targeted therapy and immune checkpoint inhibitors (ICIs) have notably improved the treatment of BRAF-mutated metastatic melanoma (MM) patients; however, resistance mechanisms dramatically impact the survival of patients. In MM cells resistant to the BRAF inhibitors, the NAD/nicotinamide phosphoribosyltransferase (NAMPT) axis is overactivated, thus becoming a driver of melanoma progression and resistance. Moreover, extracellular NAMPT, released within the tumor microenvironment, acts as a cytokine-like factor potentially regulating tumor-host interactions. Recent studies highlighted a potential correlation

between the NAD/NAMPT axis and interferon-gamma (IFN-γ)-mediated signaling. Analyzing the TCGA melanoma cohort and cell lines database we found a positive and significant correlation between NAMPT expression and global IFN-γ signaling, as well as a direct correlation with IRF1, STAT1, and the IFN-γ-induced gene CD274. Focusing on CD274/PD-L1, we revealed a direct correlation with NAMPT at protein level, as demonstrated by analyzing a melanoma tissue microarray. BRAF-mutated melanoma cell lines treated with IFN-γ upregulate NAMPT, and vice versa, pharmacological inhibitors of NAMPT activity markedly reduced the activation of the IFN-γ signaling, as well as the expression of CD274/PD-L1. PD-L1, implicated in immune evasion mechanisms in tumors, is regulated at the transcriptional level via IFN-γ/IRF1 axis, but also through the bromodomain and extra-terminal motif (BET) epigenetic factors. Our data revealed a similar molecular regulation also for NAMPT, as demonstrated by the downregulation of NAMPT expression in the presence of JQ1 and AZD5153, two BET protein inhibitors. This novel epigenetic regulation of NAMPT expression via BET proteins will be further investigated. Overall, these data highlighted a novel reciprocal regulation between NAMPT and IFN-γ/PD-L1 signaling activation, linking NAMPT-dependent metabolic reprogramming and immune regulation.

P-26-023
NAMPT inhibition impairs mTOR-dependent regulation of translation in melanoma

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The frequent emergence of drug resistance in melanoma remains a challenge. This phenomenon relies on the rewiring of multiple processes, including cancer metabolism, epigenetics, translation process, and interactions with the tumor microenvironment that are only partially understood. Activation of NAD metabolism through its rate-limiting biosynthetic enzyme nicotinamide phosphoribosyltransferase (NAMPT) has been identified as key driver of targeted therapy resistance and melanoma progression. Another major player in this context is the mammalian target of rapamycin (mTOR) pathway, which plays key roles in the regulation of melanoma cell anabolic functions, energy metabolism and protein translation. An interplay between NAD/NAMPT and mTOR signaling axes in regulating translation was revealed some years ago in a leukemic model, however no further studies fully addressed this functional connection. We showed that NAMPT inhibitors (FK866, OT-82) induce a translational arrest in BRAF-mutated human melanoma cell lines sensitive and resistant to BRAF inhibitors. The molecular mechanism involved the activation of 5′ AMP-activated protein kinase (AMPK), the inhibition of mTOR/4EPB1 and an increased phosphorylation of the initiation factor EIF2A. All these events lead to protein synthesis arrest, measured directly using Click-it chemistry based on the incorporation of an amino acid analog (AHA). Analysis using TCGA database confirmed the correlation between NAMPT and mTOR pathway/translation. In addition, we performed NAMPT immunoprecipitation following mass-spectrometry in cellular extract to identify NAMPT-interacting proteins. Data showed major enrichment of NAMPT-interacting proteins involved in RNA processing, translation, nuclear protein and metabolic processes. The interaction between NAMPT and initiation/elongation factors will be further investigated, however these data suggest a potential direct impact of NAD/NAMPT axis in translational reprogramming in melanoma.

P-26-024
C/EBP-β splicing induces chemotherapy resistance by rewiring metabolism in non-small cell lung cancer

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The transcriptional factor CCAAT/Enhancer Binding Protein-β (C/EBP-β) is involved in cisplatin-resistance in non-small cell lung cancer (NSCLC) [1]. An altered ratio between its two splicing isoforms LAP and LIP triggers metabolic rewiring in murine embryonic fibroblasts [2]. We investigated if this splicing-induced metabolic reprogramming could be linked to cisplatin-resistance in NSCLC. We performed our analysis on NSCLC cell lines selectively overexpressing LAP (LAP+) or LIP (LIP+). LAP overexpression increased drug efflux through ABC transporters and decreased cisplatin-induced cytotoxicity and DNA damage, while LIP displayed opposite effects. According to metabolomic profiles and functional assays, LAP+cells had more energy-producing metabolites, anti-oxidant metabolites and building blocks. LAP+cells showed higher fluxes of tricarboxylic acid (TCA) cycle, electron transport chain (ETC), mitochondrial ATP and reactive oxygen species (ROS), and were fatty acid β-oxidation (FAO)-addicted. The silencing of carnitine-palmitoyl transferase 1A (CPT1A), the FAO pacemaker enzyme, or the use of FAO inhibitor etomoxir restored cisplatin sensitivity in LAP+cells. The efficacy of etomoxir as chemosensitizer was validated in Hu-CD34+NSG xenografts. Single-cell RNA-Seq analysis and mass spectrometry imaging showed that LAP+xenografts had quantitative/qualitative differences in cancer cells and infiltrating immune-cells, and different spatial distribution of FAO-related metabolites that were abrogated by etomoxir (unpublished data). This work demonstrated that C/EBP-β LAP mediates chemoresistance by driving cell metabolism toward a FAO-dependent phenotype. Targeting this pathway is a novel chemosensitizing strategy in NSCLC. References: 1. Salaroglio IC et al. (2022) J Exp Clin Cancer Res, 41(1), 243; 2. Ackermann T et al. (2019) Commun Biol, 14(2), 208. The work was supported by the Italian Association for Cancer Research (IG21408; IG29250).

P-26-025
Targeting collagen in PDAC reduces aggressiveness by enhancing L1CAM expression

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Pancreatic ductal adenocarcinoma (PDAC) is a devastating and essentially incurable disease characterized by a pronounced collagen-rich fibrotic extracellular matrix known as the desmoplastic reaction. While collagen fibrils are primarily synthesized and secreted by stromal cells, the significance and function of cancer-cell-derived collagen have been largely overlooked. We have demonstrated that PDAC tumors, marked by high stroma and TGF-β1 content, exhibit reduced expression of the L1 cell adhesion molecule (L1CAM, L1), which marks a highly tumorigenic subpopulation of cancer stem cells (CSCs) capable of disseminating and forming liver metastases[1]. RNA sequencing (RNA-seq) revealed an increased expression of genes associated with the matrisome, particularly collagen biosynthesis, in cells with low levels of L1 (L1low) compared to their counterparts with high levels of L1 (L1high). Single-cell RNA sequencing (scRNA-seq) data analysis confirmed a mutually exclusive pattern between L1 and collagen. By categorizing cell identities, we observed that both stromal cells and, intriguingly, epithelial cancer cells expressed collagen-associated genes. We have demonstrated that the L1low epithelial cancer cells actively synthesized and secreted proline-enriched fibrillar collagens *in vivo*. This process serves both as a reservoir of energy for sustaining cellular metabolism and tumor progression and as a barrier to drug resistance. Notably, the treatment of L1low-derived tumors with Tranilast, a novel anti-fibrotic drug inhibitor of TGF-β, together with gemcitabine, the gold standard treatment for PDAC, reduces tumor volume and collagen content compared to untreated tumors, and decrease their ability to form liver metastases Altogether, our findings point to a novel pathophysiological role for L1 and its interconnection with PDAC aggressiveness and fibrosis. Reference: 1. Delle Cave, D. et al. (2020) Oncogene 39, 4271–4285. doi: 10.1038/s41388-020-1289-1.

P-26-026
Design and biological activity of RNA molecules targeting SHMT for cancer therapy

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To identify new therapeutic strategies and new drug targets for tumors, we are exploiting the interaction between RNA and proteins and its importance in tumorigenesis. We have identified as a therapeutic target the metabolic enzyme Serine hydroxymethyltransferase (SHMT) that catalyzes the reversible conversion of serine and tetrahydrofolate into glycine and 5, 10 methylenetetrahydrofolate. SHMT plays a key role in one carbon metabolism, a complex network fueling the precursors fundamental for highly proliferating cells. Cytosolic and mitochondrial isoforms (SHMT1/2) are found overexpressed in different types of tumors, with the SHMT2 isoform related to tumor progression and poor patient prognosis. No effective small molecule SHMT inhibitor is currently available. Starting from the demonstration that RNA molecules can efficiently inhibit *in vitro* both SHMT1 and SHMT2 enzymatic activity [Previously published in Guiducci G. et al. (2019) Nucleic Acids Res. 47, 4240–4254] we have produced novel high affinity inhibitory RNAs targeting SHMT2, as demonstrated by *in vitro* binding and inhibition studies. In parallel, we have devised a strategy to selectively send these RNAs to the mitochondria of cancer cells, causing cell death and tumor size reduction in an animal model. Nanoparticles-based delivery strategies are currently being optimized to improve selective targeting of CD-71 expressing tumor cells. These preliminary results pave the way for new therapeutic opportunities, allowing for the substitution of small molecule inhibitors, currently not entirely utilizable, with RNA-based inhibitors (iRNA).

P-26-027
The impact of TRPV1 receptor in a murine model of neuroendocrine prostate cancer

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Transient receptor potential vanilloid 1 (TRPV1) is a non-selective cation channel known to respond to diverse stimuli including changes in pH, temperature, and ligand binding. Its involvement in cellular processes such as proliferation and apoptosis has sparked growing interest, particularly on its potential implications in cancer biology. Our study aimed to explore the influence of TRPV1 receptor expression on prostate cancer development. We initially assessed TRPV1 expression across various prostate tumor cell lines, revealing heightened levels in tumor cells compared to non-tumoral prostate cell lines, with notably elevated expression in neuroendocrine subtype cells (LN-CSS and LN-FLU). Subsequent exposure of these cells to a TRPV1 agonist at different time points enabled us to delineate changes in receptor expression patterns. Further, to investigate the relevance of TRPV1 in prostate cancer pathogenesis *in vivo*, we conducted experiments utilizing TRAMP (Transgenic Adenocarcinoma of the Mouse Prostate) mice, a model of spontaneous prostate neuroendocrine tumor development. Mice were subjected to either standard diet or a diet supplemented with a TRPV1 receptor agonist for six months. Upon euthanasia, plasma levels of Prostate Specific Antigen (PSA) were measured. Additionally, we analyzed TRPV1 receptor expression in prostate tissue using quantitative PCR and western blot techniques, alongside assessing TRPV1 expression in prostate-derived tumors. Our findings collectively suggest that prolonged treatment with TRPV1 agonists alters TRPV1 receptor expression within the prostate, potentially influencing tumorigenesis pathways. These insights contribute to our understanding of TRPV1's role in prostate cancer and hint at its therapeutic potential in disease management. Financial support: Tatiana Pérez de Guzmán Foundation and ISCIII. *The authors marked with an asterisk equally contributed to the work.

P-26-028
CAF-secreted lactic acid promotes a ferroptosis resistant state in prostate carcinoma

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In prostate carcinoma, lactic acid secreted by highly glycolytic cancer associated fibroblasts (CAFs) supports a metabolic reprogramming in cancer cells by fueling oxidative metabolism and lipid anabolism. Reprogramming of lipid metabolism represents a key hallmark of ferroptosis, a form of cell death mediated by intracellular redox active iron accumulation and extensive peroxidation of phospholipids containing polyunsaturated fatty acids at cell membranes. Several reports underlined that modulation of the availability of specific nutrients is an effective strategy to sensitize cancer cells to this form of cell death. Thus, we investigated the role of stromal lactic acid in regulating ferroptosis sensitivity in prostate cancer (PCa). We found that CAF-isolated conditioned media and exogenous lactic acid protect PCa cells from cell death promoted by the ferroptosis inducers RSL3 and Erastin. Moreover, MCT1 inhibition or targeting of two extracellular pH regulators, carbonic anhydrase (CA) IX and XII, sensitizes PCa cells to ferroptosis upon lactic acid exposure. TMA analysis of tumor tissues from PCa patients revealed that the protein levels of the ferroptosis marker GPX4 positively correlate with high Gleason Grade. Accordingly, GPX4 is overexpressed in PCa cells treated with lactic acid. Both iron and lipid metabolism are potentially involved in lactic acid-mediated resistance mechanism to ferroptosis. In particular, lactic acid deregulates transferrin uptake and ferritin expression levels. Moreover, an iron chelator reverts cell death promoted by cancer cell co-treatment with MCT1 inhibitor and RSL3 in presence of lactic acid. Besides, DGAT1/2 targeting rescues ferroptosis sensitivity, suggesting that lipid accumulation into lipid droplets could mediate the resistance mechanism. Overall, microenvironmental lactic acid protects PCa cells from ferroptosis and CAIX/XII targeting might represent a therapeutic approach to sensitize PCa cells to this form of cell death.

P-26-029
Impact of dietary composition on prostate cancer development: exploring the role of DPP4

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Nowadays, prostate cancer (PCa) is the second leading cause of cancer death in the world. A growing number of authors suggest a possible relation between PCa, metabolic diseases and diet. In fact, obesity is considered a risk factor for most cancers, including prostate cancer. The aim of our study is to investigate the effect of different diets on the development of PCa. To explore this question, a TRAMP (Transgenic Adenocarcinoma of the Mouse Prostate) prostate cancer model was used to investigate the influence of a high-fat diet (HFD) on cancer progression. In this study, 24 mice were randomly divided into 4 groups (n = 6) and fed for 24 weeks with a standard diet, a high-fat diet (HFD) (45% Kcal fat), or each diet supplemented with capsaicin (CAP). The dose of CAP used to supplement the diets was an estimate of the daily human consumption of capsaicin (mild US/Europe). The tolerance of these mice to the different diets was first assessed by testing the amount of feed ingested after two days. In general, the mice ingested the same amount of each of the diets, with the exception of the HFD supplemented with capsaicin. To improve the intake of this diet, a small amount was distributed on the floor of the cages. Once intake was normalised, the mice were maintained under these conditions for 24 weeks. Dipeptidyl peptidase 4 (DPP4) is a protein involved in insulin secretion and its expression has been altered in metabolic diseases such as diabetes mellitus. Because of this, we evaluated the effect of diets on the development of prostate cancer and determined the levels of DPP4 in tumours to elucidate the role it may play in prostate cancer. Financial support: Fundación Tatiana and ISCIII. *The authors marked with an asterisk equally contributed to the work.

P-26-030
Synthetic inhibition of the mevalonate pathway inhibits tumor growth and enhances chemo- and radiosensitization in rhabdomyosarcoma cells

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Dysregulated lipid metabolism represents an important metabolic alteration in cancer. In this work, we focused on the mevalonate (MVA) pathway as a novel therapeutic option to improve standard care of highly aggressive rhabdomyosarcomas (RMS), rare cancers affecting children and adolescence that still have a low

survival rate. By an *in silico* approach, we evaluated the expression of the main enzymes of the MVA pathway using a dataset of human RMS samples. The expression levels of the transcription factor sterol regulatory element binding protein 2 (SREBP2) which promotes the expression of genes involved in MVA pathway and of the two rate-limiting enzymes 3-Hydroxy-3-methylglutaryl-CoA reductase (HMGCR) and squalene epoxidase (SQLE), were found markedly increased in RMS compared to controls. Next, we analyzed the effects of targeting the MVA pathway on human RMS lines. We found that treatment with fatostatin, statins, and zoledronic acid, respectively inhibitors of SREBP2, HMGCR, and farnesyl diphosphate synthase, impaired RMS cell viability favouring apoptosis. Furthermore, lovastatin administration via oral gavage to xenografted tumor mice was able to reduce RMS growth *in vivo*. Finally, the sensitivity of RMS lines to chemo- and radiotherapy was significantly increased after treatment with MVA inhibitors. Statin treatment was able to restore chemo- and radiosensitivity in highly aggressive RMS cell models characterized by expression of a myristoylated Akt1 form and Cav-1 overexpression. These clones showed enhanced survival capability sustained by a faster and more efficient capacity to repair DNA damages, increased expression of the Src kinase and of the antioxidant enzyme Catalase. Thus, we identified a signaling axis crucial for RMS radioresistance that could be efficiently targeted by cholesterol-lowering drugs. Overall, our data suggest the MVA pathway as novel potential target to reduce tumor growth and improve the efficacy of standard therapeutic regimens in RMS.

P-26-031
Investigating the role of PTX3 in the biology of glioblastoma

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Glioblastoma Multiforme (GBM) is the most common and aggressive brain tumor in adults, classified as grade IV tumor by WHO. Common therapies include surgical removal, chemotherapy and radiotherapy; however, relapses are inevitable. In addition, it is hypothesized that the relapses are mainly due to a subpopulation of stem cells with self-renewal properties, called glioblastoma stem cells (GSC) localized in specialized niches. These cells are resistant to conventional treatments thanks to their ability to escape apoptosis and activate DNA repair mechanisms. Pentraxin 3 (PTX3) is a soluble pattern recognition receptor belonging to the humoral arm of the innate immunity that is also involved in several aspects of tumor growth, angiogenesis, metastasis and cancer immune-regulation. To date, a correlation between PTX3 and tumor aggressiveness in GBM has been described, but studies regarding its possible implication in GSC stemness are still missing. We used human GSC BT302 cells, derived from glioblastoma specimens diagnosed according to WHO criteria, to obtain a PTX3 silenced cells. PTX3 presence and production was assessed by western blot, qPCR, ELISA and immunostainings. Proliferation, invasion and angiogenic assays were performed to analyse the effects of *PTX3* silencing. Preliminary observations revealed a wide expression of PTX3 in GSC, and specific silencing in a GSC line revealed a significant reduction of cell growth, invasiveness and angiogenic capacity in GSC after *PTX3* knock-down that results in a decreased tumorigenic capacity *in vivo*. Our data suggest that PTX3 is expressed and may play a relevant role in GSC cells. In this context, *PTX3* silencing may impair tumor features *in vitro* and *in vivo*. This set the basis for further characterization of the pro-tumoral and pro-stemness role of PTX3 in glioblastoma.

P-26-032
Long pentraxin-3 as a tumor promoter in SHH-medulloblastoma

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Medulloblastoma (MB) is an aggressive neuroectodermal tumor of the cerebellum and represents the most common brain tumor of the childhood. Despite efforts to understand MB biology and identify treatment targets, a lot remains to be investigated. Long Pentraxin 3 (PTX3), an innate immunity component implicated in tumorigenesis, has unclear roles in cancer, acting as either an oncosuppressor or a pro-tumoral factor. Currently, no data exists on the role of PTX3 in MB. PTX3 expression was analysed in MB cell lines and subgroups, by western blot (WB), immunohistochemistry (IHC) and R2 database analysis. Then, PTX3 was knocked-down, using short hairpin RNA (shRNA) and knocked-out (KO), using CRISP/Cas9 technology, in the prototypic DAOY cell line (representing the SHH subgroup). Silenced cells and KO clones were used to elucidate the role of PTX3 in SHH-MB performing both *in vitro* and *in vivo* assays. Our results and R2 database show that PTX3 is expressed at low levels across the MB-subgroups, while is overexpressed in the SHH subgroup. This was further confirmed by additional analyses on patient-derived samples using IHC and RNAscope techniques. *In vitro* assays revealed that both the silencing and the KO of PTX3 in DAOY cells significantly impaired key tumor features, including proliferation, migration and clonogenic potential. Also, metabolomic analysis revealed decreased glycolysis and TCA cycle activity in both PTX3 shRNA and KO cells. *In vivo*, PTX3-KO clones exhibited a reduced tumor growth when implanted subcutaneously in immune-deficient mice, and IHC showed lower proliferation (Ki67+ cell percentage) and diminished vascularization (CD31+ cells) in PTX3-depleted tumors. Finally, a chicken embryo chorioallantoic membrane (CAM) assay further supported these findings, demonstrating a reduction in the angiogenic response in the absence of PTX3. These data provide further substantiation for the hypothesis of a pro-oncogenic effect of PTX3 in SHH-MB.

P-26-033
Lactate promotes metabolic reprogramming through histone H3 lysine 18 lactylation (H3K18la) in prostate cancer

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Historically, lactate was regarded as a metabolic junk product of anaerobic glycolysis. Recently, it has been re-evaluated for its pleiotropic role in metabolic rewiring and post translational modifications (PTMs). It has been demonstrated that lactate is able to drive two PTMs, as lysine acetylation and lactylation, directly modulating gene transcription. p300, a histone acetyltransferase, has been indicated as a common writer for acetylation and lactylation. Lactylated histone-3 lysine 18 (H3K18lac) has been found in different tumors and it has been associated with aggressive traits, culminating with poor prognosis in patients. In keeping with this, mass spectrometry analyses, showed that in prostate cancer (PCa) cells treated with lactate, there is an increase in histone lactylation. Lys9, Lys14, Lys18 and Lys122 have been found increased upon lactate administration, with the strongest effect in H3K18Lac. We also validated H3K18lac through immunoblot analysis. The use of a selective inhibitor of p300 confirms that lactylation and acetylation are competitive for p300. Furthermore, we evaluated H3K18lac levels by targeting the two main lactate dehydrogenase (LDH) genes (LDHA and LDHB). Our findings show that both LDHA and LDHB play a role in the regulation of histone lactylation. For this reason, we are also targeting other pathways that have been suggested to be involved in lactylation, as the methylglyoxal/GSH pathway. In parallel, to identify candidate targets regulated by H3K18lac, ChIP-sequencing analyses are ongoing. Moreover, we also detected H3K18lac in pancreatic ductal adenocarcinoma (PDAC), a model showing high-rate glycolysis and intracellular lactate accumulation. In conclusion, this study aims to define the molecular pathways involved in histone lactylation, as well as their role in cancer aggressiveness. *The authors marked with an asterisk equally contributed to the work.

P-26-034
The naturally occurring estrogen receptor-activating mutation ESR1Y537S dictates ferroptosis sensitivity in breast cancer cells with acquired resistance to estrogen deprivation

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Most breast cancers are estrogen receptor-positive (ER+) and are treated with endocrine therapy, being aromatase inhibitor (AI) the standard of care for post-menopausal patients. However, resistance limits its efficacy. Despite the reprogramming of the resistant cells being largely guided by non-genetic adaptations, mutations of ESR1, the gene encoding ER, can confer endocrine resistance. Among the non-genetic drivers, we identified fatty acids (FA) metabolic reprogramming and storage to be involved in the response and adaptation to long-term estrogen deprivation (LTED), a condition that mimics AI resistance. Here we characterized FA metabolism in a LTED cell model harboring a naturally occurring ER-activating mutation (ESR1Y537S). We found that FA synthesis and uptake, together with the subsequent accumulation into lipid droplets (LD) are features of LTED, independently of ER status. However, LTED-ESR1Y537S cells succumb when challenged with nutritional stress, whereas LTED-ESR1WT cells show enhanced metabolic plasticity and resilience, a characteristic supported by LD mobilization. Since LD can sequester polyunsaturated fatty acids (PUFA) to protect cells from ferroptosis, a form of cell death caused by iron-dependent peroxidation of PUFA, we hypothesized that the inability of the LTED-ESR1Y537S cells to adapt their lipid metabolism could dictate ferroptosis sensitivity. Indeed, we observed that LTED-ESR1Y537S show enhanced expression of acyl-CoA synthetase long-chain family member 4 (ACSL4), an essential component for ferroptosis execution, and are sensitive to the ferroptosis inducers (e.g., RSL3 and erastin) whose administration causes increased reactive oxygen species and lipid peroxidation. Crucially, RSL3 resensitized cells to the endocrine selective ER degrader fulvestrant, indicating that the increased sensitivity of LTED-ESR1Y537S cells to ferroptosis can be exploited to overcome endocrine resistance in ESR1 mutated breast cancer.

P-26-035
Mitochondrial HIF-1α: shedding light on its role in BRCA1-mediated breast cancer tumorigenesis

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Tumor diversity accounts for high metabolic plasticity establishing, in different cell types, cooperative and/or competitive relationships. In this scenario delineating the relative contribution of peculiar metabolic pathways to tumor differentiation and progression might be crucial for the definition of novel therapeutic targets. Germline mutations of BRCA1 gene account for 5–10% of triple negative breast cancers (BC) and may confer up to an 80% lifetime risk of BC in carrier females. We herein based our study on the hypothesis that BRCA1 haploinsufficiency might drive metabolic rewiring in breast epithelial cells, acting as critical push toward malignant transformation. Although triple negative breast cancer (TNBC) cells shift their metabolism predominantly toward aerobic glycolysis, it is well documented that some cells, those preserving metastatic potential, keep an oxidative metabolism. To define the molecular mechanism accounting for this plasticity, we implemented a proteomic strategy to characterize the mitochondrial proteome. We compared the mitochondrial proteome of sporadic and BRCA1 mutated BC cell, using 2DDIGE coupled with LC-MS/MS and IPA analysis. Data, confirmed by fluorescence microscopy, shows that HIF-1α might localizes within mitochondria in response to BRCA1mutated status. Furthermore, HIF-1α mitochondrial localization was also detected in tissue biopsies of TNBC patients. To define HIF-1α mitochondrial interactome a cross-linking mass spectrometry (XLMS) strategy was implemented as

well. Our results enforced the hypothesis regarding the existence of a link between BRCA1 and HIF-1α mitochondrial activity, capable to trigger metabolic changes, which, in turn, sustain the high energetic status required by the malignant phenotype. Characterizing the role of mitochondrial HIF-1α in brca1-mutated BC cells may shed light to pathophysiology of hereditary BC and might depict novel therapeutic approaches.

P-26-036
flavin adenine dinucleotide synthesis parallels changes in its client flavoenzymes LSD1 and SDHA in pancreatic ductal adenocarcinoma cell lines

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Riboflavin (Rf, vitamin B2) is a water-soluble vitamin part of the B-group. It is the precursor of the flavin cofactors flavin mononucleotide (FMN) and flavin adenine dinucleotide (FAD), which enable the enzymatic activation of the cellular flavoproteome, thus ensuring a wide range of biological processes like mitochondrial terminal metabolism and nuclear epigenetics. In this scenario it is not surprising that the demand of Rf derivatives could be changed in relation with the development of different types of cancer, often dependent on mutations in the oncosuppressor p53. To understand if FAD production accompanies changes in the level of flavin-dependent enzymes relevant for metabolism and epigenetics, we compared FAD synthesis rate in HPDE expressing wtp53 with that performed by two pancreatic ductal adenocarcinoma cell lines, PANC1 and MiaPaca2, carrying gain of function mutations in p53. A significant increase in the expression of FAD synthase (FADS), particularly isoform 2, was found in tumour cells and even higher in their derived cancer stem cells (CSCs). This increase is presumably demanded by the increase in the levels of the flavoprotein subunit of complex II of the mitochondrial respiratory chain, SDHA, as well as the nuclear FAD-dependent lysine demethylase 1 (LSD1), crucial player of redox epigenetics [Nisco A et al. (2023) FEBS J 290(19):4679–4694]. Moreover, the dependence on FAD delivery and the physical interaction between LSD1 and FADS2, previously demonstrated, has been confirmed by the Gaussia princeps Complementation Assay. Thus, the process of FAD synthesis in p53 mutant cells seems to be a crucial process in cell epigenetics and a novel target for cancer therapy. Consistently a novel inhibitor of FADS activity, Chicago Sky Blue, can selectively reduce PANC1 derived CSCs' proliferation. To understand if the adaptation in FAD synthesis rate observed in cancer cells depends on p53 mutations, experiments are ongoing using p53-null PDAC cell lines.

P-26-037
Structure-based design of KDM4 protein inhibitors for a cancer therapy

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Epigenetic mechanisms in the process of tumorigenesis have attracted considerable attention in the context of the development of anti-cancer therapy. High expression levels of histone lysine demethylases subfamily 4 (KDM4s) are considered to promote oncogenesis in commonly occurring types of cancer like breast, lung, and colorectal cancer. The ability to reverse epigenetic modifications is promising in the therapy to transform a pathological state into a normal one. All the KDM4s are structurally conserved within the catalytic domain and its 2-oxoglutarate (2-OG) binding site, as confirmed by the numerous crystal structures of the members of this subfamily. Nevertheless, to achieve a higher binding potential and selectivity toward subfamily 4 members, we postulate to benefit from structure-based exploration of the histone binding site to support design compounds that could reach this less conserved region. Implementing the detailed structural knowledge of protein-ligand interactions would give rise to novel and selective inhibitor molecules. In the presented Crystallographic Fragment Screening Campaign, we used libraries of more than 500 fragments with various properties. It resulted in the identification of many hits. Some of the hits are found at the histone binding site, while additional fragments are presented in previously unknown binding regions on the surface of the KDM4D protein. Furthermore, we have identified a considerable range of functional groups that interact with the protein residues in the active site and its vicinity. The modes of binding of the fragments will be discussed in detail in this communication. Based on the identified interactions, new LEAD compounds will be designed. We hope that the presented strategy will lead to the development of a novel compound that will selectively inhibit KDM4 proteins. Funding information: This research was funded by Polish National Science Centre (grant No. 2021/43/D/NZ7/02879).

P-26-038
δ1-Pyrroline-5-carboxylate reductase (PYCR1), a new target for anticancer therapeutics

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δ1-Pyrroline-5-carboxylate reductase (PYCR1) is crucial in proline biosynthesis because it catalyzes the last step, which is the conversion of δ1-pyrroline-5-carboxylate (P5C) to proline using NADH as a cofactor. The rewiring of proline metabolism to enable cancer progression is well-established, whereby one of the hallmarks is the upregulated expression of PYCR1. These observations sparked research on the role of PYCR1 in cancer, which likely involves the production of both proline and NAD^+. Proline is required to remodel the extracellular matrix, whereas NAD^+ produced by PYCR1 can be utilized by the TCA cycle in hypoxia when the electron transport chain is inactive. Consistently, not only PYCR1 overexpression is correlated with advanced stages in highly aggressive types of cancer (breast cancer, gastric cancer, hepatocellular carcinoma), but also pycr1 knockdown inhibits proliferation. Therefore, PYCR1 has

emerged as a potential target for anticancer therapeutics; however, no suitable candidate has been identified yet. So far, the most potent PYCR1 inhibitor (3,5-Br2-PAMBPA) features IC50 of >500 nM. To develop more potent inhibitors, we implemented two strategies, high-throughput screening (HTS), and crystallographic fragment screening (XFS). Both represent important steps in rational drug design. For HTS, we utilized the European Chemical Biology Library (ECBL) comprising ~100 000 compounds and a focused library of kinase inhibitors (~2600 compounds). We identified 132 compounds from ECBL and 42 from kinase inhibitors library that are significantly more potent than 3,5-Br2-PAMBPA. In XFS, we tested 96 diverse small organic compounds and identified two promising hits. The combination of data from XFS and HTS will provide valuable starting points for the design of highly selective inhibitors targeting PYCR1, which can be further developed into anticancer therapeutics. This research is funded by Polish National Centre (grant number 2021/43/B/NZ7/01611).

P-26-039
Cytokine signatures and glutamate dynamics: unraveling the complexities of breast cancer brain metastases

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Breast cancer's predilection for cerebral metastasis presents a dire challenge, markedly impairing patient survival. Our study hypothesizes that 'brain-seeking' tumor cells exploit cytokine-induced disruptions in glutamate regulation to penetrate the brain's microenvironment, facilitating metastasis. It's well known that cytokines disrupt astrocyte-mediated glutamate regulation, a key process for neural homeostasis. We discovered that 'brain-seeking' tumor cells secrete a unique cytokine profile, deceiving the blood–brain barrier (BBB) defenses and impairing astrocytes' ability to perform glutamate clearance creating an environment ripe for invasion by elevating glutamate levels, which metastatic cells exploit as a propellant for their migration. Our study delves also into the alteration of the inflammatory process in brain seeking cells pinpointing the NF-κB pathway's role in altering tumor behavior. The overexpression of IKKβ and the parallel suppression of TAX1BP1 in the cells metastasizing in the brain culminate in the secretion of a specific pattern of cytokines but more interestingly in the upregulation of glutamate transporters EAAT1 and EAAT2. This enhances the utilization of glutamate and the consequent migratory capacity of tumor cells. *In vivo* experiments confirmed that targeting these glutamate transporters impedes the migration of cancer cells, curbing the establishment of brain metastases. This research provides new insights into breast cancer's tumor ecosystem and identifies novel therapeutic targets for brain metastases. It paves the way for innovative treatment strategies, focusing on prevention and targeted therapies to address the disease's most challenging complications. Such advancements promise significant improvements in outcomes for breast cancer patients at risk of brain metastases.

P-26-040
Curcumin reduces lactic acid-induced resistance to docetaxel by inhibiting glycolysis in prostate cancer cells

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Dysregulated cellular metabolism is recognized to be associated with drug resistance in cancer treatment. Here, we investigated how cellular adaptation to lactic acidosis affects intracellular energy metabolism and sensitivity to the anticancer drug docetaxel and the role of HK-II in this process. Pre-adapted cells to lactic acid, PC-3AcT and DU145AcT, exhibited increased growth behavior, increased dependence on glycolysis, and reduced sensitivity to docetaxel compared to parental PC-3 and Du145 cells. Molecular analyses revealed that PC-3AcT and DU145AcT cells showed activation of the c-Raf/MEK/ERK pathway, upregulation of cyclin D1, cyclin B1, and p-cdc2 (Thr161), and increased levels and activities of key regulatory enzymes in glycolysis, including HK-II. Increased glycolytic activity has been shown to reduce sensitivity to docetaxel. HK-II knockdown significantly reduced both cell growth and glycolytic activity, with a decrease of both phosphofructokinase and PDH levels. Furthermore, decreased levels of complexes I–V in the mitochondrial electron transport chain, loss of mitochondrial membrane potential with increased intracellular ROS levels, and cellular ATP depletion were found, ultimately leading to the induction of both apoptosis and necroptosis. In a xenograft animal models, curcumin, in combination with docetaxel, inhibited the tumor size and weights, downregulated the expression of the key regulatory enzymes in glycolysis, and upregulated the expression of apoptotic and necroptotic proteins. This is consistent with *in vitro* results from 3D spheroid cultures. Overall, our findings suggest that metabolic plasticity through enhanced glycolysis observed in lactate-preadapted prostate cancer cells may be one of underlying causes of docetaxel resistance, and that impairment of metabolic reprogramming by curcumin may ultimately improve treatment outcome for the patients with prostate carcinoma.

P-26-041
Alterations of rat brain nuclear phospholipids content under the combined action of cisplatin and progesterone

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Cisplatin (cis-diaminedichloroplatinum [II]) is an antineoplastic drug widely used in chemotherapy practice for the treatment of various human cancers. However, its use has been limited due to various side effects, particularly its toxicity. Research has demonstrated that administration of steroid hormone (estradiol/progesterone) in combination with cisplatin attenuates the undesirable side effects induced by cisplatin. We investigated the effects of combining the antitumor agent cisplatin with the steroid hormone progesterone on the content of total phospholipids and their individual fractions within the nuclei of rat brain cells. Our

previous research demonstrated that the characteristic properties of cisplatin and progesterone are distinctly manifested when administered separately [Previously published in: Hakobyan, NR (2023) Prostaglandins and Other Lipid Mediators.168, 106750]. Cisplatin leads to a reduction, while progesterone, conversely, causes an increase in the content of total phospholipids. However, in the case of combined administration of cisplatin and progesterone, an antagonistic interaction between these drugs becomes evident. As a result, the total quantity of nuclear phospholipids in rat brain cells is reduced decreases by 13% compared to the control. Additionally, individual phospholipid fractions exhibit different sensitivity to this combined treatment. The obtained results are analyzed and discussed in the context of antagonistic effects of studied drugs, suggesting that combined administration of the cisplatin and steroid can help to mitigate the undesirable side effects of cisplatin.

P-26-042
Deciphering the role of ceramide in EBV-positive gastric cancer

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Epstein-Barr virus (EBV) remains dormant in the host cell but occasionally switches to the lytic cycle when stimulated. However, the exact molecular mechanism of this lytic induction is not well understood. In this study, the role of ceramide was investigated in modulating EBV lytic cycle activation in EBV-positive gastric cancer cells. We found reduction of various ceramide species in EBV-infected gastric cancer cell lines compared to non-infected ones and explores the effects of exogenously added C6-ceramide (C6-Cer) on the EBV lytic cycle. C6-Cer treatment induced EBV lytic gene expression and viral production, suggesting a potential therapeutic approach to treat EBV-associated cancers. Additionally, our results suggest the molecular mechanisms underlying these effects, including the involvement of ceramidase inhibition, glycosyl ceramide synthase inhibition, ERK1/2, CREB phosphorylation, and autophagy. Our findings contribute to understanding the complex interactions between sphingolipid metabolism and viral replication, offering insights into novel therapeutic strategies for EBV-positive gastric cancer.

P-26-043
MIM1 augments proapoptotic activity of moxifloxacin toward MDA-MB-231 triple-negative breast cancer cells

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The adverse effects related to currently applied polytherapy, the unsatisfactory effectiveness of the treatment as well as epidemiological data indicate there is a need to search and develop a new method of cancer treatment. Overexpression of Bcl-2 family proteins is a common event in cancer. Earlier conducted studies indicated that Mcl-1 protein was a crucial player in breast cancer. Thus, the significant role of Mcl-1 makes the possibility of using its inhibitors. Among the identified BH3 mimetics there is one low molecular Mcl-1 inhibitor – MIM-1 (Mcl-1 Inhibitor Molecule 1). MIM-1 may selectively inhibit Mcl-1 protein and finally induce Mcl-1-dependent cancer cells death. Previously we have demonstrated that moxifloxacin – the fluoroquinolone antibiotic may induce high cytotoxic and proapoptotic effect on MDA-MB-231 breast cancer cells via Mcl-1 protein interaction as a molecular target. Therefore, in the current study we assessed the possible both synergistic activity and anticancer effect triggered by BH3 mimetic MIM-1 and moxifloxacin in a multi-component system. The obtained data from both WST-1 and image cytometry analysis show that MIM1 potentiates moxifloxacin impact on MDA-MB-231 cells viability and mitochondrial depolarization suggesting the possible synergistic effect. Summarizing, the obtained results (i) indicate that MIM1 augments proapoptotic activity of moxifloxacin as a result of Mcl-1 protein interaction and (ii) consist the basis for further in vitro as well as *in vivo* panel of experiments to confirm the anti-breast cancer activity of MIM1 and moxifloxacin especially when used in multi-component system. This research was funded by Medical University of Silesia in Katowice (grant number: BNW-1-012/K/3/F).

P-26-044
Anti-melanoma and anti-breast cancer potential of novel betulonic acid derivatives

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The search for effective cancer treatments is still ongoing. One approach is to optimize natural compounds to obtain derivatives with high cytotoxicity selectively against cancer cells. Betulonic acid (BA) is a lupane-type triterpenoid that exists in many plants. So far, the anticancer potential of BA and its derivatives has been demonstrated. The main goal of the study was to perform screening analysis of breast cancer and melanoma cell lines for sensitivity to two new derivatives of BA with acetylenic moiety. In the next step, the assay using zebrafish embryos and larvae was conducted to assess the toxicity of the most promising derivative. The study has shown that one of our derivative – N-[3-oxolup-20(29)-en-28-oyl]methylpropargilamine – exhibits a strong cytotoxic effect on MCF-7 breast cancer cells and melanoma cell lines: A-375 and COLO 829, while it has no impact on viability of normal human fibroblasts (NHF). Furthermore, analysis on *Danio rerio* have confirmed no toxicity of the compound. The results from the WST-1 test obtained for BA has indicated its significant cytotoxicity on the analyzed cancer cell lines, but also on NHF. Summarizing, the obtained results indicate a promising direction of chemical modification of betulonic acid in order to get non-toxic compounds with anti-melanoma and anti-breast cancer activity. Funding: this research was funded by Medical University of Silesia in Katowice (grant number: BNW-1-012/K/3/F).

P-26-045
The splicing factor SF3B4 is implicated in cellular senescence by regulating the stability of p21 mRNA

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SF3B4, a splicing factor implicated in various diseases including cancer, has been found to regulate mRNA expression and function. Here, we identified a novel role of SF3B4 in the regulation of p21 mRNA stability through nonsense-mediated mRNA decay (NMD) pathway in cancers. Depletion of SF3B4 resulted in reduced cancer cell proliferation, increased SA-β-Gal positivity, and elevated p21 expression in a p53-independent manner, ultimately leading to cellular senescence. We discovered that elevated SF3B4 levels in cancer cells caused to direct binding to the 3′ UTR of p21 mRNA, exerting a negative regulatory effect on its stability. Furthermore, we demonstrated that SF3B4 facilitates the degradation of p21 mRNA by the recruiting key NMD factors, such as UPF1, MAGOH and RNPS1. Consequently, the absence of SF3B4 leads to the dissociation of NMD factors from 3′ UTR of p21 mRNA and increased p21 mRNA stability. Altogether, this study provides a novel regulatory mechanism involving SF3B4 in p21 mRNA stability, in concert with NMD factors, and its final outcome in inducing cancer cell senescence.

P-26-046
Environmental lactate drives prostate cancer aggressiveness by modulating histone methylation

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Lactate is one of the major nutrients secreted by glycolytic cancer-associated fibroblasts in the prostate tumor microenvironment. It plays an essential role in tumor cells' metabolic reprogramming, culminating in the enhancement of their pro-aggressive features. Lactate is exploited by prostate cancer (PCa) cells to improve mitochondrial metabolism by fuelling the tricarboxylic acid cycle. This leads to increased levels of oncometabolites like succinate and fumarate, which not only affect various metabolic processes but also regulate gene expression through histone methylation, contributing to prostate cancer progression. We identified an activating methylation mark H3K4me3 promoted by lactate in PCa cell lines, and we also observed metabolic changes (succinate and fumarate increased levels) driven by lactate that could be important for inducing H3K4me3 activation of genes related to PCa aggressiveness. In lactate-exposed PCa cells, we identified an increase in the activating methylation mark H3K4me3. IHC analysis on tumor obtained by SCID mice s.c. injected with DU145 cells and treated with lactate show higher H3k4me3 levels with respect to untreated mice, and this correlates with a higher metastatic potential. We also appreciated the upregulation of H3K4me3 in aggressive PCa cores from TMA with respect to low Gleason score samples. Interestingly, in lactate-treated PCa cells, we also observe a positive modulation of a member of the WD40 repeat domain-containing protein family, WDR54. WDR54 acts as an oncogene in CRC and bladder cancer. However, its role in histone methylation remains unknown. By a siRNA-based genetic impairment of WDR54, we observed that WDR54 could be involved in H3k4me3 regulation, as well as in the proliferation and self-renewal of lactate-treated PCa cells. Further analysis to define the metabolic profile and the levels of histone methylation-related genes promoted by lactate in PCa are ongoing.

P-26-047
Stromal lactate sustains a prostate cancer cell-derived collagen signature through the activation of P4HA1-DDR1 axis

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Extracellular matrix is a major component of the tumor environment, promoting the establishment of a pro-invasive behavior. Such environment is rich in both tumor- and stromal-derived metabolites, particularly lactate. In prostate cancer (PCa), cancer-associated fibroblasts (CAF) are major contributors of secreted lactate, able to impact on metabolic and transcriptional regulation in cancer cells. Gene Set Enrichment Analysis in PCa cell lines exposed to exogenous lactate for 48 h revealed that genes involved in collagen synthesis and remodeling pathways were significantly enriched, underlining the importance of collagen modifications in tumor-experiencing lactate. To corroborate the importance of CAF-derived lactate, we observed that the collagen prolyl hydroxylase P4HA1 is overexpressed in CAF-conditioned media (CM)-exposed PCa cells in a MCT1 (lactate inward transporter)-dependent manner. Also, we assessed a higher proline hydroxylation – as readout of P4HA1 activity – as well as higher levels of Collagen type I in CAF-CM-treated PCa cells as well as by administrating them exogenous lactate, highlighting the role of this metabolite in sustaining cancer cell-derived collagen type I synthesis. Notably, we found that P4HA1 targeting is detrimental for lactate-induced invasiveness and transendothelial migration in vitro and in vivo as well as for collagen I deposition. Interestingly, we identified a non-integrinic collagen I receptor – discoidin domain collagen receptor 1 (DDR1) – as responsible for a tumour collagen-dependent signalling. Indeed, inactivation of DDR1 and its cognate STAT3 signaling impaired lactate-induced collagen I expression, stemness and invasiveness in lactate-treated cells. Finally, *in vivo* data revealed that targeting P4HA1-DDR1 axis decreases the number of the lung metastatic lesions in PCa-xenografted mice. These findings uncover a new metabolic aspect of PCa cell dissemination acting via the activation of P4HA1-collagen-DDR1 axis.

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P-26-048
Mitophagy monitoring in leukemia cells

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Mitophagy is the key process of mitochondria quality control. Under physiological conditions, damaged or superfluous mitochondria are dynamically removed from their network and degraded in lysosomes. Like many other processes, mitophagy can be hijacked by cancer cells to prevent apoptosis induction. In acute myeloid leukemia (AML), mitophagy was shown to promote resistance to BH3 mimetics. To investigate possible changes in the rate of mitophagy induced by drug treatment in AML cells, we tested several methods based on flow cytometry. We then used the optimized protocol to study the effect of autophagy/mitophagy inhibitors in a panel of AML cell lines as well as in primary AML cells. In parallel, changes in cell metabolism were monitored using a Seahorse device. The basal mitophagy rate was particularly high in KG-1 cells, possibly due to defective p53 signaling. Inhibition of the mitophagy adaptor p62/SQSTM1 using XRK3F2 induced a rapid decrease in the cell respiration rate followed by cell death in all cell lines tested. Cells surviving XRK3F2 treatment were characterized by slower mitochondria turnover. Autophagy inhibition using chloroquine had milder impact on cell respiration and virtually no effect on the rate of mitochondria removal. Pilot experiments confirmed that the methods used in this study are also suitable for primary AML cells. The work was supported by the Czech Grant Agency (22-03875S) and the Ministry of Health of the Czech Republic (project for conceptual development of the research organization No 00023736). *The authors marked with an asterisk equally contributed to the work.

P-26-049
The cancer cell-starving enzyme methionine gamma-lyase: insights into cellular, epigenetic and delivery issues for prospective therapeutic applications

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Among the unique metabolic signatures exhibited by cancer cells, dependence on the essential amino acid methionine for proliferation and survival has triggered extensive investigations on methionine γ-lyase (MGL) as a therapeutic tool capable of inhibiting cell growth through methionine starvation. The comprehension of the still elusive mechanistic details responsible for this cancer-specific metabolic vulnerability is of great relevance for promoting significant advances towards the application of MGL in cancer therapy. Through the use of high-resolution mass spectrometry proteomics, flow cytometry and transcript analysis, our studies on human colorectal adenocarcinoma HT29 cancer cells yield progresses in deciphering the cell death pathways (i.e. apoptotic and non-apoptotic processes) and the alterations of the epigenetic landscape associated with MGL-mediated methionine depletion. This body of information might help to establish new drug therapy regimes with compounds targeting epigenetic enzymes. In addition, we are developing a novel delivery platform for MGL based on gold nanoparticles (AuNP) to overcome the numerous challenges related to enzyme-based therapeutics and boost clinical applicability. A combination of multiple biochemical and biophysical techniques allows for an extensive characterization of MGL-AuNP conjugates in terms of particle size and size distribution, protein loading yield, impact of functionalization on protein structure and enzymatic activity.

P-26-050
Inhibition of acetyl-CoA carboxylase 2 suppresses FASN/SREBP-1 expression and cell proliferation in oral squamous cell carcinoma cells

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Oral squamous cell carcinoma (OSCC) is a highly malignant cancer in which the number of patients has been increasing in recent years. Therefore, early detection of the disease and identification of novel therapeutic targets are an urgent priority. In various cancer cells, an altered fatty acid metabolism has been reported. In human, two isoforms of acetyl-CoA carboxylase (ACC), ACC1 and ACC2 are expressed and produces Malonyl-CoA. ACC1 encodes a cytoplasmic isoform and predominantly control fatty acid synthesis, whereas ACC2 localizes at the surface of mitochondria and inhibit fatty acid oxidation. Here we examined the role of ACC1/2 in Ca9-22, a human gingival carcinoma-derived cell line using pharmacological agent and short interfering RNA (siRNA). We found that an ACC inhibitor PF-05175157 suppresses cell proliferation and causes apoptotic cell death. The knock-down (KD) of ACC1 and ACC2 using siRNA inhibited cell proliferation respectively but did not induce cell death. Interestingly, we found that only ACC2-KD Ca9-22 cells reduced FASN expression. Since SREBP-1 has previously been shown as a transcriptional regulator of FASN, we examined the expression of SREBP-1 in ACC2-KD Ca9-22 cells. We found that SREBP-1 is significantly reduced in parallel with FASN. We are currently examining if SREBP-1 regulates FASN expression in Ca9-22 cells. Finally, we searched for the potential mechanism of cell death caused by PF-05175157, but not by ACC1-KD or ACC2-KD. We found that PF-05175157 increases the phosphorylation of AMPK at Threonine 172, an upstream target of ACC, that suppresses ACC upon its phosphorylation. No change in AMPK phosphorylation was observed in ACC1-KD nor ACC2-KD Ca9-22 cells. Our results show previously unknown role of ACC2, that regulates FASN/SREBP-1 expression.

P-26-051
Exploring the role of TJP-1 in cancer progression: insights from transgenic mouse models

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TJP-1, also known as ZO-1, plays a critical role in forming the barrier between cells, regulating the movement of electrolytes and large molecules, and facilitating cellular communication by clustering membrane proteins. Its importance as a tumor suppressor is underscored by recent findings linking its deficiency or mutation to increased cell proliferation and invasiveness in breast, colon, and gastrointestinal cancers. However, the precise *in vivo* role of TJP-1 has been unclear. To address this, recent studies utilized TJP-1 transgenic mice to investigate its impact on cancer characteristics within living organisms. Genetic analysis confirmed TJP-1 overexpression in these mice, and immunohistochemistry staining validated this overexpression at the tissue level. These mice were then used to induce cancer development by injecting B16F10 tumor cells. Surprisingly, tumors in TJP-1 transgenic mice were smaller compared to the control group, indicating a potential role for TJP-1 in suppressing tumor growth. Additionally, given TJP-1's reported association with angiogenesis, tumor tissues were stained and erythrocyte counts were conducted. Results showed a decrease in erythrocyte count in tissues overexpressing TJP-1, suggesting a reduction in angiogenesis. Conversely, comparison between control groups and TJP-1 knockout mice revealed that TJP-1 deficiency increased tumor size and promoted angiogenesis, further supporting TJP-1's role in tumor suppression. These findings highlight the significance of TJP-1 expression in tumor progression and provide valuable insights into its potential as a therapeutic target for cancer treatment.

P-26-052
Investigation of the effect of ZnO nanoparticles in relation to STAT5 on autophagy mechanism in renal cell carcinoma cell lines

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STAT5 is a transcription factor that activates tumor growth and development in many types of cancer, including renal cell carcinoma (RCC), by inducing cell proliferation, migration, and invasion. High STAT5 expression causes traditional therapies for RCC, the most aggressive type of kidney cancer, to be insufficient and shows that new therapy models need to be developed. In this context, in our study, the effectiveness of the FDA-approved zinc oxide nanoparticle (ZnO NP), which has anti-cancer properties with low toxicity and high biocompatibility, was investigated based on STAT5 expression in A-498 RCC and HEK-293 cells. The effect of ZnO NP on the autophagy mechanism in normal and STAT5 overexpressed (STAT5+) A-498 RCC cells was a research topic of this study. After STAT5 overexpression was achieved by STAT5-containing plasmid transfection into A-498 cells, it was observed that ZnO NP reduced cell viability dose-dependently in these cell lines. In addition, selected concentrations of 15 and 25 μg/ml ZnO NPs suppressed cell proliferation in a time-dependent manner. It was observed that ZnO NPs caused an increase in vacuoles within the cell. Accordingly, the effect of ZnO NPs on the autophagy mechanism was investigated in both normal and STAT5+ A-498 cells following GFP-LC3 plasmid transfection. It was observed that ZnO NP treatment triggered the autophagy mechanism in normal A-498 cells, but this effect was decreased in STAT5+ A-498 cells. In addition, although ZnO NP treatment caused an increase in Atg5, Atg7, and LC3 levels in normal A-498 cells, the protein expressions were lower in STAT5+ A-498 cells. As a result, overexpression of STAT5 reduced the autophagy mechanism in ZnO NP-treated A-498 RCC cells. This project was funded by the Scientific Research Projects Unit of Istanbul Kultur University (BAP2304).

P-26-053
Compromised metabolites investigation in serum of primary myelofibrosis patients

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Primary myelofibrosis (PMF) represents a rare neoplastic proliferation of the myeloid progenitors, related to bone marrow fibrosis, compromising erythropoiesis and angiogenesis. Molecular hallmark of the PMF converges in the uncontrolled activity of the Janus kinase-signal transducer and activator of the transcription signalling pathway, which is in turn associated to an increase of oxidative and nitrosative stress. To date, little is known concerning the circulating levels of antioxidants, oxidative/nitrosative stress biomarkers, purines and pyrimidines, in patients with primary myelofibrosis (PMF). In the present study, according to methods previously set up in our laboratory, serum samples of a group of 22 PMF patients and of 30 control healthy donors were processed to obtain a protein-free extract suitable for the high-performance liquid chromatography analysis of such metabolites. Results showed that PMF patients lost drastically antioxidant defenses (ascorbic acid and reduced glutathione were 37.3- and 3.81-times lower, respectively, than those found in healthy controls), increased malondialdehyde and nitrite + nitrate levels (4.73- and 1.66-times higher than those found in healthy controls). Furthermore, they showed remarkable alterations of circulating hypoxanthine, xanthine, uric acid, uracil, β-pseudouridine, uridine and creatinine, suggesting potential mitochondrial dysfunctions. Overall, these results, besides evidencing previously unknown serum metabolic alterations in PMF patients, suggest that the determination of serum levels of the aforementioned compounds may be useful to evaluate PMF patients on hospital

admission, for adjunctive therapies aimed to recover their correct antioxidant status, as well as to monitor patients' status and potential pharmacological treatments.

P-26-054
Metabolic fingerprinting of breast cancer cell lines: discovering similarities and differences

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Metabolic rewiring is one of the hallmarks of cancer. The metabolic reprogramming process is complex and influenced by multiple factors. It depends on the type and specific features of tumours. This study aimed to create a database of the metabolomes of selected breast cancer (BrCa) cell lines derived from tumour subtypes with different aggressiveness and to identify shared and unique features to understand the metabolic reprogramming of BrCa better. We selected five cell lines commonly used for *in vitro* studies, including MCF-7 and T-47D (luminal A), SK-Br-3 ($HER2^+$), and MDA-MB-436 and MDA-MB-231 (triple negative), and two patient-derived xenografts Hbcx9 and Hcbx39 (triple negative). We used ^{1}H-NMR spectroscopy to obtain the intracellular polar metabolic profiles of these BrCa cells cultured in optimal growth conditions. The seven cell lines exhibit distinct metabolomes, reflecting the heterogeneity of BrCa tumours. Using enriched metabolites, we identified cell-specific activated metabolic pathways. Remarkably, for the four triple negative cells, different pathways are preferentially activated: inositol phosphate metabolism in Hbcx9, purine metabolism in Hbcx39, the Krebs cycle and pyrimidine metabolism in MDA-MB-231, and nicotinate and niacinamide metabolism in MDA-MB-436. Since all patients with triple-negative tumours usually receive standardised chemotherapy, this finding may provide a prospective insight towards developing more personalised therapies. In addition, despite the metabolic differences, some metabolites can be associated with the current tumour subtype classification and aggressiveness. Finally, we compared known metabolic adaptive features of cancer within the cell lines. This study provides a database of the intracellular metabolite profiles of selected BrCa cell lines that can be useful for future research studies. It also uncovered specific features that may be helpful in the discovery of new therapeutic targets.

P-26-055
The interplay between the transcriptional factor p63 and lipase MGLL affects bladder cancer progression

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Bladder cancer (BLCA) is a common cancer with a poor prognosis as its diagnosis is often late, therefore there is an urgent need to discover new targets for the diagnosis and therapy of this tumour. Several studies have reported that the transcriptional factor p63 is considered an oncogene in a number of epithelial cancers, however in BLCA p63 expression tends to drastically decrease in advanced tumours, in particular in muscle-invasive cancer. This interesting behaviour of p63 led us to suppose that this transcriptional factor is associated with the alteration of the capacity for migration and invasion of BLCA cells. To understand what happens from a molecular point of view, we aimed to identify novel direct targets of p63 in BLCA cell lines by RNAseq and ChIPseq. We found an enrichment of lipid metabolism among the genes repressed by p63 in our tumour cell lines. Alterations in lipid metabolism in cancer are becoming increasingly recognized, yet in BLCA these pathways have been little studied until now. Among the p63 repressed genes, we identified MGLL, which encodes for the enzyme of the lipolysis that converts triglycerides into free fatty acids. We validated our results in BLCA TCGA patients and we confirmed the negative correlation between TP63 and MGLL. Moreover, we found that high MGLL levels are related to a worse prognosis, contrary to TP63, whose expression is associated with a better survival. To evaluate the phenotypic effect of this interplay between p63 and MGLL, we performed silencing of p63 and MGLL in the BLCA cell lines panel. Our data suggests that MGLL confers BLCA an aggressive phenotype. Overall, these preliminary results suggest that p63 could play a role in migrative and invasive capacity of BLCA by repressing lipid metabolism pathway that is essential for cell metabolism, cell proliferation and cell motility.

P-26-056
Metabolic alterations in human glioblastoma cells during infection with oncolytic poliovirus

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Poliovirus can act as oncolytic agent against human glioblastoma multiforme (GBM), one of the most aggressive types of cancer. One strategy to improve effectiveness of virotherapy is to search for agents that enhance oncolysis. Our goal was to study the effect of poliovirus on cellular metabolic pathways as potential targets for pharmacotherapy. Human glioblastoma cell lines were infected with poliovirus strain PV2 (Sabin), and its replication was assessed by immunostaining and RT-qPCR. Rates of glycolysis and mitochondria respiratory activity were measured using Seahorse technology. Levels of cellular metabolites were quantified using gas chromatography coupled to mass spectrometry and by high-performance liquid chromatography. We found that poliovirus causes a moderate increase in levels of biogenic polyamines and significantly enhances biosynthesis of triglycerides, fatty acids, and purine nucleotides in GBM cells. Notably, biogenic polyamines are dispensable for poliovirus replication. In contrast, the levels of pyruvate and specifically rates of its transport into mitochondria modulated propagation of poliovirus. The virus-induced changes in other metabolic pathways of GBM cells were found to be not required for enhancement of its replication or cytopathic effect. This is contrary to the data for Coxsackievirus, another enterovirus whose oncolytic effect is augmented by 2-deoxyglucose. Nevertheless, pharmacologic inhibitors of metabolic enzymes could be used to improve the efficacy of oncolysis by RNA viruses. This work was supported by Russian science foundation (grant #19-74-10086).

P-26-057
Deciphering the role of miR-22 in metabolic reprogramming and tumor suppression of hepatocellular carcinoma

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Mir-22 is emerging as a pivotal player in hepatocellular carcinoma (HCC) development, where it functions as a tumor suppressor. QPCR analysis in HCC patients revealed that lower levels of miR-22 correlated with tumor malignancy and, similarly, diethylnitrosamine-induced HCC in rats showed decreased miR-22 expression. This study aims to unravel miR-22 contribution to HCC tumorigenesis, focusing on its possible role in metabolic reprogramming. HepG2 cells transfected with an anti-miR-22 showed an increased growth rate, with a minor presence of apoptosis markers, compared to controls. On the other hand, Huh-7 cells overexpressing miR-22, showed an inverse scenario, demonstrating that miR-22 acts as an oncosuppressor. QPCR and western blot analysis revealed that low levels of miR-22 likely determine the stabilization of HIF-1α. Biochemical evaluations showed that low miR-22 levels shift the metabolic framework to a more glycolytic phenotype, leading to an increase in glucose uptake rate, glycogen gather and lactate production in HepG2 silenced cells, in line with HIF-1α activation; higher miR-22 levels in Huh-7, instead, decreased glycogen storage, leaving unvaried glucose consumption, and lactate production. Moreover, lowering miR-22 levels in HepG2 affected the mitochondrial activity, as evidenced by a rise of the mitochondrial potential, lipid droplets content, and lipid peroxidation, suggesting a lower oxidative metabolism and a halt in the electron transport chain. Overexpression of miR-22 in Huh-7 ameliorated the mitochondrial functionality, as highlighted by the increase in the ATP/ADP ratio and in the spare respiratory capacity. Furthermore, the improvement in citrate synthase activity indicated a mitochondrial biogenesis. Overall, our findings underline the significant role of miR-22 in orchestrating cell metabolism reprogramming and provide a foundation for further exploration of miR-22 as a potential therapeutic target in HCC. *The authors marked with an asterisk equally contributed to the work.

P-26-058
Identification of small molecule inhibitors of UNC-51-like kinase 1 for cancer treatment

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Autophagy, vital for cellular homeostasis, degrades and recycles damaged cellular components, including misfolded proteins and organelles. Dysregulation of autophagy is linked to health disorders, and has emerged as a potential therapeutic target for cancer. In human cells, ULK1 complex initiates autophagy in response to nutrient deprivation or energy stress. The ULK1 complex consists of several key components including the serine/threonine protein kinase ULK1 (unc-51-like kinase 1), FIP200, ATG13, and ATG101. Activation of ULK1 involves an autophosphorylation event at Thr180, the mutation of which results in a significant reduction in its activity. ULK1 has garnered considerable attention as a potential target for modulating autophagy, particularly in the context of cancer progression. To this end, a high-throughput screening campaign was initiated. Starting from an autophosphorylation-based screening of 4027 small molecules we identified 22 active compounds with varying chemotypes, endowed with micromolar to nanomolar potencies. Subsequent structure-based drug design (SBDD) studies and *in-cellulo* validation experiments were conducted. A novel "crystallographic" mutant of the ULK1 kinase domain was engineered, enabling the determination of six co-crystal X-ray structures with selected compounds, revealing the molecular details of the binding modes, and laying the basis for future structure-activity relationship (SAR) studies. Preliminary investigations conducted on U87, a human glioblastoma cell line, using a commercial autophagy detection kit revealed that two out of three of the most potent compounds effectively inhibit autophagy. In conclusion, these findings offer new tools to further investigate the role of ULK1 as a druggable target for modulating autophagy for cancer treatment, laying the foundation for a drug discovery initiative. Partially Funded by the Regional Law No. 13, 6 Aug 2021, Art. 8, par. 28–30. *The authors marked with an asterisk equally contributed to the work.

P-26-059
Proteomic profiling of RICTOR/mTORC2 downregulation in different BRAFV600E melanoma cell lines

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Metastatic melanomas (MM) harboring a B-RafV600E mutation are treated with combination therapies relying on BRAF- and MEK-inhibitors (BRAF/MEKi). However, tumor cells become resistant to treatments, showing a metabolic reprogramming activating mitochondrial energy producing pathways. Bioinformatic analysis of melanoma patients' data in TCGA revealed that low levels of Rictor, an essential subunit of mTORC2 complex, correlate with an overall worse clinical outcome and with a gene expression signature suggestive of a metabolic reprogramming miming mechanisms of resistance to BRAF/MEKi of MM cells. To gain further knowledge on Rictor/mTORC2 impact in MM B-RafV600E, we performed proteomic analysis on two different MM cell lines comparing Rictor-proficient (ShCtrl) and deficient (ShR) cells. We chose M14 and A375 cell lines since their different metabolic adaptation following acquired therapeutic resistance. Proteomic analysis of M14 cells revealed 40 dysregulated proteins of which 36 highly abundant in ShR, including two protein species of NAMPT, which represents the rate-limiting enzyme of the NAD^+ salvage pathway and previously identified as a driver of melanoma targeted therapy resistance. Proteomic analysis of A375 cell line identified 51 dysregulated proteins of which 23 up-regulated in ShR. Enrichment analysis of the two sets of data by HumanCyc database revealed that Rictor downregulation in M14 cells impacts on the nicotinate metabolism, glycolysis and gluconeogenesis, Acetyl-CoA biosynthetic process, mitochondrial function and reorganization, while it influences pentose phosphate pathway (PPP), glycogenolysis, mevalonate pathway and TCA cycle in A375. In conclusion, after Rictor silencing, M14 cells support the increase of electron transfer chain (ETC) machinery and energy production, on the other hand, A375 cells strongly support the ETC fueling for an increased energy production.

P-26-060
Immune checkpoint inhibitors can directly affect primary and secondary hemostasis by targeting platelets

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Hematologic complications caused by cancer immunotherapy with immune checkpoint inhibitors (ICIs) are relatively infrequent but can cause serious consequences. One of these may be life-threatening clotting disorders with risk of thrombotic or hemorrhagic complications, the mechanisms of which are still poorly understood. The aim of our study was to identify potential direct effects of pembrolizumab and nivolumab (PD-1), as well as ipilimumab (CTLA-4) on platelet hemostatic functions *in vitro*. Aggregation, coagulation, fibrinolysis, thrombin generation and the structure of the fibrin network was analyzed in platelet-rich (PRP) plasma of healthy donor (n = 11) using microplate UV/Vis spectrometry and confocal microscopy. PRP samples were treated with pembrolizumab (75 μg/ml), nivolumab (58.7 μg/ml) and ipilimumab (85 μg/ml) or appropriate isotype controls. Upon direct contact with platelets in PRP, nivolumab and ipilimumab accelerated thrombin generation, promoted delayed platelet disaggregation, and enhanced the local accumulation of fibrinogen on platelet microaggregates. Overall, the effects of ICIs on PRP differed from that of the corresponding isotype controls. In contrast, pembrolizumab stimulated platelet aggregation and had no effect on the plasma coagulation cascade. When clotting was followed by lysis, all ICIs were found to prolong the growth of the PRP-derived fibrin clot and delay its elimination. This study shows that tested ICIs can directly affect platelets interfering with their aggregation ability and enhancing antifibrinolytic properties. We hypothesize that the detected effects of the tested ICIs may be due to their influence on the GPIIb-IIIa receptor, both through direct interaction and indirectly through modulator ligands. The observed ICI-specific effects may contribute to our understanding of the mechanisms by which ICIs affect platelets and suggest how, in a clinical setting, to reduce coagulation disorders during ICI-treatment in the future.

P-26-061
Metabolic reprogramming and mobilization of ABC transporters determine the lysosomal drug-retention capacity and cytoprotective functions of glioma polyploid giant cells

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Generative and cytoprotective potential of polyploid giant cells (PGCs) is related to their metabolic reprogramming and mobilization of drug management systems. Our observations of the long-term intracellular doxorubicin (DOX) retention in human glioma PGCs prompted us to address its links with the metabolic plasticity of PGCs and the activity of ABC transporters. A largely nuclear DOX localization was observed in drug-sensitive U87-MG cells. In DOX-resistant T98G PGCs, DOX retention was predominantly related to its lysosomal accumulation (trapping) and correlated with the prominent up-regulation of ABCB1, ABCG2 and lysosomal V-type proton ATPases, as revealed by the immunocytochemical and LC-MS/MS assays. The cooperation of both systems during the intracellular DOX redistribution in T98G cells was confirmed by the attenuating effects of chemical ABCB1 and ABCG2 inhibition on lysosomal DOX retention, followed by its nuclear accumulation. In turn, Seahorse XFp-assisted studies demonstrated a transient

activation of OXPHOS and intensified glycolysis in DOX-loaded PGCs. Physiologic relevance of the links between the metabolic reprogramming, ABC transporters and lysosomal DOX retention is illustrated by the viability of PGCs in brain tissue, their ability to form perivascular structures in vivo and to directly exchange metabolites with non-PGCs, as revealed by *in vivo* approach (Wistar rat) and microinjection (FemtoJet® 4i). Collectively, metabolic reprogramming and mobilization of ABC transporters facilitates the lysosomal DOX retention capacity of glioma PGCs, which protects their genomic stability and potentially reduces DOX bioavailability in tumor niches. This study was financially supported by Polish National Science Centre (2021/41/N/NZ3/02823) and in part by Ministry of Science and Higher Education (0161/DIA/2019/48) and Excellence Initiative Program at JU (U1U/W19/NO/28.17).

P-26-062
Breaking bad cells: the antioxidant approach to cancer treatment

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Cancer remains a significant global health challenge, necessitating the exploration of innovative therapeutic strategies. Traditional treatments often lack selectivity, causing damage to healthy cells alongside cancerous ones. In this study, we investigated the potential of melatonin, a hormone with known antioxidant and anti-inflammatory properties, to selectively target cancer cells while sparing healthy neighbouring cells. Our research focused on determining the effect of a specific concentration of melatonin on cancer cells, specifically its ability to induce endoplasmic reticulum (ER) stress and subsequent apoptosis. The concentration of melatonin we identified not only triggered ER stress in cancer cells, but also led to their apoptotic demise, while neighbouring healthy cells remained unaffected. By specifically targeting cancer cells through ER stress-induced apoptosis, melatonin offers a promising avenue for the development of more precise and less toxic treatments. We carried out the experiments with 2 types of cell culture, lung epithelial cancer cells (A549) and white blood cells (KCL). Two solutions of melatonin were prepared, one with a concentration of 0.1mg/ml and the other with a concentration of 0.02 mg/ml. The solutions were used as received. After 24 h, the ratio of dead cancer cells to live cancer cells was determined using the trypan blue method. In 24 h, a 35% reduction was observed in A549 cells under the influence of a dense solution, while KCL cells showed a reduction of about 38%. Our findings underscore the importance of further investigation into the mechanisms underlying melatonin's selective action on cancer cells. Through continued research and clinical trials, melatonin may emerge as a valuable addition to the arsenal of cancer treatment options, offering hope for more effective and targeted therapies in the fight against cancer. *The authors marked with an asterisk equally contributed to the work.

P-26-063
Cancer associated fibroblasts regulation and multivesicular body proteins

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Cancer associated fibroblast (CAF) activation is emerging as one of the challenging fields in cancer therapy. Regulation of its activation is expected to be a strong replacement of immune cells mediated cancer treatment, including those of CAR-T and CAR-NK. In addition, multivesicular body (MVB) formation related ESCRTs play a central role in many metabolic diseases including cancer. No evidence, however, has been accumulated for the effect of ESCRTS on CAF activation and cancer growth. In this respect, our group recently identified a few ESCRTs targets. Downregulation of their expressions by siRNA potentially reduced CAF activation in normal fibroblasts. Moreover, co-cultivation of the siRNA treated MRC5 normal fibroblasts cells with A549 human lung cancer cells significantly blocked the cancer cell growth only, without any noticeable damage to the normal cells. Our results would be the first linking CAF activation downstream of MVB ESCRTs with cancer specific growth regulation, although more detailed works are required for the innovation of this anticancer therapy strategy.

P-26-064
Enhancing immune checkpoint blockade therapy is achieved through inhibition of PAK4

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P21-activated kinase 4 (PAK-4) is known to be associated with tumor development and has been shown to be overexpressed in various cancer cells. While PAK4 is recognized as controlling the development of liver cancer, its biological function in controlling immune cells in liver cancer remains unclear. Our study reveals that PAK4 induces carcinogenesis, including cell proliferation, invasion, migration in liver cancer cells. In nude mouse, shPAK4 decreased tumor volume compared to controls, and c57BL6 mice, shPAK4 induced a significant reduction in tumor volume compared to controls. Furthermore, shPAK4 was observed to stimulate CD8+ cell activity and knockdown of PAK4 resulted in increased expression of PD-L1 in liver tumors. These findings suggest a role for PAK4 in regulating liver cancer cell via immune cell modulation. Further investigation into how PAK4 influences the PD-1/PD-L1 pathway and modulates immune cells in response to immune checkpoint blockade therapy is warranted.

P-26-065
Nup62 knockdown overcomes osimertinib resistance via ubiquitination of survivin in non-small cell lung cancer

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Osimertinib, an epidermal growth factor receptor tyrosine kinase inhibitor (EGFR-TKI), is the standard first-line treatment for non-small cell lung cancer (NSCLC) patients with EGFR-

activating alterations. However, most patients show acquired resistance to osimertinib, thereby resulting in a modest overall survival benefit. In this study, we found that nucleoporin p62 (Nup62) knockdown was closely implicated in overcoming osimertinib resistance in NSCLC and explored its underlying mechanism. Treatment with siRNA Nup62 and osimertinib displays potent cytotoxicity in osimertinib-resistant NSCLC cells. Combination of siRNA Nup62 and osimertinib was found to synergistically induce apoptosis through activation of caspases, which was blocked by a pan-caspase inhibitor (zVAD) in osimertinib-resistant NSCLC cells. Nup62 knockdown decreased the expression of survivin, inhibitor of apoptosis (IAP) family protein and survivin downregulation was induced through the ubiquitin-proteasome system. Moreover, a stable shRNA Nup62 mouse xenograft model verified the osimertinib-sensitizing effect *in vivo*. Furthermore, Nup62 expression was increased in lung cancer tissues and significantly correlated with patient survival and recurrence. Our results demonstrate that Nup62 knockdown overcomes osimertinib resistance through ubiquitination of survivin in osimertinib-resistant NSCLC cells, suggesting that Nup62 knockdown may offer an effective therapeutic strategy to overcome the therapeutic efficacy of osimertinib-resistant in NSLC.

P-26-066
The role of branched chain amino acid transaminase-1 in head and neck cancer patients

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Branched-chain amino acids (BCAAs); valine, leucine, and isoleucine are essential amino acids that play vital roles in physiological processes within the body, such as protein synthesis, signal transduction and energy production. The initial step of BCAA catabolism involves the transamination of the amino acids nitrogen to alpha-ketoglutarate by BCAA transaminases (BCAT1/2), resulting in the formation of glutamate and the keto acids of the amino acids. Thus, nitrogen is separated from the structure, allow the remaining carbon skeleton to utilized for energy production. Particularly, in tumor cells with high energy requirements, the catabolism of BCAAs provides cells with metabolic precursors for energy production and nitrogen for amino acid and nucleotide synthesis. Therefore, tumor cells utilize BCAAs as fuel by increasing the expression of BCAT1, a cytosolic transaminase. In our study, the mRNA and serum protein level of the BCAT1 enzyme were evaluated in the tumor and non-tumor tissues and serum samples of 81 patients diagnosed with head and neck cancer. In comparison of mRNA levels and demographic data, BCAT1 expression was found to be significantly increased in stage III and IV patients. ($p = 0.017$). Besides, a significant correlation was found between the increase in BCAT1 expression and smoking in patients ($p = 0.020$). However, there is no correlation between BCAT1 protein level and either mRNA level or demographic data ($p > 0.05$). Considering that head and neck cancer is often diagnosed at an advanced stage when medical interventions are less effective, these results may indicate the importance of metabolic reprogramming in BCAA metabolism in the progression of head and neck cancer. This study will be included in future directions to develop targeted therapies or dietary interventions on BCAA metabolism in head and neck cancer. This work was funded by Scientific Research Projects Coordination Unit of Istanbul University. Project No. TDK-2021-38309.

P-26-067
Correlation between virulence and cytotoxicity of multidrug-resistant bacteria in human cell models

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Susceptible species do not always overtake resistant species due to the presence of a fitness cost. The commonly held belief that the development of antibiotic resistance would eventually lead to this outcome is not always the case. Our hypothesis is that the pattern of certain bacterial pathogens rapidly developing resistance while others remain susceptible for prolonged periods reflects species-specific differences in the impact of resistance on bacterial virulence. The exact relationship between antibiotic resistance and virulence is still unclear, *in vivo* assessments have shown that several antibiotic-resistant strains have altered virulence. Importantly, the molecular mechanisms underlying resistance are specific to each bacterial species. Limited overlap exists in resistance mutations and mobile resistance genes across bacterial species. Alternative resistance mechanisms may not be phenotypically equivalent, as they may incur a fitness cost in a species-specific manner. Our research has definitively shown that certain combinations of bacterial species and antibiotics have significant negative trade-offs between resistance and virulence. The cytotoxicity of bacteria on human cells has a considerable effect on the change in virulence. Our findings suggest a correlation between antibiotic resistance and cytotoxicity in human cells, which requires further investigation into the underlying mechanisms.

P-26-068
Dynamic regulation of glucose transporter expression in colorectal cell lines: a proof of concept for tumor targeting strategies via nanoparticles drug delivery

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In this study, we examined gene expression variations of solute carrier family 2 and 5 mRNAs, responsible for encoding the GLUT2 and GLUT5 transporters, respectively, in three different immortalized cell lines originating from the colorectal tissue:

CaCo2 (adenocarcinoma), HCT116 (carcinoma), and CCD841 (healthy), in response to varying glucose concentrations. The three cell lines were cultured to confluence in their respective media, followed by exposure to three experimental conditions: BASAL (DMEM with glucose concentration equivalent to their standard medium), PLUS (DMEM with a glucose concentration of 10 g/l), and DEPRIVATION (DMEM without glucose). Subsequently, mRNA extraction was performed after 15 min of treatment, followed by reverse transcription and analysis of the mRNAs using RT-qPCR, with normalization against beta-actin expression, referred to healthy CCD841. The results show that under glucose deprivation conditions, HCT116 cells exhibited a significant upregulation in GLUT5 expression compared to the Caco2 (p-value <0.0137), whereas CaCo2 cells maintained lower expression levels. GLUT2 showed different expressions in Caco2 and HCT116: in conditions of glucose abundance, CaCo2 cells exhibited elevated mRNA expression referred to HCT116 (p-value <0.0001) while during deprivation, HCT116 showed overexpression (p-value <0.001) compared to CaCo2. This study represents a preliminary exploration of a proof-of-concept approach, where next experiments will involve the internalization mediated through the control of the amount of free glucose to increase or decrease nanoparticles functionalized with labeled glucose internalization. These nanoparticles, which have already been synthesized, were prepared from solid glucose using an innovative one-pot thermal method. We aim create a tissue-specific drug delivery through NPCs functionalized.

P-26-069
Integration of omics and functional data to understand metabolic markers of bladder cancer progression in advanced *in vitro* models

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Bladder cancer (BC) is one of the most common malignancies worldwide. The incomplete resection of the non-muscle invasive tumors (NMIBC) leads to frequent relapses and progression to more aggressive stages (MIBC). Therefore, a correct diagnosis and complete tumor resection is crucial to improve the prognosis. Energy metabolism reprogramming is an established cancer hallmark, and altered metabolic pathways can represent attractive clinical targets exploitable in new therapeutic strategies. Here we use 3D cultures (spheroids), that better simulate the architectural complexity of a tumor mass *in vivo*, to characterize the metabolic phenotype in a panel of six bladder cancer cell lines at different stages and grades. Using a systems metabolomic approach, integrating omics analyses (transcriptomics and metabolomics), and functional assays [including analysis of metabolic fluxes by Seahorse technology, Campioni et al. (2022), Cells 11(5), 866] with mathematical models of metabolism [Damiani et al. (2020) Curr Opin Biotechnol 63, 190–199, Di Filippo et al. (2022) PLoS Comput. Biol. 18(2), e1009337], we show that cancer progression could lead to a deregulation of fatty acids metabolism, increasing the β-oxidation in MIBC spheroids. Moreover, Raman spectroscopy, a non-invasive and label-free technology already used in a clinical setting for diagnosis of cancer, was used to validate the behavior predicted by the computational analysis of these cell line-derived spheroids, opening perspectives for improvement of BC diagnosis.

P-26-070
Metabolic signature of therapeutic resistance in pancreatic ductal adenocarcinoma cell lines under combinatorial treatment

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The precise mechanisms that cancer cells use to regulate their ROS levels, adapt their metabolism to new conditions and needs, and develop resistance to chemotherapy remain largely unknown. Besides the large number of molecules and macromolecules involved in redox balance in cancer cells, we decided to focus our attention on a membrane transporter, SLC7A11, belonging to the SLC7 family, known also as xCT. It is responsible for cystine uptake and glutamate release, a crucial membrane transport cycle for cell redox balance. Indeed, xCT is dramatically overexpressed in several human cancers and its inhibition represents a good target for therapy. For this reason, we studied the effect of erastin, a chemotherapeutic agent that inhibits the xCT function, on different pancreatic ductal adenocarcinoma cell lines, known to have very high metabolic plasticity, implicated to therapeutic resistance and poor prognosis. Despite a good response to the drug after a short exposure, analysis of long cell proliferation curves shows a proliferation rescue of cell growth under erastin treatment at late time points, demonstrating that single drug treatment is not enough to inhibit pancreatic cancer cell proliferation. Our systems biology approach combines metabolomics profiling to computational tools, such as differential correlation analysis, and can identify vulnerable points for targeted combined drug treatments. In this way, we identified four different potential inhibitors to be used along with erastin and specifically we selected two of these, alpelisib and methotrexate, that showed a strong reduction of cell growth capability. After combinatorial treatment, metabolomics profiling revealed an increase in serine levels and in its synthesis pathway, indicating an attempt to develop a resistance mechanism. These new findings show the potential role of systems metabolomics in cancer treatment drug repurposing and serine as a metabolic resistance biomarker. *The authors marked with an asterisk equally contributed to the work.

P-26-071
Lipid metabolism alterations in cells over-expressing GATA-1S: novel insights into leukemogenesis and drug resistance in myeloid leukemia

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A strong correlation has been shown between ferroptosis, altered lipid metabolism and drug resistance. GATA-1 is a key transcriptional factor controlling hematopoiesis. Two GATA-1 isoforms, GATA-1FL and GATA-1S, are described. A balanced GATA-1FL/GATA-1S ratio helps to control hematopoiesis, with GATA-1S overexpression being associated with enhanced cell survival and proliferation, thus representing a poor prognostic factor in myeloid leukemia (Crispino et al. 2017; Blood 2103–2110). Based on these observations, we wondered whether the leukemogenic potential of GATA-1S could involve alterations in lipid metabolism. Lipidomic analysis revealing lower PUFA content in K562 cells over-expressing GATA-1S allowed us to highlight reduced lipid peroxidation and enhanced ferroptosis resistance in these cells, thus shedding novel light on the pro-leukemic role triggered by GATA-1S (Trombetti et al. 2023; Antioxidants 12, 537). Our study also showed reduced ceramide levels in these cells as compared to the GATA-1FL counterpart. According to the evidence that specific sphingolipid and ceramide species differently contribute to regulate proliferative and apoptotic pathways, we examined the expression levels of enzymes involved in this metabolism. Interestingly, in GATA-1S cells we found increased expression levels of ceramidases (ASAH1 and ACER2) and decreased levels of ceramide synthases (CERS1 and CERS2) that are consistent with the reduced ceramide content detected in these cells. We also found increased levels of CERK1 and SPHK1 responsible for the formation of the pro-proliferative and anti-apoptotic compounds ceramide-1-phosphate and sphingosine-1-phosphate, respectively, thus supporting the pro-leukemic role exerted by GATA-1S. Collectively, these findings indicate that altered lipid metabolism is a mechanism through which GATA-1S exerts a pro-survival and anti-apoptotic role in myeloid cells and ultimately could lead to new treatment options in hematological malignancies. *The authors marked with an asterisk equally contributed to the work.

P-26-072
Systems biology analysis reveals MondoA regulation of mitochondrial function and metabolic stress response in B-ALL

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In cancer biology, cells undergoing rapid divisions confront metabolic proliferation stress, requiring stress response adaption to ensure survival and maintain homeostasis. We previously found that MondoA [MLXIP, Myc-associated factor X (MAX)-dimerization protein (MLX) interacting protein], a component of the MYC interactome, is overexpressed in paediatric B cell acute lymphoblastic leukemia (B-ALL) patients correlating with a lower survival rate, mediates the transformational capacity of B-ALL cells *in vitro* and its depletion markedly suppresses malignancy *in vivo*. Mechanistically, we revealed MondoA as a significant metabolic sensor maintaining malignancy, although further mechanisms remain to be elucidated [Previously published in: Sipol et al. (2022) Blood 139:1184–1197]. Using clustered regularly interspaced short palindromic repeats (CRISPR)/CRISPR-associated protein 9 (Cas9) and RNA-interference approaches, we now assessed the role of MondoA on methylation, MYC promotor binding, transcriptomics, and proteomics in NALM6 ALL cells. In the absence of MondoA, ALL cells show overall reduced MYC promotor binding, increased and redistributed genome methylation, and significant changes in transcriptomic and proteomic profiles, concordant and discordant. Pathway analyses revealed significant alterations of proteins involved in metabolism as well as in mitochondrial structure and function in MondoA knock-out cells, including downregulation of all mitochondrial respiratory complexes and upregulation of the mitochondrial permeability transition pore. In the absence of MondoA, enrichment of GO terms such as oxidoreductase activity, proton channel activity, and NADH dehydrogenase activity was observed, among others. We hypothesize that MondoA might play a pivotal role in driving the sustainability of B-ALL cell metabolism by orchestrating epigenetic mechanisms as well as maintaining mitochondrial function.

P-26-073
Angiogenic profile in patients with NSCLC

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Immune checkpoint inhibitors (ICIs) have proven to positively modulate OS, PFS and ORR in non-small cells lung cancer (NSCLC) patients. However, the diagnostic use of PD-L1 expression for the treatment of NSCLC patients with ICIs has not been found to be a predictive biomarker of effective response. Tumor angiogenesis impairs the distribution of drugs, including ICIs, and their efficacy. The aim of this project has been to study the expression profiles of molecular determinants of angiogenesis as potential new biomarkers of the efficacy of ICIs. The study has been done in plasma (in platelets) of NSCLC patients before and during ICIs treatment and at disease progression. To date, 103 patients with diagnosis of advanced NSCLC, candidates to treatment with ICIs as first or second line of treatment have been accrued by UOC Oncology and Radiotherapy, AOU Careggi, Florence. Blood was collected at baseline T0, at 2- (T2) and 4-months (T4) during treatment, and at the time of progression (Tp). The platelet-rich plasma (PRP) was generated by centrifugation and collected. Multiplex ELISA assay was performed for Ang 2, IL-8, VEGF, FGF-2, and PDGF analysis. A cohort of women (n = 48) and men (n = 55) with a mean age of about 65 years was enrolled in this trial. VEGF is the main angiogenic marker carried by platelets. After treatment, the contents of VEGF were significantly decreased in T4 and increase at Tp. The level of PDGF and Ang2 increase with the onset of progression. IL8 and FGF2 showed very low expression in platelet, and no uniform trend has been assessed between patients in the periods analyzed. In conclusion, the contents of VEGF, PDGF and Ang2 appear closely associated with lung cancer response to ICI. The data will be integrated with molecular (TMB and cfDNA), histological and clinical data by using multi omics data platform to validate the role of platelets as potential biomarker of angiogenesis and response to immunotherapy.

P-26-074
Targeting iron metabolism and ferroptosis as a potential therapeutic approach against chordoma

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Chordoma is a rare, slow-growing and aggressive tumor arising from remnants of the embryonic notochord. The first-line treatment is a surgical removal of the tumor, while it is resistant to conventional chemo- and radiotherapy. Due to its rarity, variety and growth rate, the cell line establishment is challenging and thus chordoma has been poorly studied so far. Taking advantage of the recently isolated human chordoma CH3 cell line, the aim of the study is to characterize the cells for iron metabolism and ferroptosis sensitivity. First, the expression of iron- and ferroptosis-related mRNAs was analyzed by RT-qPCR, while the proteins level by western blot and ELISA. The CH3 cells have shown to express, at detectable levels, all analyzed mRNAs and proteins, such as transferrin receptor 1 (TfR1), ferroportin (FPN) and ferritin, involved in iron uptake, export and storage, respectively. Next, the CH3 cells were treated with iron chelator desferoxamine (DFO) or iron as ferric ammonium citrate (FAC) for 24 h and analyzed for ferritin to assess the cell response to iron level. The data show that H-ferritin level decreased by half after DFO, while increased 10-fold after iron. To verify if the iron modulation affects the chordoma cell viability, the cells treated with DFO or FAC for 7 days were analyzed by MTT. The iron supplementation decreased the cell viability at 1 mM dose (74%), while DFO had an effect at 10–50 μM (46–24%). To assess the cell sensitivity to ferroptosis, the cells were treated with ferroptosis inducers. The CH3 cells showed a slight decrease of cell viability after 7-day treatment with erastin (77–53% at doses 1–10 μM), while a severe cell mortality, already at nanomolar doses after 24h-treatment with RSL3 or ML162 (21% at dose 500 nM). These data show that chordoma CH3 cells express iron- and ferroptosis-related mRNAs and proteins, respond to the iron modulation, and the cells are sensitive to iron deprivation and ferroptosis inducers.

P-26-075
New strategies to overcome therapeutic resistance in glioblastoma

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Glioblastoma (GBM) remains a significant medical challenge due to its resistance to conventional chemotherapy and radiotherapy, to great cellular heterogeneity, high regeneration rate, and presence of cancer stem cells. Furthermore, the blood–brain barrier represents a physiological obstruction to the delivery of conventional chemotherapy, reducing the efficacy of treatments. Cathepsins are lysosomal proteases involved in several physiological and pathological processes, and their key roles in modulating cell death and pharmacological resistance has recently been demonstrated. In particular, cathepsin B is a nodal regulatory protein in different types of cell death, such as apoptosis, pyroptosis, ferroptosis, necroptosis, and autophagy. Actually, overexpression of cathepsins is among the causes of GBM angiogenesis and tumour progression. Novel Pt(IV)-based drugs, Pt(IV)Ac-POA and DB178, whose efficacy has already been demonstrated on the human astroglioma cell line U251 and on the human glioblastoma cell line T98G, have shown improved anticancer efficacy, especially when conjugated with other compounds possessing synergistic effects. This work aims to assess the effects of these two drugs in cathepsin modulation on the U251 and T98G cell lines, and whether the inhibition of cathepsin B activity may be proven useful in brain cancer therapy. The immunocytochemical and biochemical results on the two cell lines highlight that both compounds maintained basal cathepsin B levels, while efficiently activating programmed cell death mechanisms, as investigated by optical and electronic microscopy. These data emphasize the effectiveness of the two platinum-based pro-drugs, which do not rouse cathepsin B in stimulating programmed cell death. For these reasons, the possible synergy with cathepsin B inhibitors

could reveal noteworthy targets for novel therapeutic approaches.

P-26-076
Traumatic brain injury alters cerebral mitochodrial respiratory chain complexes activity and redox state of coenzymes Q9 and Q10 in the rat

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To date, there is no information on the effect of TBI on the changes in cerebral mitochondrial activity and brain CoQ levels and possible variations in its redox state. In this study, we induced graded TBIs [mild TBI, (mTBI) and severe TBI (sTBI)] in male rats, using the weight-drop closed-head impact acceleration model of trauma [previously published in Lazzarino et al. (2022) Int J Mol Sci 2022, 23, 8460]. At 7 days post-injury, CoQ9, CoQ10 were measured by HPLC in brain extracts of the injured rats, as well as in those of a group of control sham-operated rats. In the controls, about the 69% of total CoQ was in the form of CoQ9 and the oxidized/reduced ratios of CoQ9 and CoQ10 were, respectively, 1.05 ± 0.07 and 1.42 ± 0.17. In the brains of sTBI-injured animals, an increase in reduced and a decrease in oxidized CoQ9 produced an oxidized/reduced ratio of 0.81 ± 0. A concomitant decrease in both reduced and oxidized CoQ10 generated a corresponding oxidized/reduced ratio of 1.38 ± 0.23. An overall decrease in the concentration of the total CoQ pool was also found in sTBI-injured [as previously published in Lazzarino et al. (2023) 12(5):985]. The CoQ-linked metabolism depends on the proper functioning of the mitochondrial respiratory chain complexes activities. Thus, brain tissue of rats experiencing mTBI and sTBI, such as brain of control sham-operated rats, was homogenized in ice-cold lysis buffer and mitochondrial activities were analyzed spectrophotometrically. Significant disturbances in mitochondrial functionality were noticed, with profound differences among graded TBI groups and between them and controls. These results demonstrate that sTBI alters the functionality of brain mitochondrial respiratory chain complexes and the brain levels of redox states of CoQ9 and CoQ10, thus adding a new explanation to the mitochondrial impairment affecting electron transport chain and oxidative phosphorylation system and the resulting energy supply following sTBI. *The authors marked with an asterisk equally contributed to the work.

P-26-077
Metabolomic data mining for early detection of endometrial cancer

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Endometrial cancer (EC), common in developed countries, lacks effective early screening methods. This study employs a 3D fluorescence analysis of blood serum and urine to detect EC at an early stage. We analyzed spectral metabolic changes in a cohort of 118 (EC patients and healthy volunteers), using constant wavelength matrices for blood analysis and total fluorescent metabolome profiling for urine. Our approach focused on identifying specific metabolic spectral changes through advanced machine learning (ML) and classification techniques. Key findings include disrupted tryptophan metabolism and oxidative stress indicators in both blood and urine samples. Statistical analysis using the Mann-Whitney test and Cliff's Delta calculations confirmed the diagnostic importance of these metabolites (approx. $p < 0.0001$). 3D Principal Component Analysis (3D PCA) and Partial Least Squares Discriminant Analysis (PLS-DA) effectively distinguished the complex metabolomic clusters. In our ML analysis, Logistic Regression (LR) and Random Forest (RF) models outperformed other algorithms. LR achieved an impressive 93% specificity and 89% sensitivity, while RF demonstrated 90% specificity and 91% sensitivity. These results were enhanced through the application of the Synthetic Minority Over-sampling Technique (SMOTE) and grid search optimization, addressing dataset imbalances and refining performance. Our research underscores the potential of non-invasive fluorescence-based techniques in EC diagnostics. Specifically, the identification of altered tryptophan metabolism in urine and blood offers a promising diagnostic avenue. The high specificity and sensitivity of our ML models in distinguishing between early stage – EC patients and healthy controls advocate for the integration of these methods into clinical practice. This advancement in computational biology and bioinformatics could significantly improve EC patient outcomes. This work was supported by the VEGA 1/0435/23 project. *The authors marked with an asterisk equally contributed to the work.

P-26-078
Deciphering the role of extracellular vesicles in ovarian cancer progression

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Ovarian cancer (OC) is still the most lethal gynecologic tumor, due to the rapid and silent development of omental metastasis. Thus, a deeper understanding of the mechanisms regulating OC progression may have crucial impact on the outcomes of this deadly disease. There is consistent evidence of an association between obesity and increased OC aggressiveness. As omentum is

rich in adipocytes, a key pro-tumor role for visceral adipose tissue has been postulated. Indeed, a cross-talk between OC and omental adipose cells has been demonstrated; however, the study of this dialog has been limited to metabolites and adipokines, although recent findings point to a key role of extracellular vesicles (EVs) in the control of tumor evolution. In the present study, we found that EVs derived from adipocytes could affect OC cell traits, inducing increased proliferation, migration and invasion. Furthermore, conditioning of OC cell lines with adipocyte-released EVs resulted in lower sensitivity to cisplatin, with reduced phosphatidylserine externalization and decreased caspase 3 and PARP cleavage. In particular, these alterations were paralleled by the activation of Akt and ERK1/2 pathways, leading to an enhancement in glucose consumption, ROS generation and lipid droplet accumulation, a metabolic signature commonly associated with tumor aggressiveness and poor prognosis. Finally, pretreatment of OC cells with dynasore successfully counteracted the EV-related effects, suggesting that dynamin-dependent endocytosis is involved in the vesicular uptake. More importantly, the pro-tumor activity of adipocyte EVs was successfully suppressed by BEZ-123 (PI3K inhibitor), U0126 (MEK inhibitor) and K604 (ACAT1 inhibitor), indicating that targeting the above molecular cascades might represent a novel treatment strategy for OC management. Further studies will be performed to identify the adipocyte EV molecular cargo responsible for the modulation of the communication with OC. *The authors marked with an asterisk equally contributed to the work.

P-26-079
Nitroglycerin attenuates lung cancer cell proliferation in association with stimulation of reactive oxygen species generation

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Lung cancer is amongst one of the most commonly occurring cancers worldwide for both genders, and is the leading cause of cancer related deaths throughout the world. The main reasons for the high incidence levels are mainly due to exposure to smoke and living in areas with high levels of air pollution. One of the most commonly used antineoplastic agent for the treatment of non-small-cell lung cancer (NSCLC) is cisplatin, which is a platinum-based drug. However, in the long run, more than half of the people who receive cisplatin develop hypertension, which in turn causes certain diseases like peripheral artery disease, chronic kidney failure, and increases the risk of stroke and heart attack. Nitroglycerin (NTG) is a drug that dissociates into nitric oxide in the cell, and is one of the most commonly used drugs for the treatment of acute hypertension. This study aims to investigate the effects of nitroglycerin on cell death mechanisms of A549 NSCLC cells, and provide information whether nitroglycerin be used as a neoplastic agent besides its use case for the treatment of hypertension. In the current study, it was discovered that cell viability decreased with an increasing doses of NTG. Upon investigating the effects of NTG on cell proliferation rate in a time-dependent manner, the results showed that a low dose of NTG (275 μM) induced cell proliferation, whereas a higher dose of 880 μM caused cell survival rate to drop. Moreover, DCFH-DA staining was performed to analyze whether NTG increased the ROS level. It was found that NTG caused oxidative stress in A549 NSCLC cells. When A549 cells were pre-treated with N-acetyl cysteine, NTG-induced cell death was reduced. Therefore, the high dose of NTG induced cell death through increasing ROS levels. In conclusion, NTG is a promising drug candidate for the treatment and control the progression of lung cancer. *The authors marked with an asterisk equally contributed to the work.

P-26-080
In vitro determination of ROS-mediated anticancer potential of MnTPPS and MnF2Met porphyrins in combination with sodium ascorbate

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Modern cancer therapies involve the use of porphyrins as photosensitizing molecules or superoxide dismutase (SOD)-mimicking agents. Modifications of substituted groups to the porphyrin ring allow for modulation their distribution within tumor tissue and efficiency of its therapeutic activity, i.e. by increasing ROS production. However, specific modifications porphyrins including substitution of the manganese (Mn) atom in the center of the ring, allow them to react with sodium ascorbate, which destabilizes the redox homeostasis of numerous tumor cells. This effect can be exploited in the design of new anticancer drugs. Our research hypothesis assumed the process of oxidation of sodium ascorbate (Asc) in reaction with two newly designed and synthesized manganese porphyrins (MnTPPS and MnF2Met), which leads to the formation of reactive oxygen species that disturb the intracellular redox homeostasis leading to cell death. In our experimental model, combination of MnTPPS or MnF2Met with Asc showed cytotoxic effects towards cancer cell line (MCF-7, T98G and AT-2) *in vitro*. Notably, we observed the increase of lipid peroxidation status, oxidative stress and reduced intracellular pool of glutathione (GSH). In addition, induction of mitochondrial damage and induction of apoptosis were observed in lower concentrations of sodium ascorbate in the presence of MnTPPS. Modification of the expression of catalase did not show unambiguous results in contrast to extracellular addition of catalase which abolished the cytotoxic effects of tested compounds. Collectively, our observations demonstrate that MnTPPS and MnF2Met in combination with Asc exert the ROS-mediated cytotoxic effect towards cancer cells. This research sheds the light on the new the potential therapeutic strategies for current oncology and stress the need for further substantial research. The present study was financially supported by Polish Ministry of Science and Higher Education (0054/DIA/2020/49 to M.R).

P-26-081
Estrogen's potential impact on chemoresistance in high-grade serous ovarian cancer

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High-grade serous ovarian carcinoma (HGSOC) is the most common ovarian cancer (OC) associated with disturbed estrogen action and usually develops resistance to chemotherapeutic agents, but the role of estrogens in the chemoresistance of OC and HGSOC remains unclear. The aim of our study was to identify potential chemoresistance biomarkers in transcriptomic data of platinum-sensitive and -resistant HGSOC tissues and to better understand estrogen metabolism and expression of selected genes in HGSOC cell lines with different sensitivity to carboplatin. RStudio was used for bioinformatic analysis of the selected transcripts from HGSOC tissues (www.cBioPortal.com). QPCR was used to analyze the expression of genes involved in estrogen metabolism, transport and action in six HGSOC cell lines with different sensitivities to carboplatin. The formation of estrogens from estrone sulfate (E1S) in HGSOC cells was analyzed by LC-MS/MS. The effects of estrogens and sulfatase inhibitor (STX64) on the proliferation of HGSOC cells and sensitivity to carboplatin were examined using the Alamar assay. Bioinformatic analysis of HGSOC tissues and transcriptomic analysis of HGSOC cell lines identified potential target genes contributing to treatment response heterogeneity. LC-MS/MS analysis showed the formation of active estrogens in most cells sensitive to carboplatin, but not in the most resistant cell COV362. Several HGSOC cells showed E1S- and/or estradiol-dependent proliferation. Ethinylestradiol (EE2) and equilin (EQ) decreased proliferation in OVCAR-4, Kuramochi, Caov-3 and COV362 cells. STX64 decreased E1-S-dependent proliferation in HGSOC cells. Based on this data *HSD17B14*, *NQO1*, CYP1B1, *SULT1E1* and *ESR1* represent candidate prognostic biomarkers for HGSOC. Estrogens EE2, EQ and sulfatase inhibitor STX64 have to be further studied in preclinical models to assess their potential for the targeted treatment of HGSOC. *The authors marked with an asterisk equally contributed to the work.

P-26-082
Regulation of HIF1α function by FBP1 and FBP2

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In cancer cells, hypoxia-inducible factor 1α (HIF1α), a master regulator of the transcriptional response to hypoxic conditions, can be affected in a non-enzymatic manner by fructose 1,6-bisphosphatase (FBP). Both isozymes of FBP, namely liver (FBP1) and muscle (FBP2) forms, can interact with HIF1α. In this poster, we present quantitative data on the interaction of FBP1 and FBP2 with HIF1α *in vitro*, in the presence and absence of natural and synthetic allosteric effectors of FBP. We also demonstrate the effectors-induced changes in the formation of the FBP-HIF1α complex in lung cancer cells, under normoxic and hypoxic conditions. Our results reveal that the amount of the FBP1-HIF1α complex depends only on the titer of FBP1, while the FBP2-HIF1α association is regulated by AMP, an allosteric FBP inhibitor whose concentration is a function of the metabolic state of the cell. In the short term, the interaction between FBP and HIF1α leads to the degradation of the former and reduced expression of HIF1α-dependent genes. The dynamic regulation of FBP2-HIF1α complex formation is strong evidence that this isozyme has a bigger role in maintaining HIF1α levels in cells than FBP1. This difference is probably caused by distinct conformational changes in FBP oligomers in response to the allosteric effectors.

P-26-083
Unsupervised machine learning algorithms for revealing interaction of single-walled carbon nanotubes with cancer cells

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Single-walled carbon nanotubes (SWCNTs) are promising materials for cancer therapy and diagnostics, but despite extensive studies, their interaction with cells and their accumulation are still the subject of much research. Raman spectroscopy is a precise technique that can be used to detect both biochemical changes in cells and the tracking of SWCNTs. The significant difference in signal between SWCNTs and cells limits the analysis. Large data sets with noisy and complex Raman spectra are difficult to analyze visually and require machine learning algorithms to provide a reliable and objective basis for research. In this work, we apply unsupervised machine learning (ML) algorithms including principal component analysis and K-Means clustering to datasets of Raman spectra from cells interacting with SWCNTs capped with dsDNA or ssDNA to reveal the mechanism of nanomaterial accumulation, distribution and metabolism in glioma cells. For this advanced analysis, we used the experimental datasets of Raman spectra partially described in: Golubewa L et al. (2021) Nanotechnology 32, 505103. Using ML algorithms, we demonstrate the time-dependent, compartment-specific accumulation of SWCNTs, showing two phases of interaction of SWCNTs with cells, including (i) vesicle transport and accumulation of SWCNT-dsDNA and SWCNT-ssDNA in the cytoplasm and (ii) metabolization of SWCNT-dsDNA in autolysosomes triggered by saturation of SWCNT concentration in the cytoplasm, leading to autophagy and cell death. The second phase is absent in SWCNT-ssDNA accumulated in the cells. In summary, our approach allows to separate the information on cellular components from that of nanotubes from large data sets of low-intensity and noisy Raman spectra and to determine the mechanism of SWCNT accumulation and metabolism in living cells without additional research. Unsupervised ML can help increase the efficiency of cancer treatment by improving the accuracy of cell-drug interaction analysis. *The authors marked with an asterisk equally contributed to the work.

P-26-084
Lactate mediates melanoma progression by rewiring its metabolism and epigenetic architecture

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Melanoma, the deadliest form of skin cancer, presents challenges due to high mortality rates and resistance to therapy. This malignancy, influenced by genetic, epigenetic, and environmental factors, involves uncontrolled melanocyte proliferation. Despite the prominence of immunotherapy, its effectiveness is hindered by an immunosuppressive TME, where lactate, plays a key role. Our study explores lactate's impact on melanoma progression and metabolic reprogramming. To investigate, we supplemented lactate to three human *in vitro* melanoma cell lines. Results revealed a significant increase in cell proliferation, migration, and colony formation with lactate supplementation. Lactate stimulated the accumulation of its import channel MCT1. We mimicked MCT1 stimulation by in parallel usage the lactate receptor agonist 3,5-DHBA, which also upregulated OXPHOS-related genes. This prompted us to assess lactate's crucial role in melanoma cell metabolic rewire. The mechanisms underlying lactate's influence on adaptive states remain unclear. We hypothesized that lactate might elicit its effect through specific epigenetic mechanisms. We evaluated two novel epigenetic modifications, H3K18lac and H3K27lac, following lactate administration on melanoma *in vitro* cell lines. Data revealed a marked increase in these modifications following lactate supplementation. Interestingly, the MCT1 selective inhibitor AZD3965 restored histone lactylation levels. In conclusion, our findings emphasize lactate's significant impact on melanoma progression and metabolic reprogramming, highlighting its pivotal role in shaping melanoma cell aggressiveness. Our study also explores posttranslational histone modifications as potential mediators between lactate-induced metabolic changes. Further research in this direction promises to advance our understanding of the complex interrelationships between metabolism, epigenetics and melanoma progression paving the way for innovative therapeutic interventions.

P-26-085

Abstract withdrawn.

P-26-086
Exploiting the metabolic dependency of triple negative breast cancer: a new therapeutic approach via inhibition of glutamine support using asparaginase

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Triple negative breast cancer (TNBC) is characterized by the absence of estrogen receptor (ER), progesterone receptor (PR) and human epidermal growth factor receptor 2 (HER2). This lack of therapeutic targets causes poor prognoses and upholds the need of novel therapeutic targets. Glutamine (Gln) plays a vital role in TNBC cell metabolism, replenishing Krebs cycle intermediates even in low-glucose conditions and it is essential for proliferative pathways, providing carbon and nitrogen. In advanced cancers, it has been associated with the induction of epithelial-mesenchymal transition (EMT). The need for new therapeutical targets in conventional chemotherapy resistant cancers pushed the research to investigate the effects of Gln-targeting drugs. Among these, asparaginase has already been used for patients' treatment for 50 years. This enzyme hydrolyzes asparagine in aspartic acid and ammonia, and it exhibits glutaminase activity, which limits Gln uptake by cancer cells. However, Gln dependence of TNBC has not yet been fully tackled and requires further investigations. On this regard, our preliminary data confirm that the expression levels of Gln synthetase (GS) in TNBC cell lines correlates with their sensitivity to asparaginase-induced Gln deprivation, after a 72 h treatment. We saw a notable reduction in DNA synthesis and cell proliferation in the low GS TNBC BT549 cell line, while this effect was significantly lower in the GS overexpressing TNBC MDA-MB-231 cell line. In BT549 cells, the addition of Gln to the culture media resulted in the recovery of DNA synthesis, underling the role of Gln in this process. The obtained data laid the basis for further studies on the role of glutamine in TNBC progression and proliferation and offers the opportunity to describe new cancer subtypes which could be selected for targeted treatments.

P-26-087
Analysis of the unfolded protein response regulation by the mono-ADP-ribosyltransferase PARP16 in a cellular model of osteosarcoma

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The mechanisms underlying the post-translational regulation of eukaryotic unfolded protein response (UPR) represent an important aspect in cell biology. The mono-ADP-ribosylation of two key UPR regulators, PERK and IRE1α, by the endoplasmic reticulum (ER) mono-ADP-ribosyltransferase PARP16 represents a critical step for the full activation of the UPR upon ER-stress.

Ineffective UPR response can represent, in certain cases, a deleterious scenario for the survival of tumor cells subjected to ER-stress conditions. We investigated the regulation of UPR upon PARP16 depletion in a cell model of osteosarcoma, a tumor in which pharmacological treatment is limited by post-therapy drug-resistance. Using gene silencing and genome editing approaches we tested the potential of PARP16 as target for the treatment of this tumor. Our analyses showed that upon ER-stress, depletion of PARP16 in U-2 OS cells by siRNA caused an altered phosphorylation of eIF2a (p-Ser51), the major substrate of the UPR-regulating kinase PERK. In the same conditions, we observed the proteolytic cleavage of PARP1 in both U-2 OS and cell lines from different tumors, confirming that PARP16 is involved in cell survival. To further characterize this aspect, we generated a PARP16^{KO} U-2 OS cell line and tested its sensitivity to ER-stress. From our results, it emerged that the pharmacological treatment with ER-stress inducers, caused a larger inhibition in the proliferation of PARP16^{KO} cells compared to control cells. Overall, the results obtained suggest that interfering with the function of PARP16 could have a potential impact on improving the treatment of osteosarcoma. These observations open the possibility to test therapeutic approaches and/or molecules that aim to inhibit the function of PARP16 in tumors with low UPR in combination with ER stress inducers and in tumors that exploit high UPR to survive with the aim of increasing its sensitivity to ER-stress, favoring the activation of cell death mechanisms.

P-26-088
The influence of glucose concentration in response of melanoma cells to PFK-II (PFKFB3/PFKFB4) inhibitors

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While therapies directed at mutated BRAF, the predominant molecular disorder in melanoma, have been established for some time now, the emergence of resistance to these inhibitors presents a substantial obstacle to their clinical efficacy. Recent findings indicating that inhibition of glycolytic pathway can induce cell death in BRAF inhibitor-resistant cells provides a compelling rationale for investigating therapeutic approaches that selectively target the glycolytic pathway in melanoma cells. Additionally, studies have highlighted a connection between cancer-specific isoenzymes (PFKFB3/PFKFB4) of phosphofructokinase II (PFK-II) and tumor aggressiveness, underscoring their potential significance in carcinogenesis. Research hypothesis assumes that targeting these isoenzymes could represent a novel approach to anti-melanoma therapy, impacting both cell growth and BRAF inhibitor resistance. The quantification of PFKFB3/PFKFB4 expression in melanoma cell lines using RT-qPCR and western blot analyses, confirming their presence in both BRAF inhibitor-sensitive and resistant melanoma cells. Subsequently, cells were subjected to treatment with specific inhibitors of PFKFB3/PFKFB4, followed by comprehensive functional analyses including proliferation, survival, apoptosis, and metabolic profile and activity. As cancer patients may exhibit different basal serum glucose levels, we have also examined the influence of glucose concentration on the response to PFK-II inhibitors. In the case of the PFKFB3 inhibitor, the glucose concentration did not affect the sensitivity of cells to the inhibitor. However, with the PFKFB4 inhibitor, significantly higher sensitivity was observed in cells placed in a low glucose concentration medium. These results not only confirm the specificity of the PFKFB4 inhibitor's action in glucose metabolism but also indicate that its level may also be important in designing future anti-cancer therapies targeting glucose metabolism in melanoma cells.

P-26-089
Design of a bispecific antibody to block iron-endocytosis-driven metastasis

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Cancer remains a leading cause of death worldwide. Metastasis is the main contributor to cancer mortality and morbidity. Metastatic breast cancer (MBC), accounts for the vast majority of the 0.6 million deaths from breast cancer each year globally. Despite the advances in therapy regimens, the risks associated with current treatments still carry severe side effects and offer limited benefit to the patient. Thus, there is a critical need for innovative drugs capable of modulating new cancer-relevant targets. We proposed to validate and develop an unprecedented approach to tackle MBC, by targeting the overexpressed CD44 hyaluronic acid (HA) and the transferrin receptor 1 (TfR1)-transferrin pathways, with a bispecific antibody (bsAb). Since metastasis relies on the cancer cells' epithelial-mesenchymal plasticity, and this requires a change in gene expression, blocking iron endocytosis will block histone demethylation required to unlock the expression of mesenchymal and metastatic genes. In this study, we investigated a subset of 4 promising molecules and validated the blocking effect on the uptake of labelled transferrin and HA using flow cytometry and confocal microscopy. Intracellular iron contents were assessed using a turn-on fluorescent probe for the selective detection of iron(II). TfR1 and CD44 expression levels were assessed in different MBC cell lines. All antibodies showed high binding affinity towards their targets and a significant reduction of iron uptake in the MDA-MB-468 cell line. We selected the best molecules to be assembled into a bsAb and identified the variable chains to use for the design, using the Knob into Hole approach. We sought to determine whether the bsAb could improve avidity and efficacy while maximizing iron uptake impairment. We strongly believe that through simultaneous disruption of iron signalling with our bsAb we will block and control the expression of metastatic genes and achieve selective target and killing of MBC cells.

P-26-090
Multimodal interaction between a tyrosine kinase inhibitor (midostaurin, PKC412) and supramolecular compound (Congo red)

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Excipients play a crucial role in enhancing the efficacy of drugs by safeguarding active compounds, facilitating delivery, and triggering physiological responses. Self-aggregating excipients, such as those forming supramolecular structures, possess the capability to interact with various drug molecules. Congo red (CR) is a notable example of such compounds. Coaggregation of drugs with Congo red shows promise in mitigating drug toxicity, enhancing targeted cell delivery and increasing drug solubility. This approach presents opportunities to improve therapeutic outcomes. This study focuses on analysing the interaction between the supramolecular carrier Congo red and the FDA-approved tyrosine kinase inhibitor, midostaurin (PKC412), within the co-aggregates. Utilizing dynamic light scattering (DLS), UV-VIS spectroscopy, fluorescence, and ultracentrifugation the co-aggregates were investigated. These biophysical methods revealed a non-trivial interaction, highlighting the potential of these compounds in targeted drug delivery. The optimal carrier-drug molar ratio was determined, and preliminary assessment of complex formation was conducted. Supramolecular aggregates offer a promising avenue for advancing therapy, particularly in anti-cancer therapy. Our results underscore the importance of further exploring the CR-PKC412 co-aggregates, emphasizing its potential anticancer effects on tumour cells. We acknowledge the financial support from the National Science Centre, Poland (grant no. K/MNT/000232).

P-26-091
Exploring the role of GPX4 in ferroptosis induction: implications for cancer therapy

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CRISPR/Cas9 genome editing, with its precision and efficiency, holds promise for cancer treatment, potentially overcoming resistance to traditional chemotherapy via ferroptosis regulation. The study aimed to confirm the hypothesis that glutathione peroxidase 4 (*GPX4*) plays a crucial role in protecting cells from ferroptotic cell death. Research involved creating cell models – mutant lines with the *GPX4* gene knocked out, inducing ferroptosis using erastin, and analyzing the obtained data. Mutant cell lines (*GPX4* KO) were created using CRISPR/Cas9 in HCT116 WT cells. Western blot and sequencing validated *GPX4*-deficient mutants (lines 10 and 11) with mutant 64 as positive control. Ferroptosis induction using erastin at IC_{50}-derived doses characterized cell lines for ferroptotic death. MTS viability assay showed increased susceptibility of *GPX4* KO mutant line to 10 μM erastin, unlike control lines with intact *GPX4*. Erastin reduced total glutathione levels in all cells, inversely correlating with dosage. Microscopic observations revealed a significant increase in reduced lipids positively correlating with the dose of erastin, indicating activation of an antioxidative system other than the glutathione shield. High expression of *FSP1* and *PRDX1* genes in *GPX4* KO cells indicated their ferroptosis-suppressing function. Expression of *TFRC, ACSL4, TRX*, and *PROM1* genes increased in *GPX4* KO cells, suggesting potential therapeutic targets. The results of gene expression have been previously published [1]. This study focused on the crucial role of *GPX4* in protecting cells from ferroptotic cell death, manifested as lipids oxidation. Analysis of lipid peroxidation and microscopic images revealed activation of an alternative antioxidative system, supporting *GPX4* KO cells. Research was supported by grant 02/040/BK_24/1056 from Silesian University of Technology. Reference: 1. Adamiec-Organisciok M et al. (2023) Pharmaceuticals 16(12), 1710. *The authors marked with an asterisk equally contributed to the work.

P-26-092
Immune profile of head and neck squamous cell carcinoma (HNSCC) patients can be used to assessment of radiotherapy response: transcriptome-based analysis

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The main treatment method for carcinogen-related HNSCCs is surgery in combination with radiotherapy alone or with chemotherapy. However, tumor heterogeneity and tumor microenvironment both affect radiotherapy success. Due to this fact, the radioresistance process is not fully understood and seems to be the challenge for current oncology. Based on the transcriptome data and *in silico* model, using available clinical data, we analyzed subpopulation of specific immune cells and immune-related genes in HNSCC patients with response (RG) and non-response

(NRG) to radiotherapy. It was observed that only three types of immune cells have shown significant differences between NRG and RG groups. Higher levels of T cells CDR memory resting, mast cells resting and B cells native were indicated in RG than NRG groups. In the case of oral cavity localisation, significantly higher levels of mast cells resting ($p = 0.0279$) and memory CD4 T cells memory resisting ($p = 0.0059$) in RG than in the NRG group were observed. Only B cells naive displayed higher levels in RG than in the NRG group ($p = 0.0059$) in the larynx localisation. No differences of all subtypes of immune cells for pharynx localizations were indicated. Next, we observed that patients with lower levels of T cells regulatory tregs ($p = 0.0297$ and 0.0209) presented worse OS. However, when tregs and mast cells resting were taken into account together, patients with higher levels displayed better OS than the group with lover levels of these both types of immune cells ($p = 0.0144$ and $p = 0.0099$). The knowledge about association between infiltration of immune cells before radiotherapy and patients' survival could help in the planning of treatment strategy with prediction of the best results with the lowest costs. This work was supported by Greater Poland Cancer Centre – grant no.: 5/12/2022/PGN/WCO/011. *The authors marked with an asterisk equally contributed to the work.

P-26-093
Proteomic profiling of plasma-derived sEVs from ovarian cancer patients and their effect on healthy ovarian epithelial cells

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Small extracellular vesicles (sEVs) are submicrometer membrane vesicles released from cell types which include a diameter range between 50 and 150 nm and carry RNA, DNA and protein molecules. These molecules can alter the behaviour of cells and the communication between and among them, affecting the chance of cancer development. The current study aims to establish the role of sEVs in the development and progression of ovarian cancer (OC) by analyzing the protein content of sEVs isolated from the blood of ovarian cancer patients and the effect that these sEVs have on healthy ovarian epithelial cell culture. sEVs were harvested from two different samples, out of these two, one is the sample of twelve blood samples from twelve patients with advanced-stage disease (FIGO III and IV) and the other is the same number of blood samples from twelve healthy women. Three different methods were used: differential ultracentrifugation (dUC), tangential flow filtration (TFF), and size exclusion chromatography (SEC). The preparation of sEVs was also characterized by nano-flow cytometry and immunoblot, and a proteomics analysis was done to check their protein content. The next step was to culture IOSE-80T cells (healthy ovarian epithelial cells) with plasma-derived sEVs of the two groups and to monitor their outcomes. Proteomic analysis of sEV samples indicated a difference in their constituent protein profiles in the OC patients and healthy individuals, OC preparations caused gene expression changes and altered cell behaviour in the healthy ovarian epithelial cells as compared to controls, implying that circulating sEV could play a role in pro-tumorigenic transformation.

P-26-094
PLGA-based nanocarriers for combined chemo- and photodynamic therapy

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The combination of different therapeutic approaches has emerged as a promising strategy in cancer treatment. In our work, we present a novel drug combination to achieve enhanced anticancer efficacy via multimodal therapy. We have prepared a multifunctional drug delivery system based on poly(lactide-*co*-glycolide) (PLGA) nanoparticles (NPs) with two drugs: colchicine (Colch) and purpurin 18 (P18). Colch, an inhibitor of microtubule polymerization, is used as a chemotherapeutic agent. The potent photosensitizer P18 is important for photodynamic therapy, which belongs to the noninvasive method of cancer treatment. The prepared NPs reached an average size of 200 ± 75 nm and showed enhanced uptake by cancer cells of different origin (MCF-7, PC-3, CaCo-2) compared to free drugs. The use of PLGA NPs with Colch led to efficient, concentration-dependent elimination of cancer cells in 2D and 3D cell line models. Significant toxicity of the PLGA NPs with Colch and P18 was detected especially in combination with illumination (the light dose of 4 $J \cdot cm^2$), when not only chemotherapeutic but also phototoxic effects were present. After photoactivation, treatment of CaCo-2, PC-3, MRC-5, and MCF-7 cells with PLGA NPs with Colch and P18 resulted in more than a 50% decrease in their viability already at concentrations of 21.8 (first three) and 44 μg/ml (MCF-7). All Colch containing PLGA NPs were also able to arrest the cell cycle of PC-3 and HeLa cells at the G2/M phase after 24 h of treatment. Our results suggest that novel PLGA based NPs have the potential for use in multimodal therapy and highlight the importance of studying nanoformulations to improve cancer therapy outcomes.

P-26-095
ACE and MT1H genes are potential candidates as biomarkers for assessment of platinum-based treatment in ovarian cancer

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Advanced ovarian cancer prognosis is mainly due to frequent occurrence of the resistance to platinum, which is the standard of care. There is an urgent need to develop chemosensitizing strategies. Ramipril (RAM), which is a well-known cardiovascular drug, exhibits properties to overcome platinum resistance in ovarian cancer. We used TCGA data to find the molecular rationale for RAM overcoming platinum resistance in ovarian cancer. The study's main steps included: (i) identification which RAM-responsive genes were expressed differently in platinum-sensitive and platinum-resistant settings (ii) finding their positively and negatively correlated genes, (iii) finding their coupled molecular pathways. ACE and MT1H which were significantly differentially expressed in platinum-sensitive and platinum-resistant patients and were associated with survival. RAM upregulates ACE gene and downregulates MT1H gene which is characteristic of platinum-sensitivity and longer OS and DFI, respectively. Moreover, their co-expressed genes were associated with sequestration of platinum, Hedgehog signalling inhibition, NOTCH inhibition DNA repair pathways. Hence, based on our bioinformatic analysis and extensive literature review, it seems likely that RAM could increase PT-sensitivity in ovarian cancer. ACE and MT1H genes are potential candidates as biomarkers for assessment of platinum-based treatment in ovarian cancer. Since they are RAM-responsive in a way that favors platinum-sensitivity, using this drug could increase platinum efficiency in both platinum sensitive and resistant setting, and hence positively impact patient survival. Further studies, starting from in vitro analyses are needed to confirm RAM utility as platinum sensitizer in ovarian cancer. This work was supported by Greater Poland Cancer Centre – grant no.: 5/12/2022/PGN/WCO/011 and by National Science Center Poland, MINIATURA 7 to Katarzyna Regulska.

P-26-096
Liposomal locked-in dendrimers as potential doxorubicin delivery nanosystem

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Despite highly developed medicine, treatment of many prevalent diseases like cancers or neurodegenerative disorders has limitations, due to the inability of the drugs to cross cell membranes. The potential solution widely investigated by researchers to increase the bioavailability of therapeutics are lipid or polymer nanosystems. This study presents the biophysical properties and cytotoxic effects of liposomal locked-in dendrimers nanosystems (LLDs). Ruthenium metallodendrimer was complexed with doxorubicin, a drug commonly applied in some type of cancers, and locked in a liposomal structure. We found that the dendrimer/doxorubicin complex coated with lipids reduces the viability of breast cancer cells (MCF-7), simultaneously weakly decreasing the viability of human embryonic kidney cells (HEK293). Confocal microscopy revealed an improved uptake of the LLDs by MCF-7 cells compared to non-complexed components. Moreover, the studied complex displayed nanometric size, and monodispersity, which made it attractive as a potential drug delivery system. This work was supported by grant 2018/30/Z/NZ1/00911 of the Project "NanoTendo", by grant 2023/07/X/NZ7/00026 of the Project "MINIATURA7", and by grant 2018/ 31/F/NZ5/03454 "Beethoven Life 1" financed by the National Science Centre of Poland.

P-26-097
Spectrum of germline BRCA1/2 gene mutations and rearrangements in 2207 patients with high-risk breast and ovarian cancer: a single center study in Turkey

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Breast and ovarian cancers pose significant health challenges, accounting for a substantial portion of cancer-related morbidity and mortality. Among the numerous genetic factors implicated in the etiology of these cancers, mutations in the BRCA1/2 genes are prominent. Mutations in BRCA1/2 confer significantly increased risk of developing breast and ovarian cancers. Women with pathogenic mutations in these genes face a lifetime risk of up to 70% for breast cancer and 40% for ovarian cancer. The frequency of BRCA1/2 pathogenic variants in the general population has been estimated to be 1:400–500. Among all patients with breast and ovarian cancer BRCA1/2 mutation rates are 4.2–6.1% and 8.3–14.7% respectively. Studies in Turkish patients with familial breast and/or ovarian cancer, revealing a BRCA1/2 mutations frequency of 16–20%. gDNA samples were obtained

from 2207 patients with high-risk breast and ovarian cancer. Illumina platform was used for NGS analysis according to the manufacturer's instructions. MLPA method was carried out for the identification of copy number variations. NGS and MLPA data were analyzed using bioinformatics pipelines to identify variants within the BRCA1/2 genes. Variants were annotated and classified according to the ACMG guidelines. Among 283 positive results, 224 were determined as pathogenic and were classified as 135 frameshift, 61 nonsense, 17 missense, and 8 splice mutations. Large deletions in the BRCA1/2 genes were identified in 31 patients with MLPA. Overall, the frequency of pathogenic SNVs/small indels/large deletions in high-risk breast and ovarian cancer patients was found to be 11.55%. This result shows that the frequency of BRCA1/2 mutations in Turkey still has a significant rate and concordant with previous findings. Although no founder mutation was observed for the Turkish population, frequently occurring mutation regions were examined. The possible roles of these regions in protein structure and function will be discussed. *The authors marked with an asterisk equally contributed to the work.

P-26-098
SGLT2 inhibition promotes mitochondrial dysfunction and colorectal cancer cell death

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Sodium-glucose cotransporter 2 (SGLT2) is a critical glucose transporter overexpressed in different cancer models, including lung cancer metastasis, pancreatic and prostate adenocarcinomas, high-grade glioblastoma, cisplatin-resistant hepatoblastoma and renal cell carcinoma [previously published in: Madunić IV et al. (2018) Arh Hig Rada Toksikol, 69(4), 278–285]. SGLT2 inhibitors (iSGLT2), canagliflozin, dapagliflozin, tofogliflozin and empagliflozin, used to treat patients with type 2 diabetes, have broad biological effects [previously published in: D'Onofrio N et al. (2021) Mol Metab, 54, 101337]. Recent studies indicate that iSGLT2 inhibits the growth of some cancer cells. However, the anticancer mechanism(s) remains to be fully elucidated. To this end, the present study was designed to investigate the effects of canagliflozin on colorectal cancer (CRC) cells viability, cell death mechanisms and mitochondrion-related redox-energetic metabolism. Results showed that treatment with canagliflozin (50 μM) for 72 h promoted cell cycle arrest ($p < 0.001$), impaired energetic metabolism ($p < 0.001$), and induced apoptotic cell death in HCT 116 and HT-29 cells. These cellular events were accompanied by sirtuin 3 (SIRT3) upregulation ($p < 0.01$), as confirmed by SIRT3 transient silencing which resulted in the attenuation of the effects of canagliflozin on cellular metabolic alterations and apoptosis. Moreover, analysis of protein-protein interaction network and western blot analysis allowed the identification of dipeptidyl peptidase 4 (DPP4) as potential common target of SGLT2 and SIRT3. Overall, results unveil the role of SGLT2/SIRT3 axis in the mitochondrial dysfunction and cell death in CRC, deepening current knowledge on the role of iSGLT2 in limiting CRC tumorigenesis.

P-26-099
Deciphering adipose triglyceride lipase (ATGL) role in hepatocellular carcinoma sensitivity to genotoxic stimuli

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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 not applicable for patients with advanced stages, for which systemic therapy is the only option. Based on this, research efforts have been addressed to better understand the molecular mechanisms of HCC in order to both improve cancer cells' sensitivity to drugs and avoid the development of resistance. Several studies showed an alteration of lipid metabolism in HCC, accompanied by ATGL down-regulation. Moreover, increasing ATGL expression in HCC cells has an anti-tumoral effect. However, any clear evidence linking ATGL or lipid droplets (LDs) to DNA damage response in HCC exists, although recent works associated drug pharmacokinetics with LDs' dynamic and content. 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 histones acetylation, typical modifications of open/active chromatin. Indeed, the accessibility of chromatin is strictly associated with the tumor sensitivity to genotoxic compounds. Our results demonstrated that the level of ATGL could affect the DNA damage degree upon genotoxic stimuli. This work was supported in part by HEAL ITALIA (PNRR – MUR PE00000019).

P-26-100
Dissecting the role of 5-hydroxymethylcytosine (5hmC) in the acquired resistance of melanoma cells to BRAF inhibitor (BRAFi) therapies

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Cutaneous melanoma is the deadliest form of skin cancer. The serine/threonine kinase BRAF is constitutively activated via mutations in about 60% of melanomas. Selective inhibitors of BRAFV600E/K (e.g., Dabrafenib, Vemurafenib) work potently against melanomas driven by oncogenic BRAF. Unfortunately, drug resistance is ubiquitous, and most patients will progress within two years of therapy. Epigenetic deregulation plays a key role in the malignant transformation of cells. Changes in 5-hydroxymethylcytosine (5hmc) levels have been observed in the development of several cancer types, particularly melanoma. Ten-eleven translocation (TET) is a family of dioxygenases consisting of three members, TET1, TET2, and TET3 that have Fe + 2 and α-ketoglutarate (α-KG)-dependent dioxygenase activities, catalyzing the 1st step of DNA demethylation by converting 5-methylcytosine (5mC) to 5hmC, and further oxidize 5hmC to 5-formylcytosine (5fC) and 5-carboxylcytosine (5caC). To date, whether alterations of TET enzymes and 5hmC levels have a role in the chemoresistance of melanoma is still unknown. This work

aims to characterize the contribution of TET-mediated DNA hydroxymethylation in the acquired resistance of melanoma cells to current BRAF inhibitor (BRAFi) therapies. Our preliminary data indicate that M14 and A375 melanoma cells resistant to Dabrafenib express lower levels of TET enzymes and, consistently, of 5hmc, than their BRAFi-sensitive counterparts. Several 5hmC readers have been identified as proteins regulating gene expression by recruiting co-activators or co-repressors; moreover, 5hmC has been associated with the activating histone marks H3K4me1, H3K4me3, and H3K27ac. Thus, a characterization of post-translational modification of histones was performed aiming at defining a relation between 5hmc and gene expression in the resistance of melanoma. This work was supported by PRIN2022 to F.C. and HEAL ITALIA: PNRR – MUR PE00000019 to M.R.C.

P-26-101
Estrogen-related receptor alpha (ERRα) axis involvement in metabolic reprogramming breast cancer

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Breast cancer (BC), a leading cause of cancer-related death in women, often exhibits resistance to conventional treatments. Here, we investigated the roles of cholesterol and mevalonate in breast cancer progression and therapy resistance. Our recent findings have revealed that these compounds activate the estrogen-related receptor alpha (ERRα) pathway, leading to increased expression of key proteins associated with tumor aggressiveness and drug resistance [1]. Furthermore, cholesterol-induced activation of ERRα promotes epithelial-mesenchymal transition (EMT) and inflammatory responses in breast cancer cells, shaping the tumor microenvironment. Additionally, high cholesterol levels enhance macrophage infiltration, angiogenesis, and cancer-associated fibroblasts (CAFs) phenotype [2]. Clinically, these findings have important implications for understanding treatment failure and cancer dissemination. We have defined estrogen-related receptor alpha gene (*ESRRA*) signature in multiple types of breast cancer, using bioinformatic analysis of patient samples. Importantly, *ESRRA* expression correlates with poor prognosis, suggesting it as a potential therapeutic target. In addition, ERRα protein expression has been validated through immunohistochemistry in female patients diagnosed with invasive BC, including Luminal ER(+) BC, TNBC and metastatic lesions, as well as in the tissue surrounding the tumor. Overall, targeting the cholesterol-ERRα axis may offer novel strategies for combating breast cancer progression and resistance to therapy.References: 1. Brindisi M et al. (2020) Cells 8, 1819. 2. Brindisi M, Frattaruolo L et al. (2022) FEBS J 6, 1481–1501. *The authors marked with an asterisk equally contributed to the work.

Cancer Biochemistry

P-27-001
Diosmin synergistically potentiates the anti-tumor effects of regorafenib against HCC via dual inhibition of PI3K/mTOR and RAS/Erk signaling pathways

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HCC is one of the most lethal malignancies. This malignancy ranks as the sixth most prevalent form of cancer globally and stands as the third leading cause of cancer-related mortality. Regorafenib represents a significant advancement in the treatment of metastatic colorectal cancer that has failed to respond to conventional therapy. Diosmin refers to a class of flavonoid glycosides that are naturally present in citrus fruits. They possess a diverse range of pharmacological activities, including anti-inflammatory and antioxidant effects. The objective of this study was to assess the possible anti-carcinogenic effects of regorafenib and/or diosmin on the HEPG2 cell line, which serves as a model for HCC. The present study employs a rigorous methodology to investigate the research question at hand. The HEPG2 cell line, representative of human HCC cell line, was subjected to individual and combined treatments with regorafenib and diosmin. The subsequent impact of these therapies on cell proliferation and apoptosis was assessed using the ELISA technique. The two medicines have demonstrated the ability to regulate the Akt/mTOR and RAF/Erk signaling pathways. The co-administration of regorafenib with diosmin at low concentrations had a synergistic effect, leading to heightened suppression of cell growth, inhibition of Akt/mTOR and RAF/Erk signaling pathways, and greater apoptosis induction as compared to the individual activities of either agent alone. Conclusion and Recommendation: The findings of this study demonstrate that diosmin plays a role in mediating the antitumor effect of regorafenib. Based on these results, it is recommended that more research be conducted to explore the potential mechanisms behind this interaction. Additional clinical trials are required to assess the effectiveness of combining regorafenib with diosmin as a therapeutic approach for HCC.

P-27-002
Haloperidol anticancer activity involves ferroptosis and heme-oxygenase modulation in breast cancer

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Breast cancer (BC) is the malignant tumor with the highest mortality in women. Iron homeostasis is essential for cellular metabolism, it's crucial for ROS production and can contribute either to cell proliferation or cell death in BC, suggesting the existence of a delicate cellular balance of pro and anti-oxidant conditions that dictates its own fate[1]. The discovery of ferroptosis, a novel form of programmed cell death distinct from apoptosis, along

with the identification of the molecular pathways activated during its execution, has led to the uncovering of novel molecules characterized by ferroptosis-inducing properties[2]. Thus, the need of investigating the effects of already approved drugs potentially involved in ferroptosis in order to overcome these difficulties. In this view, utilization of haloperidol (a first-generation typical antipsychotic commonly used worldwide) could prove useful to our purpose. Investigation of haloperidol mechanism of action and ability to induce ferroptosis was conducted in two different BC cell lines (MDA-MB 231 and MCF-7), analyzing: ferroptotic markers expression through western blot technique; ROS and lipid hydroperoxides levels together with mitochondrial dysfunction and glutathione cellular content using both spectrophotometric assays, fluorescence measurement and cytofluorimetry. Moreover, haloperidol ability to significantly induce heme-oxygenase 1 (HO-1) expression and its cytoplasmatic translocation was investigated in order to understand its involvement in the process. Ultimately, data obtained in 2D cultures were also reproduced in 3D breast cancer spheroid to evaluate haloperidol efficacy in a more complex system.References: 1. Dixon SJ, Stockwell BR (2014) Nat Chem Biol 10(1), 9–17, doi: 10.1038/nchembio.1416. 2. Consoli V et al. (2023) Antioxid Redox Signal 40(1–3), 40–85, doi: 10.1089/ars.2022.0179

P-27-003
Targeting steroid receptors and aldo-keto reductases with N(2)-substituted steroid D-ring-fused triazole derivatives: binding potential, inhibitory activity and selectivity

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Structurally modified steroid hormones with improved biological activity have been the focus of intensive research for many years, especially in the field of hormone-dependent cancer treatment. Desirable anticancer properties of steroid derivatives are associated with their ability to reduce the level of endogenous steroid hormones in cancer cells by inhibition of steroid-converting enzymes or by blocking the action of steroid receptors. With this in mind, our research group is interested in characterization of estrogen receptor (ER), androgen receptor (AR), glucocorticoid receptor (GR) and aldo-keto reductases (AKRs) as valuable drug targets. Overexpression of these proteins has been reported in breast, prostate and other cancers, responsible for many deaths among men and women worldwide. Additionally, resistance to cytotoxic drugs and other chemotherapeutics can be overcome by inhibiting the AKR1C3 enzyme. The aim of this study is evaluation of the biological activity of *N*(2)-substituted steroid D-ring-fused triazole derivatives using *in vitro* methods. We evaluated relative binding affinities of this series of steroid derivatives for the ligand-binding domains (LBDs) of ERβ, AR and GR using a yeast-based fluorescent biosensor and their inhibition potential against human recombinant AKR1C3 and AKR1C4 by fluorescence spectroscopy. Among tested compounds, one triazole showed higher inhibition potential against AKR1C3 than the known AKR1C3 inhibitor, ibuprofen, with an IC50 ~25 μM and specificity for AKR1C3 over AKR1C4, as well as high binding affinity for ERβ-LBD. Furthermore, several compounds were shown to be potent GR ligands. None of the tested compounds showed affinity for AR-LBD. Biologically active and highly selective steroid derivatives identified in this study could serve as promising scaffolds for the development of potential anticancer drugs or adjuvants to restore chemotherapy sensitivity of cancer cells, however further studies are needed.

P-27-004
Combinatory control of HIF-1α by ERK1/2 and CK1δ facilitates its symmetrical distribution during mitosis

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Hypoxia inducible factor-1 (HIF-1) governs oxygen homeostasis and enables cancer cells to adapt and survive under hypoxia. Regulation of its oxygen-labile HIF-1α subunit involves post-translational modifications, such as phosphorylation, that control its function. We have previously identified that phosphorylation of the HIF-1α C-terminus by ERK1/2 promotes HIF-1α nuclear accumulation and activity, while absence of this modification causes HIF-1α nuclear export and its localization on mitochondria. On the other hand, modification at its N-terminal domain by CK1δ impairs HIF-1 activity by hindering the formation of a functional HIF-1α/ARNT complex. To elucidate the importance of these two competing HIF-1α modifications, we applied a CRISPR/Cas9 approach to generate a HeLa *HIF1A*$^{-/-}$ cell line and introduced double phospho-site mutants of HIF-1α. Their expression under hypoxia revealed independent and additive phosphorylation outcomes that create a gradient of HIF-1α activity. Furthermore, CK1δ-mediated phosphorylation of HIF-1α triggered the mitochondrial release of non-nuclear HIF-1α, its interaction with tubulin and its, consequent, binding to microtubules especially during mitosis. Furthermore, mimicking CK1δ-mediated modification of HIF-1α or sustained CK1δ expression was essential for equal partitioning of HIF-1α to the daughter cell nuclei at the end of mitosis. Overall, our results suggest that modification by CK1δ stimulates the association of non-nuclear HIF-1α with microtubules, which may serve as means to establish the symmetric allocation of HIF-1α during cell division under hypoxia.

P-27-005
Targeting the unfolded protein response for breast and pancreatic cancer therapy

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Breast (BC) and pancreatic cancer (PC) remain difficult adversaries in the landscape of oncology, characterised by their aggressive behaviour, high metastatic potential, and limited treatment efficacy. These cancer cells are continuously challenged by limited oxygen, nutrient supply, and elevated protein synthesis that may cause induction of the UPR, a complex signalling network

activated by ER stress to restore cellular homeostasis. Accumulating evidence highlights the vital role of the UPR as a critical process that enables tumour cells to sustain malignancy. On the other hand, prolonged pharmacological activation of the UPR has been demonstrated to induce cell death in cancer. Considering this, we decided to use for our experiments, FR054, a competitive inhibitor of PGM3 enzyme. By inhibiting the hexosamine biosynthetic pathway (HBP), FR054 causes a decrease in the N-GlcNAc protein level and, consequently, an accumulation of misfolded proteins into the ER. This stress condition triggers a prolonged activation of UPR that induces ROS accumulation and cell death in BC cells. Otherwise, as demonstrated by the non-complete restoration after NAC co-treatment, in PC cells this ROS-dependent cell death mechanism is somewhat inhibited. Our transcriptional data suggests a potential key player in this inhibition: the xCT/SLC7A11 antiporter. This antiporter is responsible for mediating the uptake of extracellular cystine in exchange for glutamate participating in glutathione biosynthesis. To confirm this, we observed how the inhibition of xCT/SLC7A11 by erastin, a recognized ferroptosis inducer, significantly enhanced the FR054 effect causing a noteworthy increase in cancer cell proliferation arrest and death. In conclusion, HBP inhibition triggers UPR in both breast and pancreatic cancer cells. Notably, pancreatic cancer cells exhibit a protective mechanism, offering a potential avenue for therapeutic exploitation through a synthetic lethality approach in cancer therapy.

P-27-006
Boron functionalized probes can enrich specifically in glioblastoma and malignant pleural mesothelioma, an innovative method for boron neutron capture therapy

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Neutron capture therapy (NCT) is a new kind of emerging treatment modality aimed at improving the therapeutic ratio for traditionally difficult-to-treat tumors. NCT is a targeted treatment method for malignant cells, which involves using neutrons with specific energy levels to interact with an agent that has a high cross-section, like boron (BNCT) or gadolinium (GdNCT). The central obstacle for BNCT is to achieve selective boron enrichment solely in tumor cells. To direct this challenge, we decided to employ ALDH1A3, an enzyme that is overexpressed only in tumor cells and not in healthy tissue. This enzyme is a member of the aldehydes dehydrogenases, a class of enzymes that catalyze the oxidation of aldehydes into their corresponding carboxylic acids, utilizing NAD(P)+ as a cofactor. This reaction is essential for the good functioning of the cell, as it prevents the accumulation of damaging aldehydes, which can lead to oxidative stress, DNA damage, and apoptosis. We demonstrated that the ALDH1A3 is associated with poor prognosis in various types of cancers, including glioblastoma, and malignant pleural mesothelioma (MPM), in particular, these isoforms have been implied in advancing cancer cell survival, invasion, and metastasis. After conducting a thorough complete biochemical and biological analysis, we have discovered a promising lead compound that can be used for the BNCT to treat MPM and glioblastoma, two rare types of tumors that currently have limited treatment options. Therefore, it is of utmost importance to develop a new treatment approach for these tumors.

P-27-007
Mechanism of human DNPH1 reaction: implications for anticancer therapy

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The enzyme *Homo sapiens* 2′-deoxynucleoside 5′-monophosphate *N*-hydrolase 1 (*Hs*DNPH1) catalyses the *N*-ribosidic bond cleavage of 5-hydroxymethyl-2′-deoxyuridine 5′-monophosphate (5hmdUMP), a cytotoxic nucleotide whose erroneous incorporation into DNA restores and enhances sensitivity of *BRCA*-deficient cancers to anticancer poly(ADP-ribose) polymerase inhibitors (PARPi). DNPH1 upregulation is linked to PARPi resistance in $BRCA^{-/-}$ cancer cells, thus the enzyme is a promising inhibition target both to potentiate PARPi action and to resensitise cancer cells resistant to PARPi therapy as a result of 5hmdUMP depletion. This work reports the first one-pot, room temperature protocol for biocatalytic synthesis of 5hmdUMP from 5-hydroxymethyl uracil nucleobase, and use of this physiological substrate for steady-state and pre-steady state kinetic analysis of the *Hs*DNPH1 reaction mechanism. Isolation of the first chemical step of catalysis points to an anionic nucleobase intermediate along the reaction profile. Mutation of catalytic triad residues Tyr24 to Phe or Asp80 to Ala reduces k_{cat} 600- or 300-fold respectively relative to wild-type enzyme. Mutageneses of conserved active site residues are combined with protein NMR and pH rate profile analyses to show how a complex network of hydrogen bonds function to influence both the orientation and pK_as of ionisable groups relevant for catalysis. Kinetic analysis is supported by crystallographic data, laying the foundation for inhibitor design against *Hs*DNPH1.

P-27-008
Adenosine pathway as a potential therapeutic strategy for the treatment of chondrosarcoma: *in vitro* and *in vivo* validation

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Chondrosarcomas are rare bone tumors characterized by the production of a cartilage matrix and which are resistant to conventional radio- and chimio-therapy. Treatment consists of removal of the tumor and a rim (margin) of healthy tissue around it by surgery. In some cases, amputation might be needed. Also, it is necessary to identify new treatments. Previous results in the lab have shown a great antitumoral effect of 3-deazaneplanocin A (DZNep) in chondrosarcomas. Unfortunately, DZNep has never been tested in clinical trials. That's why we searched for other molecules which are already used in humans and may function as DZNep. The effects of five candidates molecules which were identified for their structural similarity with DZNep were investigated. We used four different human chondrosarcoma lines (SW1353, CH2879, JJ012, FS090) to mimic tumor heterogeneity. The effects of adenosine analogues on cell viability and proliferation were assayed in chondrocytes (non-

tumoral cartilage cells). Apoptosis was evaluated by PI/annexinV staining. To better mimic the tumoral microenvironment, experiments were also performed in 3D cultures (alginate beads). Finally, the antitumoral effect of the best analogues was assessed through measurements of tumor volume of JJ012 xenografts in *nude* mice. *In vitro*, only three of analogues that we tested were able to reduce cell proliferation in chondrosarcomas but not in chondrocytes, and to induce apoptosis in all tested chondrosarcoma cell lines. Furthermore, *in vivo* experiments demonstrated that two of them (cladribine and clofarabine) are able to reduce tumor growth. In conclusion, we identified two analogues of adenosine which are able to induce apoptosis in chondrosarcomas and to reduce tumor growth *in vivo*. These drugs, which are already used to treat other tumors in humans, will deserve to be included in clinical trials to treat chondrosarcomas, in drug repositioning strategy.

P-27-009
Modulation of gemcitabine efficacy in pancreatic cancer cells by oxysterols

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Oxysterols, derivatives of cholesterol, have been implicated in various pathological conditions, including cancer. While previous research has predominantly focused on breast carcinoma, our study explores the impact of nine specific oxysterols on pancreatic cancer cells *in vitro*. We utilized two human pancreatic cell lines, Paca-44 and BxPC-3 differing in *KRAS* mutation status, to examine the effects of oxysterols on cell viability and response to gemcitabine. We revealed that some oxysterols have similar impact in both cell lines, while others like 25-hydroxycholesterol, 27-hydroxycholesterol, and 5α,6α-epoxycholesterol demonstrate distinct effects. Interestingly, 4β-hydroxycholesterol showed no effect in both cell lines. Furthermore, we calculated the combination index of selected oxysterols with gemcitabine in the BxPC-3 cell line, providing insights into potential therapeutic strategies. As a result, we found a synergistic effect, e.g. for 7α-hydroxycholesterol or 5β,6β-epoxycholesterol while 7-ketocholesterol or cholestane-3β,5α,6β-triol exerted an antagonistic effect. These findings contribute to a deeper understanding of oxysterol roles in pancreatic cancer, offering a new perspective, compared to already established knowledge from published breast carcinoma studies. This study was supported by the Grant Agency of Charles University project no. GAUK 164323, the Czech Health Research Council grant no. NU21-07-00247, and the National Institute for Cancer Research (NICR), project no. LX22NPO5102.

P-27-010
The stress-responsive p53-RFX7 signaling pathway regulates tumor suppressors and mTORC1

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The understudied tumor suppressor RFX7 belongs to a family of eight transcription factors that share a highly conserved DNA-binding domain through which they can bind to *cis*-regulatory X-box motifs. Recently, RFX7 has emerged as a tumor suppressor that is recurrently mutated in lymphoid cancers and may play a role in neurological and metabolic disorders. We found that RFX7 can be activated by the well-known tumor suppressor p53. Integrative analysis of the RFX7 DNA binding landscape and the RFX7-regulated transcriptome revealed that RFX7 directly controls target genes that include established tumor suppressor genes as well as metabolic and neuronal regulators. Intriguingly, RFX7 target gene expression is dysregulated in numerous cancer types beyond hematological cancers, suggesting that RFX7 signaling is often silenced by means other than RFX7 gene mutations. Stratification of patients of in TCGA pan-cancer cohort showed that patients with higher expression of RFX7 target genes had a better prognosis. When we followed up on the RFX7 target DDIT4, we uncovered a p53-RFX7-DDIT4 signaling axis that inhibits the pro-survival kinase AKT. In addition, we found that RFX7 inhibits the energy sensor and growth regulator mTORC1 both downstream and independently of p53. In a search for RFX7 co-factors, we employed mass spectrometry analyses and found that an X-box motif in the promoter of the tumor suppressor gene PDCD4 is occupied by RFX7, ANKRA2, RFXANK, RFXAP, and RFX5. Transcriptome analyses revealed that ANKRA2 – another p53 target– is required for the regulation of direct RFX7 target genes in response to p53. RFX5 and the ANKRA2 paralog RFXANK had little effect on RFX7-dependent signaling and regulated largely distinct gene sets. Collectively, our work establishes the role of RFX7 as a ubiquitous regulator of cell growth and fate determination that together with ANKRA2 functions as a key node in the p53 transcriptional program.

P-27-011
VEGFR2R1051Q and FGFR1D647N point mutations elicits pro-oncogenic effects and higher sensitivity to specific TK inhibitors.

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Tyrosine kinase receptors (RTK) are frequently altered both in expression and activity in cancer. We performed a pan-cancer analysis, by exploiting MutationAligner and LowMACA bioinformatics resources, to identify novel putative cancer drivers and/or therapeutically actionable mutations of the kinase domain of different RTKs. We highlighted novel uncharacterized mutations in position 256 of the resulting consensus sequence. These alterations are located in the A-loop of TKD of FGFR1-4, FLT3, FLT4, PDGFRA, EGFR, VEGFR2 receptors, possibly leading

to constitutive activation of the RTKs. In order to assess if similar alterations in the tyrosine kinase domain modulate similar biological responses and druggability, we introduced the R1051Q correspondent substitution in FGFR1 (FGFR1 D647N) and we tested both mutant receptors for their biological activity. The results confirm great phosphorylation for both mutant receptors, tested through western blot although the ATP affinity (assessed using an ADP-glo kinase assay) is in the same range for both mutant and WT receptors. When it was evaluated the activity of tyrosine kinase inhibitors (TKi) including Erdafitinib for FGFR1 D647N mutant; Lenvatinib, Sunitinib and Linifanib for VEGFR2 R1051Q, both mutated receptors exhibit higher sensitivity to TKis. Supporting this data, preliminary molecular dynamic simulations of WT and R1051Q VEGFR2 was performed, highlighting a favorable conformation of the mutant receptor in the presence of the inhibitor linifanib, which stabilizes its binding, consequently increasing the sensitivity of the receptor to the drug. Our data confirm our previous hypotheses concerning the biological effect of these mutations. Future studies will allow us to extend this knowledge to other significant mutations in the same hotspot position. Indeed, PD-based analyses have the potential to accelerate the choice of patient-specific targeted drugs.

P-27-012
Evaluation of novel azomethine compound in genes involved in cell cycle and apoptosis in osteosarcoma cells

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Schiff bases containing azomethine group exhibit various activities including antifungal, antibacterial, antiviral and anticancer. The aim of our study was to investigate the activity of the azomethine group containing compound on osteosarcoma cell line (SAOS-2) in terms of anticancer activity as well as its effect on gene expression profiles involved in cell cycle and apoptosis. In this study, the newly synthesised compound B-134-0 was applied to the SAOS-2 cell line at eight different concentrations (100–0.5 μg/ml) for 24, 48 and 72 h. The anticancer effects of the compound were then determined using the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) method. The IC_{50} values of the compound in 24, 48 and 72 h of incubation were found to be 71.58 μg/ml, 54.36 μg/ml and 12.59 μg/ml, respectively. Statistical analysis showed that B-134-0 compound gave significant results on SAOS-2 cell line after 24, 48 and 72 h of incubation ($p < 0.05$). A significant correlation was found when comparing B-134-0 between 24 and 72 h incubation ($p < 0.0001$) and also between 48 and 72 h incubation ($p = 0.0001$). When the incubation times for the B-134-0 compound on the SAOS-2 cell line were examined, it was found that the highest activity was observed at 72 h. The $\Delta\Delta C_T$ method was used to analyse the expression levels of genes related to the cell cycle and apoptosis pathway in an RT-PCR device. In addition, String v11 was used to determine the interactions of the proteins involved in this pathway with each other and with other proteins. When the expression results were evaluated, it was observed that B-134-0 compound exhibited a significant decrease in the expression of *Tp53*, *RAD51*, *BRCA2*, *CASP2*, *MYC*, *MDM2*, *CDKN1A*, *ERCC1*, *ATR* and *PRKDC* genes in SAOS-2 cell line compared to the control group. In conclusion, the above compound containing the newly synthesised azomethine group has promising activity in the treatment of osteosarcoma cancer.

P-27-013
Pancreatic cancer cells adapt to compressive mechanical stress through regulation of PI3K–MAPK interdynamics

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Pancreatic ductal adenocarcinoma (PDAC), a significant challenge in cancer management, is anticipated to rank second in cancer-related mortality by 2030 in part due to its pronounced fibrosis complicating treatment. In this study, KPC (LSL-KrasG12D/+; LSL-Trp53R172H/+; Pdx-1-Cre) genetically engineered mouse model was utilized to replicate critical genetic mutations observed in PDAC. Cutting-edge techniques, including unbiased kinome activity analysis via PamGene technology, were applied to investigate cellular responses to mechanical compression in both *in vitro* and *in vivo* contexts. Firstly, shear wave elastography unveiled rigidity heterogeneity in KPC tumors, with high-rigidity subregions displaying elevated YAP protein levels and increased expression of genes associated with mechanical stress adaptation, including Rac1, Fn1, and Ctgf1. *In vitro* experiments revealed a dynamic response of primary and metastatic cancer cells to compressive stress, activating both MAPK and PI3K signaling. The study delved into MAPK and PI3K pathways, discovering activation in both upstream and downstream components. Mechanical stretching of KPC cell lines exhibited distinct dynamics compared to compressive stress. Compressive stress induced YAP/TAZ nuclear translocation, associated with changes in nuclear area, a response mitigated by MEK inhibitors but not PI3K inhibitors. Mechanical compression led to increased Fn1 and Ctgf mRNA levels, downregulated genes linked to PDAC oncogenicity (Lemd1, Gpm6b), and immunostaining of FN1 showed increase in signal intensity. This highlighted the contribution of PDAC cells to ECM remodeling, dependent on mechanical cues. Ongoing work aims to conclude Pamgene experiments, providing a comprehensive understanding of dynamic PI3K and MAPK signaling patterns in response to mechanical compressive stress. These findings bear therapeutic implications, aligning with the broader goal of integrating the mechanical context to enhance PDAC treatment outcomes.

P-27-014
The Abcc6 knockdown alters the adhesion dynamics and aggressiveness of human hepatatoma HepG2 cells

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The human Abcc6 gene belongs to the multidrug resistance-associated protein (MRP) subfamily of ATP-binding cassette (ABC) transmembrane transporters and is mostly expressed in the liver and kidney. The Abcc6 gene is primarily known for its correlation with Pseudoxanthoma Elasticum (PXE), a rare disorder characterized by ectopic calcification of elastic fibers in dermal, ocular and vascular tissues. However, recent studies have examined the potential association between ABCC6 and liver cancer. A combination of ABCC6 and the ectonucleosidases CD73 (ecto-5′-nucleotidase) and CD39 (ectonucleoside triphosphate diphosphohydrolase-1) produces purine nucleosides, as well as inorganic pyrophosphate, a mineralization inhibitor. Therefore, ABCC6 plays an important role in both PXE and Purinergic Signalling [Bisaccia F et al. (2021) Int J Mol Sci 2858]. ABCC6 is involved in the release of extracellular ATP. The amount of ATP dependent on ABCC6 transport activity modifies the cytoskeleton and cell motility of hepatoma cells (HepG2) [Ostuni A et al. (2020) Cells 1410]. RNA-seq results show that Abcc6 knockdown increases HepG2 cell adhesion to extracellular matrix (ECM)-mimicking hydrogels through the up-regulation of ITGA2 and ITGA6. The up-regulation of the epithelial marker E-cadherin and the down-regulation of mesenchymal markers Vimentin and N-cadherin suggests that Abcc6 knockdown cells have a less aggressive phenotype. Transwell invasion assays demonstrate that Abcc6 knockdown reduces the ability of HepG2 cells to infiltrate the ECM, thus reducing the secretion of MMP2 and MMP9 active forms [Matera I et al. (2023) Int J Mol Sci 16391]. The addition of adenosine and ATP restores invasion of tumor cells, indicating that ABCC6 affects tumor aggressiveness through the extracellular purinergic pathway. Abcc6 knockdown in HepG2 cells appears to induce a less aggressive phenotype, which may have therapeutic benefits for cancer treatment.

P-27-015
Exosome RNA signature associated with tumorigenic effects in esophageal squamous cell carcinoma

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Esophageal squamous cell carcinoma (ESCC) is one of the most aggressive cancer types worldwide with high morbidity and mortality. There is still a lack of effective treatment strategies for ESCC. Tumor-derived exosomes (TEXs) are nano-sized vesicles associated with cancer progression and immune tolerance (1).The establishment of exosomes' signature and cargo is vital to determine their potential therapeutic effects in distinct malignancies. To obtain molecular mechanistic insight into TEX-RNA profile in ESCC, comprehensive *in silico* analyses were performed. GSE104926 dataset was analyzed using GREIN platform. This dataset contained exosomal RNA-Seq data from ESCC patients and subjects with normal esophaguses. Accordingly, a total of 560 genes were significantly differentially expressed (adjusted p-value <0.01, log fold changes >2 applied).These genes were uploaded to iLINCS for further investigation. For pathway analyses, KEGG and David tools were utilized. By using GEPIA2, gene expression profiles were examined in ESCC and control samples from TCGA and GTEx databanks. By analyzing exosomal RNA-Seq data, we found 560 differentially expressed genes between cancer and control groups. KEGG pathway analyses revealed differentially expressed exosome signatures related to cancer pathways. Among these, the angiotensinogen (AGT) gene was significantly upregulated in ESCC group compared to control samples in all analyses. This finding was consistent with the data obtained from TCGA and GTEx datasets. Although the angiotensin system inhibitors are reported to increase survival in some malignancies, there is no publication addressing the potential role of AGT in exosomes. Owing to exosomes' regulatory roles in cellular processes and communication, the inhibition of exosomal AGT could be further investigated as one of the promising strategies in tumor therapy for aggressive malignancies including ESCC. Reference: 1. Tang et al. (2022) Cancer Letters. 548, 215823.

P-27-016
Antitumoral activity of the universal methyl donor S-adenosylmethionine in glioblastoma multiforme

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Glioblastoma multiforme (GBM) is the most frequent and highly invasive primary brain tumor, characterized by short survival times and high mortality rates. Despite aggressive multimodal treatments, the prognosis for GBM patients continues to be limited, due to the resistance of GBM cells to conventional therapeutic treatments. Recent findings have documented that some natural compounds, thanks to their multi-targeted mode of action increase the effectiveness of standard chemotherapy by overcoming drug resistance and by reducing toxicity and side effects. Among them, S-adenosyl-L-methionine (AdoMet), a naturally-occurring sulfur nucleoside widely known as the main biological methyl donor in transmethylation reactions has emerged in recent years as a promising chemosensitizing agent able to overcome drug resistance in many kinds of human cancers [1]. In the current study we reported the anticancer effects of AdoMet on human U87MG, U343MG, and U251MG GBM lines and on patient-derived GBM cells and we explored the underlying mechanisms. We found that AdoMet selectively

caused a time- and dosedependent inhibition of GBM cell viability without exerting any detectable cytotoxicity in non-tumorigenic human astrocytes and induced G2/M cell cycle arrest and apoptosis. We provided the first evidence that AdoMet downmodulated the expression and activation of proteins involved in homologous recombination DNA repair thus keeping DNA in a damaged state, as indicated by the increased γH2AX/H2AX ratio. We also demonstrated that AdoMet inhibited the expression and activation of Aurora B kinase resulting in impairment of its subcellular localization and normal spindle microtubules assembly and consequently causing GBM cells to undergo mitotic catastrophe-induced death. All together these findings suggest that AdoMet may be a good candidate for the development of novel adjuvant therapies to improve GBM treatment and patient outcomes. 1. Reference: Mosca L et al. (2020) Int J Mol Sci. 21, 85478561.

P-27-017
Effect of neuropilin-1 on radiosensitivity of human glioblastoma T98G cell line

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Radiotherapy is one of the main treatments for tumors along with surgery and chemotherapy. However, challenges still persist in effectively treating radioresistant tumors and addressing tumor repopulation post-radiotherapy. Our previous studies revealed heightened neuropilin-1 expression (NRP-1) gene in surviving tumor cells after X-ray irradiation [1, 2]. In the present study, we investigated the role of NRP-1 in the highly radioresistant glioblastoma cell line T98G cells (p53 mutant, p16 deficient). Suppression of NRP-1 gene expression by small interfering RNA significantly increased radiosensitivity to 2 Gy X-rays, but there was no effect on the expression of caspase-3 and p21. The results of flow cytometric apoptosis analysis using Annexin V and western blotting also showed no significant induction of apoptosis in NRP-1-suppressed cells. However, the expression of the p21 gene, which is involved in cell cycle regulation, increased in NRP-1-suppressed cells. p21 is involved in cellular senescence, autophagic cell death, and many other intracellular signal transductions. These results suggest that the increased radiosensitivity of NRP-1-suppressed cells may be involved in other cell death pathways other than apoptosis mediated by p21. Further studies are needed to validate the detailed mechanism and association of NRP-1 on the p21 pathway. We hope this study will lead to the improvement of radiotherapy efficacy in radioresistant glioblastoma. References: 1. Tsutsumi et al. (2006) Cell Structure and Function 31, 47–52. 2. Tsutsumi et al. (2021) Current Issues in Molecular Biology 43(3), 1203–1211. This work was supported by the Japan Society for the Promotion of Science (Project number: 23K07126).

P-27-018
Recognition and processing of 8-oxo-guanosine in DNA and RNA by AUF1 and APE1 proteins with an impact on cancer biology

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The incorporation of oxidized ribonucleotides (rNMPs) in genomic DNA, e.g. 8-oxo-guanosine (8-oxo-G), following oxidative stress exposition, has recently emerged in several works, including ours, for its propensity to destabilize the DNA backbone, thus altering DNA replication and transcription and potentially inducing the onset of cancer. Oxidation is also relevant to RNA, especially in tumoral microRNAs, whereby it acts through an epitranscriptional regulation of their functions, thus impacting malignancy progression. Currently, nothing is known about the enzymes are deputed to repairing these types of DNA and RNA lesions. Our recently published data are in support of the role of the apurinic-apyrimidinic endodeoxyribonuclease 1 (APE1), a key enzyme of the base excision repair pathway, for its ability to bind 8-oxo-G in DNA, although showing a weak incision repair activity. To guarantee an efficient repair activity, it has been hypothesized that additional processing mechanisms should be active in cells. We discovered that the AU-rich element RNA-binding protein 1 (AUF1) recognizes 8-oxo-G in DNA, stimulating the APE1 enzymatic activity on it. Contextually, we found that APE1 and AUF1 physically interact, and their interaction is modulated by oxidative stress in human cancer cells. Moreover, a depletion of APE1 and AUF1 causes an accumulation of single- and double-strand breaks, and both proteins are involved in modulating the formation of DNA:RNA hybrids of which 8-oxo-G embedded in DNA is a part. Finally, preliminary *in vitro* data have shown that both AUF1 and APE1 proteins are also able to bind 8-oxo-G on the tumoral pri-miR-221 and ongoing experiments aim to demonstrate if this binding can impact RNA cleavage and cleansing. All these results establish unexpected functions of AUF1 and APE1 in modulating genome stability concerning 8-oxo-G embedded in DNA and RNA molecules. Previously published in Malfatti MC et al. (2023) Antioxid Redox Signal 39, 411–431.

P-27-019
Mutant-p53 induces ferroptosis resistance in pancreatic ductal adenocarcinoma (PDAC)

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Pancreatic ductal adenocarcinoma (PDAC) is the most common type of pancreatic cancer. PDAC is highly lethal and projected to be the second cause of cancer death in Western societies within a decade. Therefore, new therapeutic targets to fight this

cancer are required. Ferroptosis is an iron-dependent cell death with great therapeutic value in oncology and, interestingly, it is known that the tumor suppressor protein p53 is closely associated with ferroptosis. Notably, missense mutations of the TP53 tumor suppressor gene are frequent in PDAC patients (~70%), thus generating mutant p53 isoforms with new gain-of-function (GOF) properties. We first focused on the human PDAC cell line Panc-1, which is characterized by the p53-GOF mutation R273H. We generated CRISPR-Cas9 mut-p53 Knock-Out (KO) cells and subsequent cell viability assays showed, for the first time, that Panc-1 cells harbouring mutp53 are more resistant to the effect of different ferroptosis inducers (Erastin, Ferroptocide and RSL3) compared to their p53-KO counterparts, highlighting a novel role of mutp53 in inducing anti-ferroptosis. This effect is associated to a time-dependent accumulation of reactive oxygen species (ROS). Then, we transfected p53-null human PDAC cell line AsPC-1 with 6 different isoforms of mutp53 (R273H, R282W, R248H, R248Q, R175H and Y220C) frequently detected in PDAC patients. We found that AsPC-1 transfected with different mutp53 isoforms were more resistant than mock-transfected cells towards three different inducers of ferroptosis, thus supporting the role of mut-p53 in ferroptosis resistance and suggesting that the mutp53-dependent ferroptosis resistance is not associated to a specific mutp53 isoform. Taken together, these data strongly support the hypothesis that mut-p53 has a pivotal role in ferroptosis regulation, thus paving the way to identify novel ferroptosis-regulators network and novel biomarkers for ferroptosis, capable to predict PDAC patient's response to therapies.

P-27-020
Heavy metals and carcinogenic risk assessment in free-ranged livestock of lead-contaminated goldmine communities of Zamfara State, Northern Nigeria

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The consumption of meat is of great importance as it provides a good source of proteins and significant amount of essential trace element to the body. However, contamination of meat and meat products with heavy metals is becoming a serious threat to food safety and human health. Therefore, the present study is aimed to evaluate the concentration of some heavy metals in muscles and entrails of free-ranged cattle, sheep and goats. A total of sixty (60) fresh samples of muscles, liver, kidney, small intestines and stomach of free ranged cattle, sheep and goats were collected from abattoirs of different goldmine communities of Anka, Bukkuyum, Maru and Talata-Mafara Local Government Areas of Zamfara State, Nigeria. The samples were digested using 10 ml of a mixed 70% high grade concentration of HNO_3 and 65% HCl (4:1 v/v); the mixture was heated until dense fumes disappeared forming a clear transparent solution and diluted to 50 ml with deionized water. The concentrations of Cd, Cr, Cu, Co, As, Ni, Mn, Pb and Zn were determined using Microwave Plasma Atomic Emission Spectrophotometer (MP-AES). Results obtained, shows that, goat liver had the highest level of lead, arsenic, cobalt and manganese (12.43 $\pm$ 0.31, 14.25 $\pm$ 0.32, 3.47 $\pm$ 0.86 and 12.68 $\pm$ 0.92 mg/kg respectively) while goat kidney had the highest concentration of copper and zinc (10.08 $\pm$ 0.61 and 24.16 $\pm$ 1.30 mg/kg respectively). The highest concentrations of cadmium and nickel were recorded in sheep kidney (7.75 $\pm$ 0.65 and 2.08 $\pm$ 0.10 mg/kg respectively). Cattle muscles had the highest chromium concentration than all the organs analysed. The target hazard quotients (THQs) for all the metals were below 1.0, but target risk (TR) which is a risk indices for carcinogenicity indicates an alarming result that requires stringent control to protect public health. Therefore, intensive public health awareness on the risk associated with heavy metals meat contamination should be advocated. *The authors marked with an asterisk equally contributed to the work.

P-27-021
Analysis of the expression of the exosomal gelatinases MMP-2 and MMP-9 in prostate cancer progression

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Exosomes, small vesicles 30-200 nm in diameter, have a considerable number of biomolecules inside them that are involved in intercellular communication. They are capable of transferring material promoting the transformation and proliferation of cancer cells. In turn, matrix metalloproteases (MMP-2 and MMP-9) are directly involved in the processes of angiogenesis and metastasis in prostate cancer. In the present study, exosomes from prostate epithelial cells (RWPE-1) and from hormone-dependent (LNCaP) and hormone-refractory (PC3) prostate cancer cells were characterised. In addition, analysis of the expression of both exosomal gelatinases was also addressed. The vesicle characterisation study revealed that the expression of the exosome-specific membrane glycoproteins CD9 and CD63 was significantly increased in hormone-dependent cells (LNCaP) compared to that obtained in non-tumour cells (RWPE-1) and androgen-independent prostate cancer cells (PC3). This could be due to an increase in the number of exosomes released in the early stages of the disease. In relation to the study of the expression of exosomal metalloproteases 2 and 9, there was a linear increase in their levels correlated with the increase in aggressiveness of prostate cancer. Taken together, these results support the existence of important molecular changes as prostate cancer progresses. Thus, in early stages, a greater release of exosomes is evident in order to establish the corresponding cell transformation and proliferation. And in more advanced stages, the action of exosomal MMP-2 and MMP-9, which facilitate processes such as angiogenesis and metastasis, is favoured. Research Group: Cancers of epithelial origin Funding: [1]Instituto de Salud Carlos III (ISCIII) "P118/00526" cofunded by European Regional Development Fund (ERDF), "A way to make Europe"; [2]Universidad de Alcala, Vicerrectorado de Investigación y Transferencia-Financiación Puente a Grupos Consolidados "UAH-GP2022-1", both to A.M.B.

P-27-022
Raman-SERS spectroscopy analysis of exosomes from hormone-dependent (LNCaP) and hormone-refractory (PC3) human prostate cancer cell lines

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Prostate cancer (PCa) is the second most common cancer in men and the fifth leading cause of cancer death in men. PCa is a multifactorial disease that can present in a slow-growing (indolent) or aggressive form. Prostate-specific antigen (PSA) has been used in recent decades as a biomarker and diagnostic tool for PCa. However, PSA is a poorly specific screening method, as the parameter can be altered by other factors, such as benign prostatic hyperplasia or prostatitis, leading to a large number of false positives and unnecessary biopsies. All this makes it necessary to search for new biomarkers for the development of more sensitive and non-invasive diagnostic techniques. Lately, exosomes have acquired great relevance due to the role they play in cellular communication involved in pathological processes. Specifically in PCa, exosomes can promote androgen independence and metastasis. In the present study, exosomes from culture media of hormone-dependent (LNCaP) and hormone-refractory (PC3) prostate cancer cell lines were isolated and analyzed by Raman-SERS spectroscopy to establish whether there are molecular differences between exosomes from both stages of the disease. Raman-SERS analysis of isolated exosomes from CaP cell lines reveals measurable molecular differences between hormone-dependent (LNCaP) and hormone-refractory (PC3) human CaP cell lines. Likewise, the comparison of the results obtained by Raman-SERS between lysed and unlysed exosomes from PC3 or LNCaP reveals the existence of differentiating molecular factors between the outer and inner components of the exosomes. Our results support the analysis of exosomes as potential biomarkers of disease progression to a more aggressive stage. Funding: 1Instituto de Salud Carlos III (ISCIII) "P118/00526" cofunded by European Regional Development Fund (ERDF), "A way to make Europe"; 2 Universidad de Alcala, "UAH-GP2022-1", both to A.M.B.

P-27-023
AKR1C3 as a therapeutic target in the treatment of aggressive tumors, with poor prognosis and/or resistant to traditional therapies

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Aldoketo reductase 1C3 (AKR1C3) is a metabolic enzyme that performs oxidoreduction reactions on a variety of substrates, such as endogenous steroids, prostaglandins and exogenous compounds. It is mainly expressed in prostatic tissue, but more generally it shows endocrine organ expression in adrenals, breast and uterus too. Its principal function consists in regulating the steroids' availability for the respective receptors. From a pathological perspective, AKR1C3 overexpression is involved in the development of several malignant diseases, e.g. breast cancer, castration resistant prostate cancer (CRPC), endometrial cancer, acute myeloid leukemia (AML), some of whom have currently poor prognosis. It is also involved in mechanisms of some anticancer drugs resistance, as anthracyclines and nitrogen mustards. Since its key role in resistance occurrence for different aggressive tumors with poor prognosis, AKR1C3 is here studied on one hand as a target of inhibitors that could be co-administered with drugs currently used in therapy, towards which resistance arises (e.g. abiraterone, a CYP17A1 inhibitor, or enzalutamide, an androgen receptor (AR) inhibitor, both used to treat CRPC), and on the other hand as a target of dual inhibitors that could bond both AKR1C3 and other target, such as CYP17A1 or AR or DHODH (dihydroorotate dehydrogenase, targeted to treat AML). Starting from our potent and selective AKR1C3 inhibitors previously developed [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] and from the pharmacophore scaffold of either CYP, AR or DHODH, new compounds were synthetized and tested for their ability to inhibit AKR1C3 as well as the other targets. *In vitro* enzymatic assays on purified proteins and cell-based assays were performed. The results obtained highlight the potential usefulness of targeting AKR1C3 and either CYP, AR or DHODH to treat AKR1C3-overexpressing cancers.

P-27-024
Decoding TRPM7 activity in breast cancer models: investigate how TRMP7 channel is involved in breast cancer modulation and pharmaceutical response

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Transient receptor potential melastatin-related 7 (TRPM7) is a divalent cation characterized by an ion channel function and a serine-threonine kinase activity. It is a critical ion channel that plays an important role in calcium and magnesium homeostasis [1]. Over the past two decades, the correlation between TRPM7 and cell proliferation, survival and thus growth and progression of various types of tumor cells, including breast cancer, has been highlighted [2,3]. The initial goal of this project is to study TRPM7 channel activity in several breast cancer cell lines (SKBR3, MCF-7, MDA-MB-231) and subsequently evaluate changes in TRPM7 during pharmaceutical treatment. qPCR and immunofluorescence analysis demonstrated the presence of TRPM7 in all cell lines analyzed. Fluorescent Ca^{2+} dyes, such as Fura-2, were used to evaluate calcium signaling in the breast cancer cell line. In addition, to investigate the actual role of TRPM7

in calcium homeostasis, some experiments were performed with channel blockers such as 2-APB Overall, the data suggest that TRPM7 may be involved in the pathophysiology of mammary adenocarcinoma cells. Further experiments are underway to understand the relationship between TRPM7-mediated calcium signaling and drug resistance phenomena. References: 1. Cheng XY et al. (2022) Eur J Pharmacol 931, 175180. 2. Cordier, Clément et al. (2021) Cancers 13(24), 6322. 3. Liu, Hengrui et al. (2020) Cancers 12(1), 131.

P-27-025
Effects on mitochondrial function of a novel hClpP protease activator with potential anticancer activity

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Mitochondrial chaperones and proteases are overexpressed in most tumor types and are involved in cell metabolic reprogramming that allows evasion of apoptosis and increased tumor survival. The human caseinolytic protease XP (hClpXP) is an effector of mitochondrial proteostasis. It plays an important role in the protein quality control; moreover, it is upregulated in many primary and metastatic human tumors and, in some cases, it correlates with shortened patient survival. hClpXP is a soluble mitochondrial matrix protease complex, formed by the protease ClpP and the chaperone ClpX. As part of the mitochondrial unfolded protein response system (UPRmt), ClpXP canonically degrades misfolded or aggregated proteins in mammalian mitochondria, to maintain organelle function and integrity. Both hClpP inhibition and activation result in tumor cell death, so it is unknown whether inhibiting or activating hClpP is the preferred therapeutic strategy. Clinical trials showed that the imipridone ONC201 compound determined in a few cases a regression of the primary thalamic lesion in patients affected by Diffuse Intrinsic Pontine Glioma (DIPG). DIPG is a rare, aggressive tumor that affects children aged 4–7 years and is the leading cause of death for pediatric brain tumors due to its infiltrative nature and inoperability. Imipridone ONC201, by binding to the hClpP protease subunit and activating it, negatively influences oxidative phosphorylation and mitochondrial metabolism, thus exerting its cytotoxic effects in DIPG cell lines and other cancer models. Unfortunately, some patients have acquired resistance to this drug, thus raising the need to develop additional effective molecules. Using purified recombinant hClpP in an *in vitro* assay, we analyzed the hClpP-activating capacity of a novel imipridone-related compound, termed THX6; we also evaluated its effect on mitochondrial functions in cultured SU-DIPG-36 cells.

P-27-026
Development of new dual VGFR2/MTA inhibitors for treatment of drug refractory/ metastatic cancer forms

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Although most cancer cells are sensitive to antineoplastic drugs, tumors often hide small populations of inherently resistant cells that are generally responsible for tumor relapse. These cells can avoid drug-induced apoptosis through various mechanisms and continue to proliferate regardless the antitumor therapeutic protocol adopted. Furthermore, most resistant cancer cells are able to evade attacks from immune system cells and spread to healthy tissues, promoting metastatic dissemination. Activation of VEGFR2, which stimulates the formation of new intratumoral vessels, is considered an essential process for promoting metastatic dissemination. For this reason, VEGFR2 inhibitors are often used to treat metastatic forms of cancer in combination with microtubule-targeting agents (MTAs). These drugs exhibit different pharmacokinetics and can induce unsustainable side effects when administered in combination. To overcome this problem, we propose novel dual VEGFR2/microtubule inhibitors that are expected to possess similar cytotoxic activity but lower non-specific toxicity. These new molecules are engineered by linking some well-known MTAs to Axitinib, a potent and highly selective VEGFR2 inhibitor. These compounds were tested on melanoma and kidney cancer cell lines and IC50 toxicity values and cell cycle alterations were evaluated. Some compounds showed promising behaviour and, for most of them, high inhibition potential on VEGFR2 was confirmed by in vitro investigation. We decided to perform co-cultures studies by using HDF fibroblasts in presence of A375 melanoma colonies in order to check any effect on tumor microenvironment. By now, the 'MC5' compound, that showed well-preserved MTAs and VEGFR2 inhibitory behaviour, is cytotoxic on both melanoma cells and cancer associated fibroblasts (CAFs). More will be done to describe their cytotoxic behaviour and structural optimisation will be performed on the most promising molecules.

P-27-027
Preclinical progress in ruthenium-based candidate drugs as anticancer agents: focus on triple negative breast cancer and metastatic potential

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Our research team has recently developed a new ruthenium (III) complex, named AziRu, showing interesting biological properties and effective antiproliferative activity as a candidate metallo-chemotherapeutic drug. To allow further progress for prospective biomedical applications, the novel AziRu complex was proposed

as a molecular platform for the design of original nucleolipid nanoaggregates and, more recently, of new lipophilic analogues[1,2]. Based on compelling preclinical outcomes concerning the progress of our novel ruthenium-based metallotherapeutics[3,4], we are focusing research efforts on challenging indications for the treatment of invasive neoplasms such as the triple negative breast cancer (TNBC). In this context, we are exploring for the first time the biological effects of a new complex designed as a lipophilic analogue of AziRu, named PalmiPyRu. Herein, we highlight PalmiPyRu potential to act as a multimodal anticancer agent, on TNBC cell growth and proliferation as well as migration and invasion. Moving in this direction, by both advanced preclinical models and computational approaches we show the multimodal action of ruthenium-based complexes in metastatic phenotypes as well as novel prospective biomolecular druggable targets implicated in the dynamic regulation of cell death/cell survival processes. The here obtained preclinical findings let us suppose a potential targeting of the complex pathways network controlling invasive and migratory cancer phenotypes. References: 1. Ferraro et al. (2020) Cells 9(6), 1412. 2. Riccardi et al. (2022) Biomaterials Adv 139, 213016. 3. Piccolo et al. (2021) Cancers doi: 10.3390/cancers13205164. 4. Ferraro et al. (2023) Int J Mol Sci 24(7), 6473.

P-27-028
AKR1B10 and CBR1 inhibitors to improve the efficacy of anthracyclines in cancer treatment

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Resistance to chemotherapy is one of the most widespread obstacles to cancer treatment, resulting in a loss of efficacy of cytotoxic agents that consequently leads to prolonged exposure without any therapeutic benefit. Anthracyclines, mainly represented by doxorubicin and daunorubicin (DNB), are natural compounds which find application in cancer treatment; their therapeutical action can be reduced by several cytosolic detoxifying enzymes, such as the Aldo-keto reductase family 1 B 10 member (AKR1B10) and carbonyl reductase 1 (CBR1). These enzymes can reduce the C13-carbonyl group of anthracyclines, leading to the formation of alcoholic metabolites, that not only show a reduced antitumoral activity, but are also associated with strong cardiotoxicity. Consequently, the inhibition of these enzymes could increase the efficacy of anthracyclines, improving their antitumoral potential and reducing side effects. A series of thiazolidinone derivatives were tested *in vitro* for their capacity to act as inhibitors of human recombinant AKR1B10 and/or CBR1. The A549 cells, a human non-small cell lung adenocarcinoma cell line overexpressing both AKR1B10 and CBR1, were also used to evaluate the efficacy of selected compounds. Among tested compounds we identified molecules able to *in vitro* inhibit at different extent and with different selectivity the two enzymatic targets. Most promising compounds resulted also able to potentiate the cytotoxic effect of DNB in cultured A549 cells. Our results strongly support a relevant involvement of AKR1B10 and CBR1 in detoxification and DNB resistance in the A549 cells model, making the two enzymes relevant targets to reduce anthracyclines chemoresistance.

P-27-029
Proteomic profiling of PD-1 interactome by APEX-mediated proximity labeling

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The programmed cell death-1 (PD-1)-mediated signaling pathway plays a pivotal role in inhibiting anti-cancer immune response, representing an attractive therapeutic target in several cancers[1]. Within the tumor microenvironment, PD-1 engagement by its main ligand PD-L1 prevents effector anti-cancer activities of T cells, leading to tumor immune escape[2]. Anti-PD-1/PD-L1 neutralizing antibodies have revolutionized cancer immunotherapy, although only a fraction of patients develops durable anti-tumor responses[1]. The intracellular signaling pathway downstream of PD-1 is largely unknown[1]. With the aim to provide new mechanistic understanding of the PD-1/PD-L1 pathway, we are setting-up the engineered ascorbate peroxidase (APEX)-based proximity labeling technique (APEX-PLT) combined to mass spectrometry[3]. This elegant approach will allow us to identify with high spatial and temporal resolution the proximity proteome of PD-1 in living T lymphocytes, to identify new PD-1 interactors. In the first step of our project, we generated the pcDNA3.1 vectors encoding either the PD-1-flag-APEX fusion protein or the flag-APEX control protein, and we stably transfected them in Jurkat T cells. The flow cytometric analysis of cell transfectants using anti-flag antibodies demonstrated that both fusion proteins are successfully expressed in transiently transfected Jurkat T cells. Moreover, immunofluorescence analyses revealed that the PD-1-flag-APEX fusion protein localizes at the plasma membrane, as expected, while the control flag-APEX protein has a diffuse cytoplasmic localization. We are currently setting up the APEX-PLT assay in either unstimulated or stimulated Jurkat transfectants. The results will provide comprehensive information about the T-cell suppressive interactome of PD-1 and will help uncovering new potential candidates of anti-cancer therapy. References: 1. Iwai Y (2017) J Biomed Sci 24(1), 26. 2. Jiang X (2019) Mol Cancer 18(1), 10. 3. Nguyen TMT (2020) Biochemistry 59(3), 260–269.

P-27-030
The use of molecular docking and SAR analysis of synthetic compounds for classifying them into activators and inhibitors of polyamine oxidation and revealing corresponding characteristic features

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The polyamine (PA) metabolism is involved in cell proliferation and differentiation. Increased cellular PA levels are observed in

different types of cancers. Products of PA oxidation induce apoptosis in cancer cells. These observations open a perspective for enzymes of PA catabolism to serve as a target for anticancer drug design. The agents enhancing PA oxidation can be considered as potential anticancer agents. In this work, we studied the influence of new compounds (ligands) from various chemical groups on the PA oxidation. The goal of the present study was to discover how distinct interacting with the enzyme result in different types of ligand modulation of the enzyme activity and thus to classify substances of various chemical structure into activators and inhibitors of PA catabolism. Yeast polyamine oxidase (Fms1) crystalline structure was used for molecular docking of ligands to develop homology modeling of binding of the tested compounds with PA catabolic enzyme. In vitro experiments were carried out for evaluation of the action of the tested compounds upon PA oxidative deamination in a cell-free test-system from regenerating rat liver. The key amino acid residues (His67, Trp174, His191, Tyr450, Gly487) were discovered in the structure of Fms1 to be significant for binding of the tested compounds with the enzyme. 19 out of 51 screened compounds were activators and 17 were inhibitors of oxidative deamination of PA. Taken together the results of molecular docking and in vitro investigations allowed to build a recognition model with characteristic descripts depicting activators and inhibitors. The understanding of how the structure determines the binding mode of compounds with PA catabolic enzyme may help in explanation of their structure-activity relationship and thus promote structure-based drug design. This study has been supported by the RUDN University Strategic Academic Leadership Program. *The authors marked with an asterisk equally contributed to the work.

P-27-031
The impact of some nitrogen-containing, heterocyclic and cyclic compounds on cell proliferation of some tumor lines

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The algorithm for primary testing of new synthetic compounds for biochemical and biological activity has been tested. The influence of individual nitrogen-containing heterocyclic compounds on the PA metabolism was evaluated through extensive screening using models of enhanced physiological regeneration and proliferation of tumor cells, specifically human lung carcinoma SkBr3, human breast cancer A549, and cervical cancer HeLa cell lines. The investigated substances predominantly activated PA catabolism in a non-cellular test system based on regenerating liver tissue, demonstrating potential anti-tumor activity. The most active compounds in terms of amino oxidase activation were A3 and A16 (tilorone). Substance A16 significantly inhibited PA synthesis. Substance A16 also proved to be the most potent inhibitor of proliferation in the A549, SkBr-3, and HeLa tumor cell lines during cultivation. The IC50 for substance A16, after 72 h of incubation with A549, SkBr3, and HeLa tumor cells, was significant: 0.381 $\pm$ 0.078·10-6M, 0.176 $\pm$ 0.021·10-6M and 0.126 $\pm$ 0.011·10-6M, respectively. This recommends further investigation of A16 as an anti-tumor agent. Using 3D computer modeling (docking) of the binding of low-molecular-weight heterocyclic compounds of original synthesis with the enzyme polyamine oxidase, five key amino acid residues of the enzyme necessary for ligand integration were identified: Asp211, Tyr204, His191, Trp174, Gly487. The general tendency indicated that a strong interaction with Asp211 or Tyr204 was rather typical for activators. Quantitative correlations of topological indices with amino oxidase activity for the tested compounds demonstrated the importance of the Balaban index for this type of activity. This study has been supported by the RUDN University Strategic Academic Leadership Program.

P-27-032
Nicotinamide phosphoribosyltransferase (NAMPT) is over-expressed and abundantly released from malignant pleural mesothelioma becoming a potential biomarker

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Malignant pleural mesothelioma (MPM) is an aggressive and incurable cancer of the pleural surface. Chronic inflammation, oxidative stress, and persistent aberrant signaling due to asbestos exposure led to mesothelial cells transformation over years. Despite increasing studies on MPM biology, continue efforts to identify novel biomarkers and tumor vulnerabilities to be targeted are needed. Nicotinamide adenine dinucleotide (NAD) biosynthesis is essential to support tumor energetic needs, as well as to regulate NADPH-mediated detoxification system. The enzyme nicotinamide phosphoribosyltransferase (NAMPT), in addition to possess a key function in NAD generation, can be secreted in the extracellular space (eNAMPT), where it behaves as a mediator of inflammation, regulating tumor-host interactions. NAD/NAMPT axis emerges deregulated in several tumors; however, no data are available in MPM. Bioinformatics analysis on TCGA database showed that NAMPT is the main expressed NAD-biosynthetic enzymes in MPM, and its expression correlates with hallmarks gene sets related to inflammation, metabolism, and signaling. RT-PCR and western blot analysis on MPM cell lines and mesothelioma primary cells vs normal mesothelium (Met-5A, or primary mesothelial cells) confirmed that NAMPT is overexpressed in MPM. Preliminary data showed that NAD levels are similar in the comparison between tumor and normal tissue, while NADP levels are increased in MPM cell lines. Lastly, we revealed significantly increased serum eNAMPT levels from a cohort of 115 MPM patients compared to healthy donors. eNAMPT is strongly released in pleural effusions from the same MPM patients, mainly in MPM with the most aggressive sarcomatoid phenotype. Overall, these data support the hypothesis of

an impact of NAD/NAMPT axis in MPM biology and highlight a potential role of eNAMPT as biomarker with a functional activity like a damage-associated molecular pattern (DAMPs)/cytokine in MPM that will further be investigated. *The authors marked with an asterisk equally contributed to the work.

P-27-033
Study of a multi-target ruthenium (III)-based agent as ferroptosis-inducing metal complex in breast cancer cells

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Ferroptosis represents a new mode of non-apoptotic regulated cell death pathway powered by iron-dependent accumulation of reactive oxygen species (ROS) and lipid peroxidation[1]. Several ruthenium-based chemotherapeutics act with a multimodal action triggering multiple cell death pathways and are capable of causing oxidative stress at the cellular level. Moreover, knowing that ruthenium shares many physico-chemical similarities with iron in biological environment, here we report the effects of the involvement of ferroptosis and iron-dependent oxidative stress in the mechanism of action of our new Ru(III) nucleolipid complexes, that we have largely demonstrated to be very effective in counteracting growth and proliferation of human breast cancer cells (BCC), by triggering both apoptotic and autophagic pathways[2–4]. Special attention was focused on iron cellular homeostasis regulation following PalmiPyRu complex *in vitro* treatments on a panel of BCC lines. Analysis of cellular proliferation revealed our candidate drug as very effective against both ER+ and triple negative BC phenotypes, displaying micromolar IC_{50} values. The evaluation of the expression of the main iron-related proteins, i.e. ferritin and transferrin receptor, through immunoblot analysis and fluorescent confocal microscopy, suggested our Ru-drug as able to interfere with cellular iron homeostasis, making cancer cells more responsive to chemotherapeutics. Assessment of labile iron pool and ROS levels revealed impairment of the detoxification mechanisms causing ROS accumulation. Interestingly, our results reveal that our Ru-drug, as ferroptosis-inducing complex, could represent an additional weapon for the treatment of breast cancer resistant to conventional therapies. References: [1]Piccolo et al. doi: 10.3390/cancers16061220. [2]Ferraro et al. doi: 10.3390/ijms24076473. [3]Piccolo et al. doi: 10.3390/cancers13205164. [4]Piccolo et al. doi: 10.1038/s41598-019-43411-3.

P-27-034
Primary breast tumor spheroids express an organized extracellular matrix limiting doxorubicin efficacy

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Tumor microenvironment and specifically the extracellular matrix (ECM) modulates cancer cell behavior and response to chemotherapy. Collagens are the main components of ECM, and many collagen proteins are overexpressed in tumors, giving the characteristic stiffness of the tumor microenvironment. We developed an *in vitro* model to mimic the *in vivo* microenvironment and specifically the interaction of tumor mass with the surrounding ECM. Breast carcinoma in rats was induced with a single dose of 7,12-dimethylbenzatracene (DMBA), intraperitoneally administered in 5 weeks old female Wistar rats. The explanted tumor mass was treated with recombinant collagenases (Class I and Class II) and thermolysin, and a heterogenous cell population, such as epithelial-tumor cells and fibroblast, was isolated. Tumor spheroids were generated in low attachment plate and after 6 days of culture, confocal microscopy revealed the presence of a collagen matrix. Proteomic analysis identified a complex matrix organization on 3D spheroids compared to 2D cell culture. Therefore, to investigate in vitro the ECM involvement in the modulation of Doxorubicin effects, spheroids were treated with recombinant collagenases for the digestion of the ECM to reduce the thick and dense collagen matrix around them. Doxorubicin cellular uptake studies highlighted the role of ECM as a barrier, limiting drug penetration, and consequentially, collagen degradation increases doxorubicin cellular internalization. Moreover, treating spheroids with collagenases prior to Doxorubicin incubation has been shown to significantly enhance drug cytotoxicity effects. Overall, isolated primary cancer cells were used as reliable sources to generate *in vitro* 3D spheroid that mimic the tumoral ECM. These spheroids have a distinct ECM organization, which enhances chemoresistance, impairing the efficacy of anticancer drugs.

P-27-035
S-Adenosyl-L-methionine inhibits the growth of human renal carcinoma cells by reducing autophagy and promoting apoptosis

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S-Adenosyl-L-methionine (AdoMet) is a naturally-occurring sulfonium compound playing a primary role in cellular metabolism. AdoMet has been shown to exert cell-type specific antiproliferative effects and is involved in tumor suppression through the regulation of multiple cell processes, such as cell cycle regulation, proliferation, and apoptosis. Renal cell carcinoma (RCC) is among the top ten most common cancers, accounting for 2–3% of human cancers and causing approximately 100 000 deaths per year worldwide. Multiple targeted therapies have been developed

to treat RCC but chemoresistance still remains a challenge. Therefore, new therapeutic strategies with higher efficacy and fewer side effects are needed. Interestingly, many studies have recently reported important relationships between autophagy inhibition and RCC [1]. In this study, we investigated for the first time the antitumor activity of AdoMet in RCC and explored its underlying mechanisms. AdoMet caused a dose- and time-dependent inhibition of 786-0 RCC cell viability with an EC50 value of 500 μM, after 72 h treatment. In contrast, non-cancerous human kidney HEK-293 cells were affected only minimally maintaining more than 80% cell viability under the same treatment conditions thus suggesting a selective anti-proliferative action of AdoMet toward tumor cells. AdoMet induced apoptosis of RCC cells through caspase cascade activation, PARP-1 cleavage, and increase of Bax/Bcl-2 ratio and arrested cell cycle progression in S-phase by reducing cyclin D and A and increasing cyclin E. Finally, AdoMet inhibited autophagy by inducing p62 accumulation, Atg7 downregulation, and decrease of LC3 II/I ratio. In conclusion, these results strengthen the evidence already present in literature on the anticancer potential of AdoMet suggesting the use of this compound as an autophagy inhibitor in adjuvant therapies aimed at improving RCC treatment and patient outcomes. Reference: 1. Jones TM et al. (2020) Cancers 12, 1185.

P-27-036
Inhibition of the HMGB1/RAGE signaling suppresses epithelial-to-mesenchymal transition and cell invasion in 3D triple-negative breast cancer models

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Triple-negative breast cancer (TNBC) remains a highly aggressive subtype with limited treatment options and poor prognosis. Epithelial-to-mesenchymal transition (EMT) plays a crucial role in driving the invasive and metastatic behavior of TNBC cells, with high-mobility group box 1 protein (HMGB1) and its receptor for advanced glycosylation end products (RAGE) emerging as interesting regulators of this process. This study sought to investigate the impact of recombinant HMGB1 (rHMGB1) on cell invasion in TNBC using a 3D collagen-encapsulated spheroid model. Additionally, the potential of metformin and cannabidiol (CBD) in inhibiting HMGB1/RAGE-induced invasion was evaluated. Metformin, a well-known anti-diabetic drug, and CBD, a non-psychoactive component of cannabis, have shown promise in targeting cancer progression. In this study, both metformin and CBD demonstrated efficacy in suppressing cell invasion and reducing the expression of mesenchymal markers in TNBC cell models. Furthermore, the potential of metformin and CBD as inhibitors of HMGB1/RAGE signaling highlights their therapeutic relevance in combating the metastatic progression of TNBC. These findings underscore the importance of investigating metformin and CBD as targeted therapeutic agents in TNBC, with implications for improving patient outcomes in the management of this aggressive breast cancer subtype. Further research is warranted to elucidate the underlying mechanisms and explore the clinical translation of these promising results.

P-27-037
BOP1 protein and morphometric parameters of the nucleoli as a new tool for the determination of renal clear cell carcinoma grading

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Clear cell renal cell carcinoma (ccRCC) is the most common subtype of kidney cancer. Its malignancy is determined based on the size and shape of the nucleoli in the immunohistochemical image and relies on the subjective analysis of an experienced pathomorphologist. The objective algorithms for differentiating evaluation of ccRCC tumor grade are lacking. Here, we aimed to identify nucleolar proteins whose expression correlates with nucleolus morphology, to find objective biomarkers of ccRCC tumor grade. To verify the hypothesis, LC-MS/MS proteomic analysis of nucleoli isolated from normal cells (RPTEC/TERT1) and ccRCC cells (786-O and Caki-1) was performed. The proteins with the most differential expression between cell lines were selected to evaluate correlations between their expression and nucleolar morphology using RCC cell lines of different tumor aggressiveness: 786-O (primary tumor) and Caki-1 (skin metastasis). Cells seeded on coverslips were fixed after 48 h and stained using: (i) Diff-Quick for morphometric measurements (area, perimeters, Feret diameter, number of nucleoli); (ii) immunocytochemistry (ICC) to verify the fluorescence intensity of the tested protein. Imaging was performed on a ZEISS LSM800 confocal microscope using ZEN 3.7 software. Morphometric measurements were performed using ImageJ software. Among 233 proteins with impaired expression in ccRCC, GLTCSR2, DDX21 and BOP1 were selected for further analysis. ICC intensity of all three proteins was increased in RCC cell lines when compared with RPTEC cells. BOP1 fluorescence intensity correlated with tumor aggressiveness. Moreover, we also observed statistically significant correlation between the number of nucleoli in the nucleus and the arithmetic mean fluorescence intensity of the BOP1 protein. In conclusion, BOP1 protein expression is a promising potential biomarker of ccRCC tumor aggressiveness. Financed by National Science Center, Poland grant 2019/35/B/NZ5/00695.

P-27-038
Involvement of paraoxonase-2 enzyme in human osteosarcoma cell proliferation, migration and chemosensitivity

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Human osteosarcoma (OS) represents the most recurrent primary bone neoplasm, predominantly affecting young people. OS is characterized by an aggressive behavior, often leading to a poor prognosis, mainly due to enhanced chemoresistance and

outstanding tendency to metastatic dissemination (1). In this scenario, the elucidation of cellular mechanisms featuring OS tumorigenesis, and the identification of new molecular therapeutic targets, become crucial. In this study, we focused on paraoxonase-2 (PON2), a ubiquitously expressed intracellular protein, mainly localized in the endoplasmic reticulum (ER) and associated with the inner mitochondrial membrane. PON2 functions as an antioxidant enzyme, by counteracting reactive oxygen species release, thus displaying an antiapoptotic effect and preventing the formation of atherosclerotic lesions. Enzyme overexpression was reported in a wide variety of solid tumors, promoting tumor cell aggressiveness (2). Preliminary immunohistochemical analyses were carried out to explore PON2 expression in tumor and normal bone tissue specimens, obtained from OS patients and healthy subjects, respectively. In order to investigate enzyme contribution to cancer cell phenotype, shRNA-mediated gene silencing was used to achieve PON2 knockdown in OS cell lines and the impact of cell proliferation, migration and chemosensitivity was further assessed. Obtained results showed a significant PON2 overexpression in OS with respect to control samples. Data collected from cell-based assays demonstrated that enzyme downregulation was associated with a significant decrease of proliferative capacity and migration ability, as well as with an enhancement of sensitivity to chemotherapeutic treatment. The sum of this evidence seems to suggest a promising role for PON2 as molecular biomarker and therapeutic target for OS. References 1. Kansara M et al. (2014) Nat Rev Cancer 14, 722–35. 2. Campagna R et al. (2024) Biomolecules 14, 208.

P-27-039
Discovery of benzimidazole-indazole based inhibitors targeting mutant FLT3 kinases for the treatment of acute myeloid leukemia

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The FLT3 gene encodes a receptor tyrosine kinase expressed in hematopoietic stem cells. Mutations in FLT3, which are observed in 30% of acute myeloid leukemia (AML) cases, result in aberrant activation of the receptor's kinase, leading to the proliferation of immature myeloblast cells. Although small molecule inhibitors targeting FLT3 kinase have been approved, new inhibitors are still needed due to side effects and drug resistance caused by kinase domain mutations such as D835Y and F691L. This study presents novel benzimidazole-indazole based inhibitors that are designed to target mutant FLT3 kinases by optimizing various chemical moieties around the core structure. Compound 22f showed potent inhibition of FLT3 and FLT3/D835Y, with IC_{50} values of 0.941 and 0.199 nM, respectively. It also demonstrated significant antiproliferative activity against an AML cell line (MV4-11 cells) with a GI_{50} of 0.26 nM. Notably, 22f exhibited single-digit nanomolar GI_{50} values against mutant FLT kinase-expressing Ba/F3 cell lines, including FLT-D835Y (GI_{50} = 0.29 nM) and FLT3-F691L (GI_{50} = 2.87 nM). Molecular docking studies showed that the compound is a type 1 inhibitor in the homology model of the active conformation of FLT3 kinase.

P-27-040
Investigating intra- and extra-cellular functions of Transglutaminase 2 by use of inhibitors with different localization in breast cancer cells

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Transglutaminase 2 is involved in breast cancer with several processes, such as epithelial-mesenchymal transition, aggressiveness, and metastatization. This protein displays intracellular and extracellular roles, which we have investigated using membrane-permeable and impermeable inhibitors. The alteration of transcriptome following the treatment of two triple-negative breast cancer cell lines allows us to determine the modulated genes, pathways, and networks. Integrin signaling and p53 were commonly affected by each inhibitor, while other pathways were specific. AA9, entering the cell, induced apoptosis in MDA-MB-436, affecting cadherin, Wnt, gastrin, and cholecystokinin receptors (CCKR) signaling, with RHOB and GNG2 as relevant players, while it decreased glycolytic enzymes by impact on the Warburg effect. In MDA-MB-231 cells, AA9 significantly modulated genes belonging to HIF-mediated hypoxia, AKT, and mTOR pathways. These effects suggest an anti-tumor efficacy exhibited by inhibiting intracellular TG2 functions. Generally, these effects suggest an anti-tumor efficacy exhibited by inhibiting intracellular TG2 functions. In contrast, NCEG2 increased the expression of ATP synthase and DNA replication-related proteins, indicating that inhibition of extracellular functions could encourage cell division as pro-cell replication action. This study underlines opposite effects following the treatment with inhibitors having different cell localization that will need to be considered for the applications of anti-tumor strategies.

P-27-041
FTIRM analyses as a tool to explore cisplatin sensitivity of oral squamous cell carcinoma cell lines after PON2 knockdown

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Oral squamous cell carcinoma (OSCC) is the most frequent and aggressive variant of head and neck cancer. The management of this malignancy is complicated by the chemoresistance, which can be primary or can quickly arise during the chemotherapeutic treatment, leading to a 5-year survival rate lower than 50%. For these reasons, the identification of novel biomarkers that could

be utilized for targeted therapy is urgently required. The intracellular enzyme paraoxonase-2 (PON2) belongs to the multigene family and is known to exert a negative regulation towards pro-oxidative stimuli due to its capacity to reduce intracellular reactive oxygen species. PON2 is upregulated in several malignancies and there is an increasing evidence that it contributes to tumor progression and chemo- or radio-resistance. We recently demonstrated that PON2 is upregulated in OSCC compared to normal tissue and an inverse correlation was detected between enzyme levels and tumor size, suggesting a potential role of PON2 in the early phases of oral tumorigenesis [previously published in: Campagna R et al. (2023) Hum Cell 36, 1211–1213]. Moreover, we demonstrated that an *in vitro* knockdown of the enzyme was able to enhance the chemosensitivity of OSCC cells towards cisplatin (CDDP) [previously published in: Campagna R et al. (2022) Int J Mol Sci 24, 338]. In order to deepen the efficacy of CDDP following PON2 gene silencing, a Fourier-Transform Infrared Microspectroscopy (FTIRM) investigation was coupled with a statistical combined approach to evaluate the time-dependent changes appearing in the OSCC cell lines HOC621 and HSC-3. Besides the oxidative stress spectral biomarkers which were previously reported, differences in nucleic acids composition and in other diagnostic markers related to carbohydrates were detected demonstrating the effects induced initially by the PON2 knockdown and subsequently by the CDDP treatment in a broader perspective, making FTIRM a powerful investigation tool.

P-27-042
Shedding light on breast cancer cell signaling: promising epigenetic regulation of signaling genes by microRNAs

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Breast cancer (BrCa) is a heterogeneous malignancy with a significant incidence and mortality rate globally. The molecular classification of the tumor is pivotal for the prognosis of patients as diverse signaling pathways are triggered. MicroRNAs (miRNAs) are small non-coding RNAs that perform epigenetic regulation by binding to complementary mRNA sequences. Aiming to uncover miRNA-mRNA regulatory axes with an impact on significant signaling pathways in BrCa, we performed treatments of BrCa cell lines with two proteasome inhibitors. The MCF-7, BT-474, SK-BR-3, and MDA-MB468 cell lines belonging to the major molecular subtypes of BrCa, luminal A, luminal B, $HER2^+$, and triple negative, were treated with Bortezomib and Carfilzomib to trigger cell signaling. After treatment of cells with the IC_{50} of each inhibitor, cell viability was assessed, apoptosis was documented, and total RNA was extracted. Poly(A) RNA selection using oligo-dT magnetic beads, and size selection of small RNA were performed from treated samples and relative controls. Next, high-throughput sequencing approaches were implemented for the identification of differentially expressed genes, and extensive bioinformatics analysis was performed. Treatment with Carfilzomib altered gene expression mostly in the MCF-7 and BT-474 cell lines, while Bortezomib appeared more effective in the SK-BR-3 and the MDA-MB468 cell lines. Interestingly, breast cancer, estrogen signaling, MAPK, PI3K-Akt, NF-kappa B, and other signaling pathways were enriched. Furthermore, the majority of the common genes that appeared regulated are upstream regulators in each signaling pathway. Finally, both identified in other cancer types, and predicted regulatory interactions between miRNA-mRNA pairs consisting of *BRCA1*, *CCND1*, *INSR*, *MRAS*, *MYB*, *PIK3R1*, *PRLR*, *TP53*, miR-193b-5p, miR-22-5p, miR-22-3p, miR-21-3p and others were identified. The identification of miRNA-mRNA interplay in this context aids in untangling the cancerous environment.

P-27-043
IRAK1 contributes to chemo-immuno-resistance and correlates with poor prognosis in non-small cell lung cancer

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The high expression of ABCB1 (P-glycoprotein) and ABCC1 (multidrug resistance-related protein 1), both involved in the efflux of chemotherapeutic drugs, and the low expression of ABCA1, a transporter promoting the immuno-recognition of the tumor by Vγ9Vδ2 T-lymphocytes, determine a chemo-immuno-resistant phenotype in non-small cell lung cancer (NSCLC)[1], but the underlying molecular pathways are unknown. A CRISPR-KO kinome library on the top chemo-immuno-resistant NSCLC NCI-H2228 cell line unveiled interleukin 1 receptor-associated kinase 1 (IRAK1) in the top 6 kinases producing the shift from chemo-immuno-resistance (ABCB1/$ABCC1^{high}$, $ABCA1^{low}$ phenotype) to chemo-immuno-sensitivity (ABCB1/$ABCC1^{low}$, $ABCA1^{high}$ phenotype). IRAK1-silencing in NCI-H2228 cells transcriptionally decreased ABCC1 and ABCB1, by inhibiting NF-kB/AP-1 activity, lowered the IC50 to cisplatin and docetaxel, and in parallel increased ABCA1 by activating the liver transcriptional X receptor alpha (LXRα), increasing ABCA1-mediated efflux of isopentenyl pyrophosphate (IPP), an activator of Vγ9Vδ2 T-cell-mediated immune-killing. IRAK1-overexpression in chemo-immune-sensitive NCI-H1975 cells produced the opposite effects. IRAK1-silenced NCI-H2228 tumors implanted in Hu-CD34+ NSG mice were re-sensitized to cisplatin compared to wild-type tumors and had qualitative/quantitative differences in the immune-infiltrate evaluated by single-cell transcriptomics and immunophenotypic profile. Moreover, the transcriptomic profile of 110 NSCLC patients showed that IRAK1 was significantly more expressed in tumors than in non-tumoral lung tissues. According to the median value of IRAK1 in tumors, patients categorized as $IRAK1^{high}$ had worse progression-free survival and overall survival. Our results indicate that IRAK1 contributes to chemo-immuno-resistance by modulating ABC transporters and represents a new biomarker of clinical outcome in NSCLC patients. Reference: 1. Salaraoglio IC et al. (2022), J Exp Clin Cancer Res 41, 243. *The authors marked with an asterisk equally contributed to the work.

P-27-044
Hepatic fibrogenesis in TP53KO organoids within a pro-carcinogenic microenvironment

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Hepatic fibrogenesis is a pathological outcome of chronic liver injury, characterized by the excessive accumulation of extracellular matrix proteins, leading to hepatocarcinogenesis. However, the lack of suitable models for mimicking precancerous fibrogenesis in the early stage is an important obstacle to creating effective treatment strategies. Here, we employ human pluripotent stem cell-derived hepatic organoids (eHEPO) to mimic early liver fibrosis, focusing on CRISPR/Cas9-mediated TP53 loss within a pro-carcinogenic microenvironment (pc-ME). Transcriptome analysis reveals that our TP53KO-eHEPOs model describes enrichment in the inflammatory, ECM modification, early hepatic fibrosis, and tumorigenesis pathways. The early hepatic fibrosis phenotype change confirmed by histological staining includes: atypical cell morphology, pseudo-glandular-tubular rosettes, steatohepatitis-like inflammatory areas, and hepatocytes with ballooning-like characteristics. In addition, TP53KO-eHEPOs represent augmented Masson's trichrome staining. Furthermore, we confirm that a well-known fibrosis-related TGF-b pathway activation, myofibroblast and fibrosis marker PDGFRB, IL-33 expression levels, and precancerous markers GPC3, and MUC1 are significantly augmented in TP53KO organoids. This trend is followed by inflammation-related HIF1A, IFNA, and STAT3 signal pathways enrichment. In conclusion, this model offers insights into liver fibrosis and hepatocarcinogenesis, facilitating the study of TP53 and inflammatory conditions' impact on hepatic progenitor cell transformation, and providing a platform for early-stage drug development and candidate identification.

P-27-045
Combination treatment of metformin and TRAIL enhances apoptosis in human colorectal cancer cells

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Resistance to chemical drugs, which is common in colorectal cancer (CRC), causes tumor recurrence and metastasis. Therefore, new strategies to improve this are urgently needed. Tumor necrosis factor (TNF)-related apoptosis-inducing ligand (TRAIL) is a promising therapeutic candidate with anticancer potential, but combination treatment is often necessary to overcome various resistances depending on cancer cell type and increase anticancer efficiency. We found that among human CRC cell lines, HCT116 cells have high sensitivity to TRAIL, whereas SW620 cells have low sensitivity to TRAIL, requiring additional sensitizers. In this study, treatment with TRAIL alone rarely induce apoptosis in SW620 cells, but combined treatment with TRAIL and Metformin significantly increased apoptosis. TRAIL activated caspases through the extrinsic pathway, but also increased resistance to apoptosis through the AKT/mTOR pathway. On the other hand, Metformin reduced the inhibitory effect of XIAP by blocking the AKT and NF-kB pathways, and CHOP activated by ER stress induces apoptosis through the Death-Receptor pathway. However, SW620 cells treated with Metformin alone induced cell cycle arrest, a state in which cell proliferation and growth were blocked, but did not ultimately lead to apoptosis. These results were also confirmed in a xenograft model, where the combination of relatively low doses of Metformin and TRAIL increased susceptibility to cell death and enhanced anticancer effects. Therefore, it is expected that the combination treatment of TRAIL and Metformin may provide a potential treatment strategy for TRAIL-resistant CRC, such as SW620 cells. *The authors marked with an asterisk equally contributed to the work.

P-27-046
DNA and topoisomerases as molecular targets of novel 2-substituted acridone derivatives

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Acridone derivatives have been explored for their broad range of biological activity, including antibacterial, anti-inflammatory, antiparasitic, antiviral, antimalarial, antitubercular, antiallergic, fungicidal and anticancer activities. They have been reported to interact with proven molecular targets in cancer, including topoisomerases 1 and 2, telomerase and protein kinases [Yadav TT et al. (2022) Eur J Med Chem 239, 114527]. Another target of small organic molecules with anticancer activity is DNA because of its crucial role in cell division. In the case of acridine/acridone derivatives, this interaction is well-established and is primarily achieved through intercalation of the azaheterocyclic chromophore between the base pairs of DNA [De Almeida, SMV et al. (2017) Biomed Pharmacother 96, 1538-1556]. The aim of our research was to study the interaction of six novel 2-substituted acridones with calf thymus DNA (ctDNA) and monitor their inhibition activity against topoisomerases (Topo) 1 and 2α. We have employed spectroscopic methods (UV-Vis, fluorescence and circular dichroism) and thermal denaturation studies of ctDNA to elucidate the binding mechanism between the studied derivatives and ctDNA. The inhibition capacity against Topo 1 and 2α was studied through agarose gel electrophoresis using superspiralized and catenated DNA as substrates, respectively. The results of the spectroscopic studies confirmed a non-covalent interaction between ctDNA and the studied derivatives, which seem to preferably bind into the minor groove of the double helix with partial intercalation of the acridone moiety. Five of the studied compounds exhibited considerable inhibitory activity against Topo 1, while two derivatives were effective against Topo 2α as well. Overall, the results have shown that the studied acridone

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derivatives could represent promising anticancer agents and are suitable for further research. This study was supported by VEGA Grant No. 1/0037/22. *The authors marked with an asterisk equally contributed to the work.

P-27-047
RITA-induced apoptosis is associated with Bcl-2 cleavage and its dissociation from the mitochondria

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Targeting of p53-Mdm2 interaction is of great interest as a part of cancer treatment strategies. Beside various Mdm2 inhibitors, like Nutlin and its analogs, the small molecule RITA targeting p53 has been developed to disrupt the p53 interaction with Mdm2. Although the effect on the interaction was not unambiguously proved, RITA has been shown to induce p53-dependent apoptosis in various cancer cells, including acute myeloid leukemia (AML). We have shown previously [Wolfová K et al 2023, BBA-MCR 1870(7):119501] that AML cell sensitivity to RITA correlates with changes in phosphorylation of nucleolar proteins nucleophosmin and nucleolin. Apoptosis was further accompanied with c-Myc and Mcl-1 downregulation, DNA damage, absence of cell cycle arrest, p21 induction, and caspase-3 activation. In the present contribution, we analyzed pro- and anti-apoptotic members of the Bcl-2 family, p53, and phosphorylation status of the nucleolar proteins in cytoplasmic, mitochondrial and nuclear fractions from RITA-treated AML cells. Our results demonstrate dynamic redistribution of the investigated proteins among individual cell fractions. Moreover, apoptosis-associated cleavage of the Bcl-2 protein is documented. Similar results obtained with several Bcl-2 inhibitors are presented as well. In particular, the role of nucleolar proteins in the effect of BH3 mimetics on AML cells is highlighted. The work was supported by the Czech Grant Agency (22-03875S) and the Ministry of Health of the Czech Republic (project for conceptual development of the research organization No 00023736).

P-27-048
Innovative strategy to predict the development of testicular cancer in adulthood for children with undescended testicle

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Testicular germ cell tumor (TGCT) is the most common cancer in males between 15 and 35 years old; despite displaying a very high survival rate, the problems that TGCT patients face are mainly two: (i) lack of specific markers that allow an early diagnosis, (ii) chemo- and radio-therapy to which they are subjected result in loss of fertility. One of the primary causes of this tumor is cryptorchidism, the absence of one or both testicles in the scrotum at birth, altering the testicular structure and modifying the germ-stem cell niche. For this reason, the purpose of the study was to examine in depth the cellular and biological mechanisms that trigger the transformation of pre-neoplastic testicular cells into tumoral cells, by taking advantage of primary testis cells derived from cryptorchid and TGCT patients. Specifically, we cultivated *in vitro* primary cells and de-differentiated them into stem cells using a protocol optimized in our laboratory. We therefore analyzed: (i) the main markers of EMT and stemness, (ii) the most important pro-cancer markers, and (iii) the proliferation rate, comparing the cells obtained from cryptorchid patients with TGCT patients to check if there was a trend of similarity between the two samples. Finally, we treated cells *in vitro* with the main hormones involved in the hormonal cascade of male development to mimic the biological conditions of patients during puberty and adulthood. With this *in vitro* predictive model, we aim to identify those cryptorchid patients who are prone to develop TGCT in puberty or adulthood, together with the discovery of the biological mechanisms that are involved in the transform of testicular cells into tumor cells under the action of hormones. The final ambition is to discover a molecular marker that can define the predisposition to develop TGCT and act as a sentinel to prevent the onset of the tumor, allowing cryptorchid pediatric patients to be followed constantly in clinical follow-up.

P-27-049
Sonodynamicly-excited C60-Berberine nanocomplex induces oxidative damage and apoptosis in HeLa and LLC cell spheroids

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Recently, sonodynamic therapy (SDT) has emerged as a promising non-invasive approach for treating cancer by activating sensitizers with ultrasound (US). In this context, we investigated C_{60} fullerene (C_{60}) utilized as a nanocarrier for the aromatic drug Berberine (Ber) – a potential sonosensitizer. The preferential accumulation of C_{60} and the proapoptotic effects of Berberine make the C_{60}-Berberine nanocomplex (C_{60}-Ber) a good candidate for a direct induction of the intrinsic apoptotic cell death giving a good perspective for a selective, noninflammatory cancer treatment strategy. The primary objective of this research targeted the biochemical effects of SDT towards HeLa and LLC cell spheroids. Human cervix carcinoma (HeLa) and mouse Lewis lung carcinoma (LLC) spheroids were treated with a 20 μM C_{60}-Ber for 24 hours, followed by irradiation with 1 MHz, 1 W/cm^2 US. To evaluate the efficacy of the proposed treatment on cancer cells spheroids, assessments of cell viability, caspase 3/7 activity, ATP levels, and ROS levels were conducted. The results revealed that US irradiation alone had negligible effects toward LLC and

HeLa spheroids. However, spheroids irradiated with US in the presence of the C_{60}-Ber exhibited a significant decrease in viability (38% and 37% for HeLa and LLC cells, respectively) and ATP levels (66% and 67%, respectively), along with a notable increase in caspase 3/7 activity (430% and 189%, respectively) and ROS levels (247% and 208%, respectively). In addition, the anticancer effects of C_{60}-Berberine nanocomplex surpassed those of C_{60}, Ber, or their combination (C_{60} + Ber) in both cell lines. Intensified ROS generation, ATP level drop relate to targeting mitochondria and caspase 3/7 activity increase points the apoptotic pathway induction. These findings suggest that SDT with the C_{60}-Berberine nanocomplex offering targeted approach to induce cancer cell apoptosis as a potential approach for cancer treatment.

P-27-050
The inhibitors of histone lysine demethylases together with olaparib sensitize head and neck cancer cells towards cisplatin

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Cisplatin is the standard treatment for loco-regionally advanced head and neck squamous cell carcinomas (HNSCC) however its effectiveness is not satisfactory. In order to improve outcomes, the use of combinations of cisplatin with other compounds is a viable option. Cisplatin kills cancer cells by causing extensive DNA damage, and efficient DNA repair contributes to cisplatin-resistance. DNA damage response (DDR) is a complex process, which relies on the action of many proteins, including histone modifying proteins. Reversible histone methylation/demethylation by histone methyltransferases and histone demethylases (KDMs) together with the action of poly(ADP-ribose) polymerase (PARP1) significantly contributes to proficient DDR. KDMs are implicated in the regulation of homologous recombination (HR) pathway, which is BRCA-dependent. On the other hand, olaparib - a PARP1 inhibitor, causes synthetic lethality in BRCA-mutated cancer cells. Thus, by impairing the HR pathway, KDM inhibitors could possibly synergize with olaparib in cell death induction initiated by cisplatin. The aim of this study was to assess the effect of the combinations of KDM inhibitors (ML324, CPI-455, GSK-J4 and JIB-04, which inhibit KDM4, KDM5, KDM6 and pan-KDM, respectively) with olaparib on cisplatin-induced apoptosis. FaDu and SCC-040 cells were grown in standard conditions (DMEM + 5% FBS, 37°C, 5% CO_2) and treated with the studied compounds for 24–72 h (depending on the assay). The effect on cell viability was evaluated with the resazurin assay. Muse Annexin V & Dead Cell Kit was used for apoptosis detection, and DNA damage was analyzed by measuring the level of H2A.X activation by flow cytometry. Gene expression was analyzed by qPCR. FaDu cells were more sensitive to the induction of H2A.X phosphorylation and apoptosis by the studied compounds. The triple combinations of cisplatin, olaparib and either GSK-J4 or JIB-04 showed the highest potency, pointing to possible therapeutic benefit.

P-27-051

Abstract withdrawn.

P-27-052
A transcriptional fingerprint based on MGRN1 gene expression predicts outcome of melanoma patients

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Patients of melanoma, the deadliest skin cancer, are staged with the Tumor-Node-Metastasis classification (TNM) according to the American Joint Committee on Cancer. In situ skin melanoma (low TNM grade) is curable and has high survival rates but advanced stages have worse outcomes and shorter lifespan. Reliable prognostic markers are lacking, which complicates the handling of patients. The MGRN1 ubiquitin ligase modulates the phenotype of melanoma cells, with effects on pigmentation, shape and motility, and a less aggressive phenotype. MGRN1 knockdown also causes aberrant cell cycle progression and genomic instability. The effects of MGRN1 deficiency on the transcriptional landscape of melanomas and the prognostic of patients remain poorly known. The TCGA project analyzed the genomic landscape of >450 melanoma cases. We used the TCGA and other melanoma datasets to compare MGRN1 expression in malignant or premalignant clinical samples and normal tissue, and to analyze the relationship of MGRN1 expression and patient survival. MGRN1 was overexpressed in melanoma vs normal skin or nevi and was mutated in a significant number of melanomas with a mutually exclusive pattern with TP53 mutations. Kaplan-Meier analysis showed longer survival for carriers of melanoma with low vs high MGRN1 expression. We also found that MGRN1 expression reliably complemented the prognostic information of TNM staging. Gene set enrichment analysis of melanomas with low or high MGRN1 expression revealed differential expression of gene sets associated with cell cycle, DNA damage and repair processes. These data were validated upon repression or knockdown of MGRN1 in melanoma cells, where we found comparable gene expression and a consistent phenotype. Therefore, MGRN1 modulates the expression of genes involved in major cancer related pathways and provides a prognostic biomarker that might complement the current TNM staging of melanomas. Supported by grant PI22/00404 from ISCIII, cofinanced by EC.

P-27-053
Mahogunin Ring Finger 1, a possible melanoma biomarker, is required for normal cell cycle progression and genomic stability

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In mouse melanocytes, the MGRN1 E3 ubiquitin ligase is required for genomic stability, as shown by DNA strand break (DSB) accumulation upon MGRN1 repression or knockout. Low expression of MGRN1 in human melanoma is associated with longer survival of patients and differential expression of

gene sets involved in DNA metabolism and repair (see poster 53828). To analyze the molecular bases of this association, we repressed or abrogated MGRN1 expression in HBL human melanoma cells (NRAS, BRAF and NF1 wildtype). MGRN1 depletion augmented the DSB burden and altered cell cycle progression, consistent with transcriptomic profiles. Loss of MGRN1 also promoted replication fork stalling, suggesting induction of replicative stress. The ataxia telangiectasia and Rad3-related (ATR) kinase is essential for correct DNA replication and for the replicative stress response. MGRN1-deficient cells displayed impaired ATR signaling, as shown by lower activation of the effector kinase CHK1 and p53 following treatment with hydroxyurea. Proteomic analysis revealed the interaction of MGRN1 with the single-stranded DNA coating protein RPA, responsible for recruiting ATR interacting partner (ATRIP)-ATR complexes to stalled replication forks. MGRN1 was also found to interact with the cell cycle kinase CDK2, which phosphorylates ATRIP to increase ATR signaling. The MGRN1-CDK2 interaction appeared to stabilize CDK2 by impairing its proteolytic degradation. Conversely, siRNA-mediated repression of CDK2 or inhibition of its kinase activity, augmented MGRN1 levels, suggesting reciprocal CDK2/MGRN1 regulation. Moreover, chromatin-bound CDK2 levels detected by fractionation with CSK buffer also decreased upon MGRN1 repression. Thus, MGRN1 might act to promote ATR-mediated stabilization and/or restart of replication forks, likely in a CDK2-dependent manner, to prevent fork collapse and DSB formation. This model is currently being tested. Supported by grant PI22/00404 from ISCIII, cofinanced by EC.

P-27-054
From oral lichen planus to oral squamous cell carcinoma: unraveling pathways for improved diagnosis and treatment

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Oral squamous cell carcinoma (OSCC) is a common type of head and neck malignancy which develops in the oral mucosa, representing about the 90% of all mouth cancers. It is the culmination of genetic alterations and can arise from a pre-existing oral potentially malignant disorder (OPMD) or *de novo*. Among OPMDs, Oral Lichen Planus (OLP) is an autoimmune inflammatory disease with a malignant transformation rate ranging from 0.44% to 2.28%. Although its etiology is currently unknown, immune dysregulation plays a well-established role in OLP. Specifically, autophagy has emerged as a fundamental catabolic process in mediating T cell response and regulating T cell immunity, causing a dysregulation of apoptosis in oral keratinocytes. Considering this data, the study aims to investigate these biochemical pathways, which can provide specific biomarkers to assess the risk of OLP transforming into OSCC. The research goals are the identification of biomarkers predicting OLP progression into OSCC, through immunofluorescence (IF), and the development of a nano drug delivery system for a topical drug administration. IF is performed on biopsy tissues from OLP and healthy patients to target autophagy-related proteins. As it concerns drug delivery, Poly lactic-co-glycolic-acid (PLGA) nanoparticles (NPs) are synthesized by using the solvent evaporation technique. Drug release kinetics is measured by spectrophotometric techniques and HPLC is employed to assess drug recovery from cell culture medium and lysate. Cytotoxicity of PLGA is determined through the MTT test, while Dynamic Light Scattering and Scanning Electron Microscope are used to ascertain the size, the population homogeneity and the morphology of the NPs. Through the comparison of biomarkers detected in the biopsies of patients with OLP with those that will be found in the biopsies of OSCC, this research aims to achieve a significant advancement in understanding the pathophysiology of OSCC.

P-27-055
Biochemical and structural characterization of NuMA/β-catenin complexes involved in Wnt3a-dependent self-renewal

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The Wnt pathway is a fundamental process that regulates cell differentiation and proliferation during embryonic development and maintains tissue homeostasis in adult life. Under physiological conditions, Wnt ligands bind to Frizzled/Lrp6 receptor complexes, inhibiting the destruction complex and leading to the stabilization of β-catenin, which in turn activates the transcription of Wnt genes. Consistently, mutations in Wnt genes, causing the aberrant expansion of the staminal compartment, are observed in several cancers, primarily colorectal cancer. Intestinal stem cells undergo oriented planar cell divisions governed by Wnt3a-ligand secreted by niche Paneth cells. However, the precise mechanism by which the Wnt3 signals are transduced in mitotic intestinal stem cells to promote self-renewal remains largely unexplored. The dynein-adaptor NuMA (Nuclear Mitotic Apparatus protein) plays essential roles in spindle assembly and division orientation. We have found evidence suggesting that upon Wnt activation, mitotic NuMA enters a complex with β-catenin and the Lrp6 coreceptor. Biochemical reconstitution of this mitotic complex from recombinant expression in mammalian cells suggest that NuMA might interact directly with β-catenin. Our data supported a model in which the spindle orientation machinery centered on NuMA is recruited at localized Wnt3-contact sites to promote asymmetric cell divisions.

P-27-056
Enhancing triple-negative breast cancer treatment: sulforaphane-doxorubicin liposomal formulation for targeted therapy and chemoprotection

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Breast cancer stands as the most lethal cancer among women worldwide. Therapies targeting the triple-negative breast cancer (TNBC) subtype, characterized by the absence of known molecular targets, predominantly rely on systemic chemotherapy, which carries significant side effects. To mitigate these challenges, drug carriers and combination therapies are under development to enhance therapeutic efficacy and mitigate systemic toxicity. This study aims to explore the efficacy and mechanism of action of a novel formulation combining isothiocyanate – sulforaphane (SFN) with the cytostatic drug doxorubicin (DOX), encapsulated within liposomes, for the treatment of TNBC. Three liposomal formulations containing DOX (DOX-lip.), SFN (SFN-lip.), and a combination of DOX and SFN (DOX + SFN)-lip. were developed and characterized. Safety and efficacy assessments of the liposomal formulations were conducted using *in vitro* and *in vivo* models. The developed formulations exhibited promising outcomes in reducing TNBC primary tumor growth and providing chemoprotection against DOX-induced toxicity. The study findings underscore the potential of sulforaphane as a valuable component within liposomal doxorubicin for TNBC chemotherapy. Previously published in: Pogorzelska, A.et al. (2023) Biomed Pharmacother., 161, 114490.

P-27-057
Harnessing acetylsalicylic acid to counteract pancreatic stellate cell-mediated tumor promotion

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Pancreatic cancer is a highly aggressive type of cancer due to its unique tumor microenvironment. Active pancreatic stellate cells (PSCs) are abundant in the PaCa microenvironment and can promote cancer aggressiveness by secreting growth factors and cytokines. The daily use of acetylsalicylic acid (ASA), the active component of aspirin, has been linked to low cancer incidence in various cancers, including pancreatic cancer however, there is currently no study indicating its role on pancreatic stellate cell-mediated cancer aggressiveness. Therefore, we aimed to investigate the effect of ASA effect on PSCs and thereby aggressiveness of pancreatic cancer. PSCs were evaluated for active and passive states using α-smooth muscle and Oil Red O stainings. Aspirin doses of 1.25 and 0.625 mM that were not toxic for cells were selected for further experiments. PANC-1 and BxPC-3 PaCa cell lines were treated with the CM collected from non-treated (NT) PSCs and 24 h ASA-treated PSCs. Then, evaluated changes in cell viability, migration, and invasion using SRB assay, wound healing, matrigel invasion assays, and colony formation assays, respectively. Furthermore, the expression levels of EMT markers were compared following exposure to CM from both NT PSCs and ASA-pretreated PSCs. Moreover, the difference in CM collected from PSCs after ASA pretreatment was elucidated by ELISA assay and changes in released IL-6 levels were measured. The study revealed that PaCa cells exhibited increased proliferation, migration, and invasion when exposed to CM from NT PSCs, while these aggressive characteristics decreased when incubated with CM from ASA-treated PSCs. In summary, ASA treatment decreased the cancer-promoting abilities of PSCs by possibly changing their secretome. Further research is needed to reveal the exact mechanism of ASA on PSCs.

P-27-058
Development of small molecule compounds for improved medical radiation protection

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Ionizing radiation (IR) has widespread applications in medicine for diagnostic, interventional, and therapeutic purposes, and its use in clinical settings is continuously increasing. Given that the use of IR involves a risk of harmful effects to healthy tissues surrounding, there is a critical clinical need for pharmacological agents that can reduce radiation-induced toxicity against normal tissue. The aim of this study was to develop a mini-library of novel candidate radioprotectors decreasing radiation-induced cytotoxicity through inhibition of apoptosis. Herein, we used molecular modelling to synthetize piperazin-based modulators of apoptosis that target the BH3-only Bcl-2 pro-apoptotic proteins family. Structure and purity were assigned by standard analytical methods (NMR, HPLC-MS and elementary analysis). The newly synthesized analogues were subjected for their *in vitro* antiproliferative activity against a panel of 9 human cancer cell lines (Jurkat, MOLT-4, A549, HT-29, PANC-1, A2780, HeLa, MCF-7, SAOS-2), and one non-cancer cell line (fetal lung fibroblasts - MRC-5). The obtained antiproliferative rates revealed that two compounds (NŽ37 and NŽ58) demonstrated standalone cytotoxicity at 100 μM, leading to their exclusion from subsequent experiments investigating the radioprotective effect. Further investigation of the radioprotective effect was conducted using the MOLT-4 leukemia cell model followed by flow cytometric quantification of Annexin V/propidium iodide-binding. Results

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showed higher viability rates for MOLT-4 cells irradiated with selected compounds compared to those exposed to radiation alone (after 24 h). Application of developed derivatives at 100 μM for 2 h before irradiation with 2 Gy increased MOLT-4 cell viability up to 143% (NŽ46/47) compared to reference cells exposed solely to ^{60}Co-gamma irradiation (considered as 100% viability). This work was supported by grant project NU23-08-00256 of the Ministry of Health. *The authors marked with an asterisk equally contributed to the work.

P-27-059
Investigation of the anti-cancer effect of *Myrtus communis* extract against human lung adenocarcinoma cell line by *in vitro* and *in ovo*

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According to the data of the International Agency for Research on Cancer (IARC), lung cancer ranks first among the cancer types with the highest incidence and mortality rate. Due to the side effects of chemotherapeutics, researchers have turned to natural treatment agents. Although many studies have been conducted on the various medicinal properties of *Myrtus communis* (MC), which has high biological activity, the number of studies on the anticancer activity of its essential oil and MC components is quite limited. In this study, the cytotoxic effect of ethanol extracts obtained from *Myrtus communis* leaves (MCE) on A549 cells and their effect on migration ability were examined *in vitro*. The antiangiogenic effect of MCE was examined *in ovo*. BEAS-2B was used as the healthy control group. MCE was obtained using the Soxhlet device. The cytotoxic effect of MCE on A549 and BEAS-2B cells was measured by Sulforhodamine B assay. The effect of MCE on migration on A549 and BEAS-2B cells was measured by Scratch assay. The antiangiogenic effect of MCE was examined with the *in ovo* Chick Chorioallantoic Membrane (CAM) Assay. Embryo images were collected at 0 and 24 hours and total vessel length, total vessel area and number of vessel branching were analyzed with IKOSA software. Statistical analysis was done with GraphPad Prism 8. MCE induced cytotoxicity and inhibited migration in A549 cells in a dose-dependent manner compared to the control group and BEAS-2B cells. According to the *in ovo* CAM assay results, 3.125 mg/ml and 6.25 mg/ml concentrations statistically reduced the total vessel length, total vessel area and number of vessel branching compared to the control group. These results showed that MCE had anti-cancer effect against A549 cells and inhibited the migration ability of A549 cells. In addition, the fact that MCE has been shown to have an anti-angiogenic effect suggests that MCE may be an effective therapeutic or adjuvant strategy in cancer treatment.

P-27-060
Carbonic anhydrase 9 (CA9) expression in response to rectal cancer treatment

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Neoadjuvant treatment resistance develops in locally advanced rectal cancer patients due to the adaptation to hypoxia. Angiogenic factors regulated by hypoxia are under the control of hypoxia-inducible factor-1a (HIF-1α). One of the genes in which HIF-1α increases gene expression is carbonic anhydrase (CA9). Our aim was the gene expression and protein levels examination of the HIF-1α and CA9 that could be helpful in monitoring the response to neoadjuvant treatment in locally advanced rectal cancer. 25 patients with locally advanced rectal cancer were examined in the study. Both gene expression and protein levels of HIF-1α and CA9 were analyzed in fresh tissue specimens and blood samples before and after neoadjuvant therapy. The relation between tumor regression grade and tumor stage with the expression level of these detected markers were evaluated in compliance with the American Cancer Joint Committee (AJCC). Higher blood CA9 expression levels ($p = 0.013$) and lower blood HIF-1α protein levels ($p = 0.043$) were found in patients ($n = 10$, 40%) who responded completely or nearly completely to treatment. After neoadjuvant treatment, tissue CA9 ($p = 0.021$) and blood HIF-1α ($p = 0.040$) protein levels decreased significantly. Relation between HIF-1α and CA9 gene expressions in tissue was found statistically significant but it was moderate ($r = 0.480$, $p = 0.018$). The relation between HIF-1α and CA9 protein levels in blood was statistically strong ($r = 0.753$, $p < 0.001$). Detecting a positive correlation between tissue HIF-1α and tissue CA9 gene expression and also between blood HIF-1α and blood CA9 protein levels suggested that CA9 is under transcriptional control of HIF-1α. Blood CA9 gene expression and blood HIF-1α protein levels were associated with the treatment response. Tissue CA9 and blood HIF-1α protein levels decreased after neoadjuvant treatment. These genes are considered as markers and help to predict the effects of applied treatment.

P-27-061
Activation of TYRO3 receptor promotes tumor recurrence by enriching stemness of cancer cells in triple negative breast cancer

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TYRO3 receptor has been shown to be overexpressed and contributes to tumor growth, metastasis and chemoresistance in several types of cancer. Phosphatidylserine (PtdSer), an "eat me" signal exposed on outer membrane of apoptotic cells, is required for maximal activation of TYRO3 after binding to PROS1 and GAS6. In triple negative breast cancer (TNBC),

chemotherapeutics agents such as doxorubicin and docetaxel are widely prescribed but the relapse after treatment is still frequent. Meanwhile, when cancer cells undergo apoptosis by those agents, the exposed PtdSer on apoptotic bodies may activate TYRO3 receptor on other live cancer cells. The aim of our study is to investigate the role of apoptotic cell-induced TYRO3 activation on endowing cancer cells with stem-like properties, contributing to recurrence. By exploiting two TNBC cell lines (MDA-MB-231 and 4T1) which express high levels of TYRO3, we tested the effect of PtdSer-exposing apoptotic cells on activating stemness of cancer cells via spheroid formation assay, qPCR and immunoblottings and we found that spheroid formation and stemness markers increased after apoptotic cells were treated to live cancer cells. Moreover, knockdown of TYRO3 inhibits apoptotic cell-induced spheroid formation capacity and expression of stemness markers. When TNBC xenograft tumor were exposed to apoptotic cells by intra-tumoral injection, followed by tumor removal surgery, we confirmed that apoptotic cells contribute to early recurrence of secondary tumor. Neoadjuvant treatment with TYRO3 inhibitor reduced recurrence in mice receiving apoptotic cells via intratumoral injection. Taken together, we propose that although treatment of TNBC by conventional chemotherapy can inhibit tumor growth, it also results in enrichment of cancer stem cells and tumor recurrence presumably through TYRO3 activation.

P-27-062
Suppression of cell growth by the phosphorylation-deficient human Mcl-1 mutant gene

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Myeloid cell leukemia 1 (Mcl-1) is an immediate-early gene expressed during phorbol ester-induced differentiation of the human myeloid leukemia cell line, ML-1. Mcl-1 belongs to an anti-apoptotic member of BcL-2 family proteins. Kato et al. reported that spontaneous apoptosis of human neutrophils was accompanied by Mcl-1 degradation, and cyclic adenosine monophosphate (cAMP) agonist stabilizes Mcl-1 and delays spontaneous neutrophil apoptosis (FEBS Lett. 2006). Apoptosis induction system using human embryonic kidney (HEK) 293 cells treated with tumor necrosis factor-α (TNF-α) and cycloheximide (CHX) was utilized to investigate the molecular mechanism by which cAMP agonists stabilize the Mcl-1 protein. Dibutyryl-cAMP (db-cAMP), an analog of cAMP that stimulates cAMP-dependent protein kinase (PKA), inhibited apoptosis of HEK293 cells and the degradation of Mcl-1 induced by TNF-α plus CHX treatment. Furthermore, the PKA inhibitor, H89 cancelled the inhibitory effect of db-cAMP on Mcl-1 degradation, suggesting that phosphorylation of Mcl-1 by PKA could be involved in the protective effect of cAMP against Mcl-1 degradation induced by TNF-α plus CHX. In addition, a potent proteasome inhibitor, MG-132 suppressed TNF-α plus CHX-induced degradation of Mcl-1 in HEK293 cells, indicating proteasome-mediated degradation of Mcl-1. The proteasomal degradation of Mcl-1 is regulated by phosphorylation and subsequent ubiquitination. NetPhos3.1 was used to search for PKA phosphorylation sites in human Mcl-1 polypeptide, and four amino acid residues (S150, S159, S178, T266) were predicted to be the phosphorylation sites of PKA. Surprisingly, transfection of the phosphorylation-deficient human Mcl-1/4A mutant gene caused the detachment of HEK293 cells indicating apoptosis and inhibited colony formation. Overexpression of Mcl-1 has been reported in various human tumors, including blood and solid tumors, and therefore, the human Mcl-1/4A mutant gene is a potential cancer therapeutic agent. Reference: [1]Kato et al. (2006) FEBS Lett 580(19):4573-4761 https://doi.org/10.1016/j.febslet.2006.07.034

P-27-063
Elacridar decreases the activity of P-gp transporter in 2D and 3D models of ovarian cancer cell lines resistant to paclitaxel

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High mortality in ovarian cancer remains a significant challenge in oncology, with drug resistance posing a major obstacle to effective treatment. P-glycoprotein (P-gp), a member of the ABC transporters family is implicated in multidrug resistance (MDR) in ovarian cancer. Some known inhibitors of the ABC proteins are tested for possible use in therapy. One of them, elacridar, is a third-generation inhibitor of P-gp that is under clinical trials. Here, we tested elacridar on ovarian cancer cell lines in two-dimensional (2D) and three-dimensional (3D) cell culture conditions. To study the effect of elacridar on P-gp activity we used drug-sensitive cell line A2780 and paclitaxel-resistant sublines A2780PR1 and A2780PR2 that are expressing high levels of P-gp. The cells were cultured in 2D conditions and as 3D spheroids, to mimic the tumor microenvironment. We assessed P-gp activity using calcein acetoxymethyl ester (calcein-AM), a fluorescent substrate for this transporter. The cells treated with calcein-AM in the presence or absence of elacridar and were observed under the inverted fluorescent microscope. The resistant cell lines with no inhibitor added had no visible substrate, indicative of high activity of the P-gp transporter. A high calcein-AM accumulation was observed in the drug-sensitive cell line, indicating a lack of P-gp activity in these cells. High calcein-AM accumulation was noted in drug-resistant cell lines treated with elacridar, proving the effectiveness of P-gp inhibition. The results were conclusive for both 2D and 3D models. These findings confirm elacridar as a potential therapeutic agent in ovarian cancer that may prove useful in designing more effective treatment strategies to overcome MDR in ovarian cancer.

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P-27-064
The paradoxical role of VEGFR2 in restraining the tumorigenic and metastatic potential of ovarian cancer cells

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Anti-tumoral drugs targeting the VEGF/VEGFR2 axis have shown highly variable responses in clinical trials for ovarian cancer (OC) patients. Here we showed that differential levels of VEGFR2 modulate the growth and invasive potential of high grade serous ovarian cancer (HGSOC) cells. Despite counterintuitive, the silencing of VEGFR2 or its inhibition in OVCAR3 cells promote tumor growth and dissemination *in vivo*. *In vitro* data confirmed that VEGFR2 silencing promotes the acquisition of an invasive phenotype by loosening cell-ECM contacts, reducing the size and the signaling of focal adhesion contacts (FAs). This is translated into a reduced FAK activity at FAs, ECM-dependent alterations of mechanical forces through FAs and YAP nuclear translocation. Consistent with this, low expression levels of VEGFR2 in OV7 cells are associated with increased cell proliferation and motility as well as with high expression of EMT markers, when compared to OVCAR3 cells. Together, the data show that low expression, silencing or inhibition of VEGFR2 in HGSOC cells alter mechanotransduction and lead to the acquisition of a pro-proliferative/invasive phenotype which explains the need for a more cautious use of anti-VEGFR2 drugs in ovarian cancer.

P-27-065
Antitumoral effect of DIDS in prostate cancer cells PC3: a new function for an old drug

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Prostaglandin E2 (PGE2), an inflammatory mediator overexpressed in many tumors, increases principal tumor-associated phenotypes such as proliferation, migration, invasion, and loss of cell adhesion. We have found in prostate cancer cells that most of these PGE2-induced cancer-related features are due to intracellular PGE2 (iPGE2). To exert its functions, PGE2 must be taken up again through the prostaglandin transporter PGT and bind to its intracellular receptors. In the present work, we have used DIDS (4,4'-diisothiocyanostilbene-2,2′-disulfonic acid), a PGT inhibitor, to study the possible prevention of the protumoral effects of iPGE2 in PC3 prostate cancer cells by analyzing: cell proliferation (by BrdU incorporation), migration (by wound healing assay), invasion (by Transwell chamber assay), adhesion to collagen I, cell viability, and cell death. Our results shown that DIDS inhibited all these tumor phenotypes and increased the expression of 15-hydroxyprostaglandin dehydrogenase (15-PGDH), an enzyme that metabolizes PGE2. Furthermore, pretreatment with SW033291, an inhibitor of 15-PGDH, prevented the antitumoral effects of DIDS and increased iPGE2 levels. These results suggest that the antitumoral effect of DIDS acts by modulating iPGE2 levels: i) blocking the reuptake of PGE2, ii) increasing the expression of 15-PGDH, thus reducing the levels of iPGE2. In summary, the control of iPGE2 levels might be a new therapeutic strategy in the treatment of prostate cancer.

P-27-066
Potential inhibitory growth effect and BRM suppression via epidrugs in salivary gland adenoid cystic carcinoma

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Distant metastasis and high rates of recurrence are the foremost obstacles in the managing treatment and palliative care of salivary adenoid cystic carcinoma (SACC), a rare aggressive type of head and neck malignancy. Conventional treatments including surgical resection following radiotherapy, and/or chemotherapy are used to ensure tumor-free margins. However, the hidden growth mode of cancer by unexplored factors affects disappointing curative outcomes referred to the needed finding crucial stimulants and their specific molecular targets. Currently, the strong overexpression of BRM; the core subunit of SWI/SNF chromatin remodeling complexes encoded by SMARCA2, was found. This abnormality is closely related to homeostasis imbalances of epigenetically regulated machinery and cancer development. However, the relationship between these aberrations is still obviously obscure. This study aims to decipher the functional role of BRM by suppressing SMARCA2 via potential epidrugs including histone deacetylase inhibitors (HDACi), DNA methyltransferase inhibitor (DNMTi), and RNAi technology in the human UM HACC-2A cell line. HDACis: Panobinostat and Trichostatin A have shown the highest potential to inhibit cancer cell development and reduction of BRM expression when compared to untreated cells. These compounds seem to have shown higher robustness than Vorinostat, the drug used in the clinical trial phase II of SACC and DNMTi class, 5-AzaC. In addition, the epidrugs-treated cell transcriptomes reveal that these drugs disrupt several hijacking pathways of tumor progression. Reductions of genes enrichment in cell division, growth receptors, protein kinase cascade, metastatic activity, and immune checkpoint are positively correlating with BRM suppression. Collectively, our findings may serve as a promising novel approach for the development of future treatments of SACC.

P-27-067
The influence of metabolic substances treatment of salivary gland adenoid cystic carcinoma (SACC) cell line: to understand tumor development

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Adenoid cystic carcinoma (ACC) is a rare malignant tumor with high metastatic potential and poor survival rates. Unlike other carcinomas, ACCs grow slowly and have lower rates of spreading to nearby lymph nodes. Current therapies, including surgery and radiation, frequently do not lead to satisfactory results, and there are no effective systemic treatments approved for advanced or metastatic ACC. Urgent basic research is needed to develop improved diagnostic and therapeutic approaches. SMARCA2, implicated in various diseases including cancer, undergoes mutations, overexpression, or epigenetic silencing. While BRM expression is generally decreased in cancers, its overexpression is linked to chemoresistance in pancreatic and ovarian metastatic cancers. Tumor invasion and subsequent metastasis are critical aspects of cancer progression. Our previous study indicated that ACC is featured by the BRM overexpression. Here, we investigate the effect of metabolic substances on BRM alteration in the SACC cell line and its overall impact on cell properties. Proliferation assays in the human ACC cell line (UM-HACC 2A) revealed that metabolic substances like brain extract and glucose notably boosted SACC cell viability, enhancing tumor clonogenicity and migration. RNA sequencing analysis further elucidated the transcriptional changes induced by these substances, highlighting their influence on key pathways such as cellular metabolism, cell cycle regulation, nervous system development, and growth factor signaling. Through ChIP analysis of our samples and comparison with clinical samples, we aim to identify target genes implicated in SACC progression, with the ultimate goal of improving therapeutic interventions and patient outcomes.

P-27-068
Rac1-SUMO1 interacts with Caveolin-1 to promote breast cancer progression

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It is estimated that 1 in 8 women will be diagnosed with breast cancer during their lifetime, making it the most common female tumor. Most deaths related to breast cancer are due to the development of chemoresistance and metastases, processes linked to the epithelial-mesenchymal transition (EMT). Activating factors of the EMT process have been associated with higher mortality rates in cancer patients. Metastasis involves cancer cells or clusters of cells with mesenchymal traits breaking away from the primary tumor mass, invading the extracellular matrix (ECM) and endothelium, and spreading to secondary sites. This invasion process requires the formation of cellular protrusions by converting monomeric globular actin (G-actin) to polymerized filamentous actin (F-actin). The small GTPase RAC plays a crucial role in reorganizing the actin cytoskeleton to create these cellular protrusions, facilitating the mobility of mesenchymal-like cancer cells. Post-translational modifications (PTMs), such as SUMOylation (Small Ubiquitin-like MOdifier), are essential for regulating protein activity. SUMOylation can impact various cellular processes and has been implicated in tumorigenesis by affecting the PTM of key proteins like tumor suppressors and oncoproteins, including c-JUN, cFOS, MDM2, and the GTPase RAC1 [Previously published in: Castillo-Lluva et al. (2010) Nat Cell Biol. (11):1078-85]. Our group has shown that SUMOylation of RAC1 is crucial for the invasion of breast cancer cells [Previously published in: Lorente et al. (2019) J Cell Sci. 22;132(20)]. To identify the mechanism through which RAC1-SUMO1 promotes the invasive capacity of BC cells, we conducted mass spectrometry analysis and identified Caveolin-1 (CAV1) as a new interacting protein with RAC1-SUMO1. Our research demonstrates that CAV1 contains a SIM motif that is essential not only for the interaction between RAC1-SUMO but also for CAV1's role in promoting breast cancer progression.

P-27-069
Understanding the impact of protein corona on the biological effect of gold nanoparticles in 2D and 3D breast cancer models

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Gold nanoparticles (AuNPs) are emerging as elective candidates for selective breast cancer damage. However, their use in clinical practice remains limited due to an incomplete understanding of the factors at the bio-nano interface both *in vitro* and *in vivo*. Upon exposure to biological fluids, proteins rapidly adsorb to AuNPs and form protein corona (PC), which can modify the biological identity of NPs. To this end, we hypothesized that the PC governs AuNPs-breast cancer interactions. To test this hypothesis, PC was formed by incubating AuNPs (sphere- or star-shaped) in the cell culture medium (supplemented with 10% fetal bovine serum) of SK-BR-3 and MCF7 breast cancer cell lines at 37°C and at different incubation times. PC formation was assessed by dynamic light scattering (DLS), zeta potential measurements, UV-Vis spectrophotometry, SDS-PAGE electrophoresis, Cryo-EM, and bicinchoninic acid (BCA) assay. Mass spectrometry (MS)-based proteomic analysis was used to assess PC composition. In 2D cultures, both differently shaped gold nanoparticles without PC significantly reduced the viability of cancer cells by altering the expression of apoptotic proteins. Interestingly, PC reversed these effects. Similarly, the presence of PC affected the uptake of AuNPs by decreasing the level of internalization in breast cancer cells. The presence of PC influenced the biological effects of AuNPs in 3D breast cancer models by increasing the DNA content and reducing LDH (lactate dehydrogenase) release. Preliminary proteomic data revealed unique protein patterns based on the shape of AuNPs. Bioinformatic analyses are underway to identify proteins related to the uptake and biological effects of AuNPs. These findings are expected to have implications for the future development of AuNPs-based anticancer therapies

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P-27-070
Interplay of FAK, BMP, and mTOR signaling pathways regulates heparan sulfate glycosaminoglycan homeodynamics in mesenchymal stem cells

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Heparan sulfate glycosaminoglycans (HS-GAGs) are polysaccharides that are present in a wide range of physiological and pathological processes. The aim of this study was to examine the regulation of HS-GAG biosynthesis in bone marrow-derived mesenchymal stem cells (BM-MSCs) and the mechanisms involved. Through quantitative analysis, it was determined that HS-GAG biosynthesis follows a specific kinetic model, with levels reaching a plateau after approximately 48 hours. When treated with heparinase III (HepIII), the homeostatic regulation of HS-GAGs was disrupted, leading to a linear increase in their levels. The presence of intact HS-GAGs played a role in facilitating the assembly of the matrix (fibronectin, collagen I) and the dynamics of cell adhesion during the early stages (1–8 h), which were negatively affected by HepIII treatment. Transcriptomic analysis did not reveal any differentially expressed genes, suggesting that the regulatory mechanism is non-transcriptional. The saturation pattern of HS-GAG levels was found to correlate with the focal adhesion kinase (FAK), bone morphogenetic protein (BMP), and mammalian target of rapamycin (mTOR) signaling pathways. Inhibition of these pathways resulted in a decrease in HS-GAG biosynthesis, indicating cross-talk between them. FAK inhibition downregulated the BMP and mTOR pathways, while BMP inhibition affected the BMP and mTOR pathways. mTOR inhibition disrupted HS-GAG biosynthesis and also impacted the BMP and FAK pathways. Notably, the stimulation of HS-GAG biosynthesis by BMP2 was mediated through the mTOR pathway, and could be negated by mTOR inhibition. These findings provide insight into the tightly regulated homeodynamic mechanism for HS-GAG biosynthesis in BM-MSCs, which is governed by the interplay between the FAK, BMP, and mTOR signaling pathways, ultimately impacting matrix assembly and cell adhesion processes.

P-27-071
CCL8 and IL-8 mediate the crosstalk between endothelial cells and triple-negative breast cancer (TNBC) cells, resulting in the aggravation of TNBC

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Triple-negative breast cancer (TNBC) is an aggressive type of breast cancer with a poor prognosis for which no effective therapeutic measures are currently available. The present study aimed to investigate whether interactions with endothelial colony-forming cells (ECFCs) promote aggressiveness of TNBC. We showed that co-culture increased the invasive and migratory phenotypes of both MDA-MB-231 and ECFCs. Through a cytokine antibody array and RT-PCR, we revealed that co-culture induced secretion of the CCL8 from ECFCs and that of IL-8 from MDA-MB-231 cells. CCL8 was crucial for ECFC-induced IL-8 secretion and invasion of MDA-MB-231 cells as well as for MDA-MB-231-enhanced MMP-2 secretion and angiogenesis of ECFCs. We suggest c-Jun as a transcription factor for CCL8-induced IL-8 expression in MDA-MB-231 cells. IL-8 was important for co-culture-induced CCL8/MMP-2 upregulation and invasion of ECFCs. Notably, our findings reveal a positive feedback loop between CCL8 and IL-8, which contributes to the aggressive phenotypes of both ECFCs and TNBC cells. Using an MDA-MB-231 cell-based xenograft model, we show that tumor growth and metastasis are increased by co-injected ECFCs. Increased IL-8 was observed in tissues with bone metastases in mice injected with conditioned media from co-cultured cells. High IL-8 levels are correlated with poor recurrence-free survival in TNBC patients. Together, these results suggest that CCL8 and IL-8 mediate the crosstalk between ECFCs and TNBC, leading to aggravation of tumorigenicity in TNBC.

P-27-072
Research on deriving the key molecules of gender-specific cancer malignancy mechanism

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Gender differences in cancer can be expressed as gender differences for extrinsic or intrinsic factors, such as exposure to cancer-causing external environments, cancer-related gene expression and regulatory mechanisms under the influence of sex chromosomes or sex hormones, and biological responses induced by stress in cells and tissues. Therefore, it is essential to consider gender specificity in the diagnostic procedures for cancer and the evaluation of cancer treatment. In this study, 314 genes showing statistically significant differences in expression depending on sex hormone estrogen treatment were identified by comparative analysis of the MCF7 cell line, a female-derived cell line, using total

RNA sequencing. In these genes, the roles of protein coding genes accounting for 50.8% and non-coding genes accounting for 38.5% were analyzed and verified using Gene Set Enrichment Analysis (GSEA) and DAVID Gene Functional Classification Tool in order to determine gender-specific significance (-log2FC- > 0.5849, p-value ≤ 0.05). Five key molecules for cancer malignancy were identified and explored in theses analysis. Research on these key molecules and related mechanisms such as reactive oxygen species pathway, cholesterol homeostasis, and hedgehog signaling derived from this study will contribute to providing new strategies for anticancer treatment that take gender specificity into account. Funded by NRF-2022R1F1A1076029.

P-27-073
An antibody-based strategy targeting oncogenic mERG in prostate cancer

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Prostate cancer is a leading cause of cancer death worldwide and the most common cancer in men. A gene fusion involving the ETS transcription factor ERG and the 5′ region of the TMPRSS2 gene occurs in more than half of prostate cancers. This fusion results in ERG overexpression that drives prostate cancer growth. However, ERG requires additional cooperating factors to drive prostate cancer progression. Recently, we have shown that lysine 362 of ERG is methylated by EZH2, a histone-lysine N-methyltransferase highly expressed in advanced prostate cancers. This unique modification occurs within the internal autoregulatory domain and enhances ERG transcriptional and oncogenic activity. Thus, the selective blocking of methylated ERG activity could represent a valid strategy to treat and prevent progression of ERG-positive prostate cancers. Transcription factors are widely considered undruggable targets for conventional small-molecule drugs. For this reason, we generated a monoclonal antibody that selectively recognizes the methylated form of ERG (mERG). To allow its intracellular delivery, we shrank the antibody into a single-chain variable fragment (scFv) and used a DNA expression construct for its delivery. *In vitro* experiments demonstrated that upon DNA transfection, our anti-mERG scFv efficiently and specifically blocks mERG oncogenic activity in prostate cancer cells. Additionally, preliminary experiments in xenografts mouse models showed that the *in vivo* delivery of the DNA encoding for the anti-mERG scFv significantly reduces tumor growth without causing any toxic effect. These findings collectively underscore the potential of our antibody-based strategy as a promising therapeutic option for the treatment of ERG fusion-positive prostate cancers.

P-27-074
Melanoma agressiveness is determined by properties of melanin pigment

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Melanins are a group of natural, macromolecular pigments that in humans are present mainly in skin, hair, retina and inner ear. Photoprotection is one of the most important roles of melanin. Melanins absorb a broad spectrum of photon's energy, including UVA (320-400 nm) and UVB (280–320 nm), and effectively dissipate it into heat. Recent studies suggest that melanins, may exhibit both not only photoprotective but also phototoxic behavior, which can be utilized in novel therapeutic strategies. Photoreactivity of melanins involves the generation of reactive oxygen species including singlet oxygen, leading to cell apoptosis through the oxidation of DNA and other cellular components. The aim of this study was to investigate the photoprotective and photoreactive properties of melanins derived from different melanoma cell lines and melanocytes. Melanoma cell lines with different level of malignancy, such as A375, WM9, WM115, MNT-1, SKMEL-188, were analyzed along with melanocytes from hiPSC (human induced pluripotential stem cells) used as a control. Melanin pigments were identified based on their EPR (Electron paramagnetic resonance) spectra. Their photoreactive properties were determined using EPR oximetry, EPR spin-trapping and time-resolved singlet oxygen phosphorescence. Melanoma melanins were found to photoproduce ROS (reactive oxygen species) with a higher efficiency compared to hiPSC, especially those derived from A375 and SKMel-188 cells. In cellular experiments, where HaCaT (human immortalized keratinocytes) cells were used as one-layer skin model, the presence of melanoma melanins decreased viability of HaCaT cells, while presence of hiPSC did not, suggesting decreased photoprotection exerted by the former. The results obtained in this study suggest that photoreactivity of melanoma-derived pigments correlate with their phototoxicity and the malignancy potential of melanoma cells.

P-27-075
Evaluating flavonoids in pancreatic cancer therapy: targeting dual-specificity tyrosine-phosphorylation-regulated kinase 1B (DYRK1B)

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Pancreatic cancer presents significant treatment challenges, characterized by high mortality rates and limited effectiveness of current therapies such as surgery, radiation, and chemotherapy. Despite the use of various chemotherapy drugs, the majority of patients with pancreatic cancer develop metastasis, and the cancer cells often exhibit strong resistance to chemotherapy, resulting in poor outcomes. Studies have shown that pancreatic cancer cells can escape chemotherapeutic attack by entering a quiescent state during cell division. In this state, they express elevated levels of dual-specificity tyrosine-phosphorylation-regulated kinase 1B (DYRK1B), which contributes to their survival by inducing quiescence and reducing reactive oxygen species (ROS), thereby evading drug effects. Our research focused on developing inhibitors of DYRK1B as a novel therapeutic strategy to force

cancer cells out of quiescence and make them more susceptible to chemotherapy. We discovered a promising DYRK1B inhibitor, Fv03, through computer simulations and enzyme-based assays. In tests on PANC-1 pancreatic cancer cells, which express high levels of DYRK1B and are resistant to the chemotherapy drug gemcitabine, Fv03 effectively killed these cells. Fv03 also decreased the phosphorylation of p27Kip1, indicating successful inhibition of DYRK1B's activity. This inhibition increased ROS levels in PANC-1 cells, causing cell death. However, Fv03 did not exhibit a synergistic effect when combined with gemcitabine. Future efforts will focus on optimizing Fv03 to improve its efficacy in blocking DYRK1B and to elucidate the molecular mechanism by which it promotes cancer cell death. This study lays the groundwork for further exploration of DYRK1B as a target for pancreatic cancer treatment and opens up new avenues for developing effective therapies for this devastating disease.

P-27-076
Novel benzisoxazole-estradiol chimeras induce mitochondrial dysfunction and apoptosis in cancer cells

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Despite many novel and promising therapeutic approaches, chemotherapy is still administered as the first-line treatment when fighting cancer. Both benzoxazoles and benzisoxazoles are among the most important candidates in drug design and discovery since they display versatile properties and excellent pharmacological activities. In our work, we aimed to evaluate the anticancer activity of some novel benzoxazole–estradiol, and benzisoxazole–estradiol chimeras. We tested the performance of these chimeras on various non-cancerous and cancer cells, also on multidrug-resistant cancer cells. Cell viability was assessed by 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide assay. Modulated gene expression was examined by quantitative polymerase chain reaction. Fluorescent microscopy techniques were utilized to quantify intracellular reactive oxygen species (ROS) and mitochondrial damage. We noticed an interesting structure-function relationship, namely that 3'-substituted benzisoxazole-estradiol chimeras carrying a nonpolar substituent exhibited excellent antiproliferative properties, while 2-substituated benzoxazole-estradiol hybrids carrying the same substituent did not. The most promising benzisoxazole derivatives displayed great tumor specificity, with selectivity indexes ranging from 10 to 40. By measuring the expression of apoptosis marker genes, we found that the selected steroid derivatives upregulate the expression of p21 and BAX in cancer cells. Based on the literature data, we hypothesized that mitochondrial dysfunction might be the molecular mechanism behind this action. By staining the cells with appropriate dyes, we confirmed that treatment with the selected benzisoxazole–estradiol derivatives results in accumulated intracellular ROS and decreased mitochondrial membrane potential. Our results indicate that mitochondrial dysfunction is a core molecular mechanism behind the observed outstanding anticancer action of benzisoxazole-estradiol chimeras.

P-27-077
Unveiling the therapeutic potential of cladosporols in prostate cancer: a comprehensive experimental approach

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Chemoprevention, employing natural and synthetic compounds, shows a promising strategy against cancer. Cladosporols, secondary metabolites from *Cladosporium tenuissimum*, exert potent inhibition over cancer cell proliferation via PPARγ-mediated gene modulation [previously published in: Rapuano R. et al. (2021) Biophys Acta Gen Subj 1865, 129973]. Our study focused on cladosporols anticancer properties in human prostate cancer cells, given the high mortality rates associated with metastatic and androgen-resistant prostate cancer. To evaluate the impact of cladosporol treatment on PC-3, the most aggressive cell type, we conducted cell proliferation and migration assays. Cladosporols reduced both PC-3 cell growth and migration, as confirmed by modulation of expression of pivotal proteins associated with these processes [previously published in: Rapuano R. et al. (2024) Biochem Pharmacol, 116097]. Importantly, cladosporols also induced apoptosis and downregulation of enzymes involved in lipid biosynthesis, emphasizing their potential therapeutic significance. Moreover, we demonstrated that cladosporols exerted an inhibitory effect on adipocyte differentiation and secretion of factors from cladosporol-treated 3T3-L1 preadipocytes thus inducing a significant arrest in both proliferation and migration of PC-3 cells and hence highlighting the multifaceted impact of cladosporols on the intricate interplay between tumor and stroma. These effects were also evident even in dynamic experimental conditions, substantiated by a microfluidic bioreactor, essential to mimic physiological conditions, providing a more accurate understanding of cellular responses under continuous oxygen and nutrient flow. Our data, highlighting the strength of cladosporols inhibitory effects, revealed their potential in managing prostate cancer as promising therapeutic tools, paving the way for future targeted chemopreventive interventions.

P-27-078
Discovering next-generation anticancer drugs through the design and characterization of selectively targeting antimicrobial peptides

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Antimicrobial peptides (AMPs) are potential next-generation drugs, since they are natural peptides, produced by all organisms, active against multiple classes of pathogens. As they show a broad spectrum of biological activities, strong efforts are in progress to bring AMPs into clinical use. Development of AMPs as highly effective/selective anticancer drugs would potentially reduce the side-effects of current chemotherapy. To this aim, we explored the anticancer activity of mutants derived from a natural AMP, named chionodracine (Cnd), produced by an Antarctic fish inhabiting an extreme environment. Based on its scaffold, we previously designed a mutant peptide (KHS-Cnd) along with its

shorter forms active against ESKAPE/fungal pathogens. [Previously published in Olivieri et al. (2018) RSC Adv 8, 41331-41346; Bugli et al. (2022) Int J Mol Sci 23, 2164.] Herein, we assessed the effects of three short forms of KHS-Cnd and their respective myristoylated (myr) peptides on the human cervical cancer cell line HeLa. MTT assays showed that myr-peptides possessed selective cytotoxicity towards HeLa cells (IC_{50} of 32–47 μM), whereas non-myr peptides failed to kill cancer cells. Interestingly, all designed shorter peptides, including myr-peptides, did not affect the viability of two primary human fibroblast cell lines. Therefore, the improved activity of myr-peptides may be attributed to the specific interaction of the myr region with the cancer cell membranes, which exhibit distinct features compared to those of healthy cells. Moreover, scanning electron microscopy and TUNEL assay demonstrated that myr-peptide, with the lowest IC_{50} and no hemolytic activity, induced apoptosis in HeLa cells as well as a state of advanced necrosis. These findings indicate that modifying natural AMPs, such as by myristoylation, may be a promising strategy to develop selective anticancer drugs.

P-27-079
Exosomal EPHA2 and EHD1 proteins promote lung cancer metastasis

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The cause of cancer-associated death is due to metastasis. The mechanism of exosomal proteins-mediated metastasis is unclear. Here, we used animal experiments to demonstrate that highly invasive lung cancer cells, CL1-5, can promote the metastatic ability of low invasive lung cancer cells, CL1-0. Also, we showed that the exosome of CL1-5 cells could increase the cell mobility of CL1-0 cells. To prove that the increase of cell migration and cancer metastasis in CL1-0 cells were contributed by exosomes secreted by CL1-5 cells. We used GFP-CD9 to track the exosomes of CL1-5 and found that CL1-0 could receive the exosomes secreted from CL1-5 in the co-cultured condition. To exclude the effect of cell contact, we co-cultured the cells in a transwell system with a smaller pore to prevent the cells from penetrating the membrane. The results showed that CL1-0 indeed received the exosomes derived from CL1-5 and had higher cell migration ability. To identify which proteins affect cell migration, we used the LC-MS/MS to analyze the exosomal proteins secreted from CL1-0 and CL1-5. The EH-domain-containing protein 1 (EHD1) and Ephrin receptor A2 (EPHA2) protein expression were higher in exosomes secreted from CL1-5 than those from CL1-0. Knockdown of EPHA2 and EHD1 downregulated the cell migration. Intriguingly, the exosomes secreted from EPHA2-knockdown or EHD1-knockdown CL1-5 cells would lose the function of increasing migration ability compared to the parental cells-secreting exosomes. We demonstrate that EPHA2 and EHD1 in the exosomes of highly invasive CL1-5 cells will be transmitted to low-invasive CL1-0 cells to promote cell migration and cancer metastasis. *The authors marked with an asterisk equally contributed to the work.

P-27-080
Cardiotrophin 1: a novel SASP marker in senescent MCF-7 cells

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Cellular senescence is a permanent arrest of the cell cycle in response to many stress factors and is characterized by excessive secretory activity called senescence-associated secretory phenotype (SASP). Studies have shown that senescent cells have paracrine protumorigenic effects on the cancer microenvironment via SASP. Therefore, developing new treatment methods in cancer targeting SASP has recently become important. However, understanding how senescence contributes to cancer is difficult due to the absence of standardized SASP markers. IL6 is defined as a hallmark marker of SASP, consistently exhibiting increased expression in normal senescent cells. It has been demonstrated that cardiotrophin 1 (CTF1), a member of the IL6 family, secreted from breast cancer cells has a role in cancer metastasis. In this study, we induced senescence by incubating three different cancer cell lines, MCF7, A549, and HeLa with doxorubicin and analyzed senescent cell secretome for IL6 and CTF1. The treatment of cells with 300 nM doxorubicin significantly increased the number of senescence-associated beta-galactosidase positive cells indicating doxorubicin induced senescence in all three cell lines. In studies where we analyzed the secretome content, while the secretion of IL6 increased in senescent HeLa and A549 cells, IL6 could not be detected in the secretome of senescent MCF7 cells. On the contrary to this finding, CTF1 secretion increased in senescent MCF7 cells, while CTF1 level could not be measured in the secretome of senescent HeLa and A549 cells. It was also found that CTF1 expression increased in senescent MCF7 cells. CTF1 expression was detected in senescent HeLa and A549 cells, but no change was found in senescent cells compared to the control group. Taken together these findings indicate that IL6 cannot be considered as a SASP marker for every senescent cell and that CTF1 can be considered as a novel SASP marker for senescent MCF7 cells. This study was supported by the Scientific Project Unit of Gazi University (Grant code: TYL-2023-8901).

P-27-081
Anti-tumoral potency of Tanshinone IIA on malignant pleural mesothelioma

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The lack of an effective treatment for malignant pleural mesothelioma (MPM) necessitates the search for new targeted and/or adjuvant drugs. Tanshinone IIA (Tan IIA), a natural compound isolated from the roots and rhizomes of the Danshen plant (*Salvia miltiorrhiza*), is currently used in the treatment of various diseases such as cardiovascular, diabetes, stroke, arthritis, and sepsis. Recently, Tan IIA is emerging as a notable candidate due to its broad anti-tumor potential in various cells, including lung cancer, breast cancer, cervix cancer, ovarian cancer, leukaemia, glioma, osteosarcoma, hepatocellular cancer, gastric carcinoma, colorectal cancer, and prostate cancer. In this study, the anti-carcinogenic effect of Tan IIA on MPM and mesothelial cells was investigated for the first time. Our findings showed that Tan IIA caused cytotoxicity and led to G2/M arrest; these effects were

achieved through suppression of ERK1/2 pathway, cyclin D expression, and stimulation of p21 and p27 expressions in a p53-dependent manner. Moreover, the dose-sensitive increase of JNK and p38MAPK phosphorylations and the expression ratio of Bax/Bcl2 proteins indicated that mitochondrial apoptosis was induced in Tan IIA-exposed cells. Additionally, selectivity index calculations revealed that MPM cells exhibited higher sensitivity to Tan IIA than non-tumorous mesothelial cells. These results shed light on the possibility that this compound may also be applicable in the treatment of this cancer.

P-27-082
Blocking TGF-β inhibits cell proliferation and invasiveness in malignant pleural mesothelioma: a new prognostic marker and pharmacological target?

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Malignant pleural mesothelioma (MPM) is a rare and deadly tumor associated with asbestos exposure, characterized by a poor prognosis and a challenging therapeutic approach. As previously published in: Turini S et al. (2019) Int J Mol Sci 20,150, asbestos fibers evoke the release of several cytokines, including transforming growth factor-β (TGF-β), that drives epithelial-mesenchymal transition (EMT) in mesothelium. EMT is a crucial event in tumor progression, involved in metastatization and aggressiveness. The aim of this study is to explore the role of TGF-β in cell proliferation, migration, and invasiveness of MPM, via TGF-β inhibition of commercially available human biphasic mesothelioma cell line (MSTO-211H) and primary human MPM cell lines: MM404 (epithelioid), MM421(biphasic) and MM432 (sarcomatous). We performed TGF-β siRNA knockdown experiments in our cell models and incubated the same cell lines with anti-TGF-β antibody. The results highlight that TGF-β inhibition, through TGF-β knockdown or anti-TGF-β incubation, significantly reduced MPM cell proliferation, as measured by MTT assay, as well as real-time PCR and western blotting evaluations of proliferating cell nuclear antigen (PCNA). Moreover, TGF-β siRNA transfection increased mRNA levels of epithelial markers and decreased mesenchymal ones, showing that mesenchymal-epithelial transition (MET) occurred. Besides, by scratch assay, we observed a reduction in the migration rate of our cell models. By flow cytometric analysis, we saw a cell cycle block, so confirming the effect of TGF-β in cell proliferation. Finally, we evaluated TGF-β inhibition in MPM spheroids, reporting a slowed growth rate and/or size reduction after anti-TGF-β antibody incubation, as well as an attenuated invasive capacity. Taken together, these findings suggest that TGF-β blockade has a negative impact on cell proliferation and invasiveness in MPM, proposing TGF-β as a new prognostic marker and/or promising pharmacological target.

P-27-083
Deciphering cell-autonomous effects of VEGF signaling in melanoma

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The introduction of targeted therapies and immunotherapy represented the most significant advances in the treatment of melanoma. However, the onset of resistance remains a challenge to overcome. Vascular endothelial growth factor A (VEGFA) is a key promoter of both tumor angiogenesis and immunosuppression and an attractive target for combinatorial cancer therapy. We have recently demonstrated that VEGFA removal improved the antitumor efficacy of BRAF inhibition, through the activation of M1-macrophages and CD8+ T cells infiltration that boosted immune checkpoint blockade. Despite this, we observed *in vivo* that melanoma syngeneic models may show different sensitivity to anti-VEGFA antibody. Interestingly, we found that efficacy of anti-VEGFA treatment correlates with VEGFR1 expression in tumor cells, suggesting that VEGFA, may drive tumor growth also in a cell-autonomous fashion. In order to understand the role of VEGFR1 expression in melanoma cells, we evaluated VEGFR1 expression pattern across diverse cancer types using Cancer Cell Line Encyclopedia database. Data-mining showed that VEGFR1 transcripts were more highly expressed in melanoma cancer cell lines compared with other cancer types. We then screened a panel of human and murine melanoma cell lines and we confirmed VEGFR1 expression in the majority of cell lines also at protein level. Importantly, we observed *in vitro* melanoma cells failed to respond to recombinant VEGFA, while sh-RNA-mediated inhibition of VEGFR1 decreases proliferation, suggesting an autocrine mechanism. Moreover, tracking of individual cells by video microscopy over 24 h revealed a significant reduction in cell migration upon VEGFR1 silencing. We also observed that silencing of VEGR1 significantly reduced the invasive properties of tumors spheroid in a 3D model. Collectively, these findings suggest that VEGFA might promote autocrine growth of tumor cells expressing functional VEGFR1, contributing to melanoma progression and spreading.

P-27-084
The MCPIP1 protein – a key protector against neoplastic transformation

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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. The main aim of our research is to study the importance of MCPIP1 on neoplastic transformation of normal, epithelial kidney cells and hepatocytes. To examine the effect of MCPIP1 in normal cell line TCMK-1, was transduced to overexpress mutated, inactive form of MCPIP1 (pLIX

D141N) and control (pLIX PURO). We analyzed the levels of CSCs markers by western blot and qPCR. Next, cells were injected subcutaneously into NOD-SCID mice to check if mutation of MCPIP1 will induce tumor growth *in vivo*. Mice with MCPIP1 knock-out in hepatocytes were stimulated with DEN to induce hepatocellular carcinoma. After 9 months, mice livers were evaluated by qPCRs and IHC stainings. We have shown that pLIX D141N cells were characterized by higher levels of the c-Met receptor, vimentin, Twist, CD44 and c-Myc, which are the markers of epithelial to mesenchymal transition and cancer stemness. Next, we checked whether MCPIP1 mutation in normal cells predisposes them to proliferate when administered to mice. We found that, D141N mutation caused the growth of large tumors with higher expression of Cd44, Klf4 and Myc and number of CTCs. Mice with MCPIP1 KO in hepatocytes developed more liver tumors than control mice after DEN administration with increased expression of EMT and CTCs markers. We believe that the MCPIP1 protein may be a marker of tumor initiation and play a key role in neoplastic transformation by regulating the changes in cell phenotype and levels of CSCs markers. This study was supported by National Science Center grants no. 2017/26/E/NZ5/00691, 2022/47/B/NZ5/02724

P-27-085
Development of 3D printable cell-array for *in vitro* modeling of breast cancer

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Breast cancer is the second most common cancer and the second leading cause of cancer death in women. In advanced stages of the disease, breast cancer can spread and metastasize to the bone, contributing to malignant progression. The role of tissue stiffness and remodeling of the tumor microenvironment are relevant in influencing cancer progression and invasiveness, but they are still poorly understood. In this study we aimed to investigate the effect of the bone tissue stiffness on breast cancer cell behavior, using 3D cell-biomaterial systems [Buonvino S. et al. (2021) Int. J. Mol. Sci. 22] to modeling the *in vivo* conditions. For this purpose, we developed a 3D-printable cell-array, that is a tunable and reproducible platform on small scale, where each compartment could mimic physiological cancer environment in shape and rigidity close to bone tissue. In this system, we observed that in highly metastatic breast cancer line MDA MB 231, embedded in PEG-silk fibroin (PSFs) hydrogel spheres [Buonvino S., Arciero I. et al. (2023) Materials Today Bio 23, 100862; Ciocci M. et al. (2018) Int.J. Biol. Macromol. 108, 960-971] into the array's cavities, the increasing stiffness promotes trans-differentiation into osteoblast-like cells and the production of breast microcalcifications. Moreover, we tested this 3D model also as a platform to evaluate the cell response to the therapy, in particular investigating the drug sensitivity of the cancer cells to chemotherapeutics, observing a decrease in drug resistance over time. Supported by Medilife S.p.A and Regione-Lazio "Intervento per il rafforzamento della ricerca e innovazione nel Lazio - incentivi per i dottorati di innovazione per le imprese e per la PA- L.R. 13/2008".

P-27-086
Antiproliferative effects of a new bis-indolinone on ovarian cancer cells

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Knoevenagel adducts exhibiting two indole nuclei linked to an aromatic core have allowed the identification of derivatives endowed with interesting antiproliferative activity profiles. We focused our study on the effect of a new bis-indolinone compound on the ovarian cancer cell line (IGROV1). Our compound showed remarkable selectivity against IGROV1, without affecting healthy human fibroblast cells. The technique of quantitative phase imaging of live cells was performed to investigate the effects on cell proliferation, cell morphology and motility. Moreover, to elucidate the antitumor mechanism of action of the new bis-indolinone compound, we studied its effect on the modulation of histone acetylation and genes expression. We focused on the expression of cyclin D1 and RAD51, involved in DNA repair process. Our results showed that this compound causes proliferation arrest. The antiproliferative effect is characterized by a growth arrest in the G0/G1 phase, leading to a decrease in the population in S and G2/M. The treatment induces a reduction in motility, in particular decreases individual cell displacement and velocity at a higher rate in IGROV1 compared to normal fibroblast cells. Epigenetic analysis showed histone hyperacetylation, leading to downregulation of cyclin D1 and a5 subunit of integrin b1 gene transcription. It's known that cyclin D1 downregulation increases cell sensitivity to radiation *in vitro* and *in vivo* reducing RAD51 recruitment to damaged DNA. Furthermore, we have shown that 24 hours treatment with our compound increases radiosensitivity by causing greater DNA damage, similar to DNA damage induced by cyclin D1 gene silencing. This new bis-indolinone compound is a promising anticancer tool that could be used in association with chemo- and radiation-therapy. Financed by the European Union - NextGenerationEU through the Italian Ministry of University and Research under PNRR - Mission 4, Component 1, Investment 4.1 (DM 118/2023). *The authors marked with an asterisk equally contributed to the work.

P-27-087
Unveiling transcriptional and metabolic rearrangements in the transition from 2D to 3D growth conditions of preclinical cancer models

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Cancer is a heterogeneous disease that needs suitable models to simulate its complexity. To this end, three-dimensional (3D) models, provide a good compromise between biological complexity and feasibility of handling as compared to animal models and bi-dimensional (2D) monolayer cell cultures. Among these, spheroids obtained from immortalized cell lines are organized cellular aggregates in which gradients of nutrients, oxygen and waste products contribute to simulate the intra-tumor heterogeneity [Zanoni M et al. (2016) Sci Rep, 6, 1-11]. In this work we have compared 2D (monolayer) and 3D (spheroids) models of breast and bladder cancer cell lines, studying their proliferative rate, respiratory and glycolytic metabolism and performing transcriptomic analysis. The results show that within the spheroids the number of viable cells does not increases over time and the percentage of cells in S-phase of cell cycle decreases compared to 2D cultures. The transcriptomic analysis corroborates these findings proving that the 2D-3D transition induces a modification of the whole transcriptome, especially in genes involved in cell proliferation, survival, and differentiation pathways, that are downregulated in 3D models. The analysis of metabolic parameters demonstrates that the transition to the 3D architecture is not necessarily linked to an increase of the glycolytic pathway as could be supposed considering the physiological formation of a hypoxic core, and this metabolic switch seems to be predicted from the metabolic phenotype in 2D condition. Since all these observations refer to average values among all cells of the spheroid, not addressing the relevant problem of spatial diversification of cells within it, we are adapting a cycling immunolabeling method (IBEX, Radtke AJ et al. (2022) Nat Protoc, 378–401) to spheroids' cryosections enabling to spatially resolve the differences in the expression of metabolic, proliferative and differentiative markers within the spheroids.

P-27-088
Targeting of human aldehyde dehydrogenase 1A3 in solid tumours to develop molecules to use in boron neutron capture therapy and as diagnostics tools

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The activity of a class of enzyme named aldehyde dehydrogenases (ALDHs) correlates with poor outcome for solid tumours, because their over expression sustaining cell proliferation and chemoresistance of cancer stem cells (CSCs). Accordingly, potent and selective inhibitors of ALDHs may represent a novel CSC-directed treatment paradigm for $ALDHs^+$ cancer types. We focus our attention on the Isoenzyme ALDH1A3 belongs to enzymatic superfamily of 19 different isoforms involved in the oxidation of a plethora of aldehydes to respective carboxylic acids, through a $NAD(P)^+$-dependent reactions. We initially studied its role in two different type of tumour that have low survival rate at five years of diagnosis. Glioblastoma that is the most aggressive primary brain tumour and malignant pleural mesothelioma (MPM), for both of which effective treatments and efficient tools for an early-stage diagnosis are lacking. Thanks to the structural analysis of human ALDH1A3 model, we identified potent and selective inhibitor towards ALDH1A3 able to hinders cancer cells growth, invasiveness and stemness. In addition, we patent a curcumin-based fluorescent molecule, that illuminates CSCs thank to its selective ALDH1A3 binding, and *in-vivo*, accumulates only in glioblastoma cancer cells, allowing us the identification of the growing tumour mass. Using our developed tools, we are able to start studies regarding the molecular mechanism underlying role of ALDH1A3 in the malignancy of these two types of cancer. At the end, thanks to our structural drug design approach, we are selecting new boron fluorescent probes that bind only ALDH1A3 and selectively accumulate in tumour growing cells. Our results allowed us to obtain extremely powerful and novel molecules that can be developed a promising approach for improving precise tumour treatments of patients affected by ALDH1A3-positive cancers using boron neutron capture therapy (BNCT).

P-27-089
Investigating the anticancer advantages of pairing *Rumex obtusifolius* herb extract with 5-fluorouracil against breast cancer *in vivo*

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Currently, chemotherapy drugs used in anticancer therapy have many side effects. That is why research is currently being conducted to find new effective treatment methods. One of the methods used is based on the combined therapy of herbal compounds and chemotherapeutic drugs. The use of this method makes it possible to assess metabolic pathological changes in the tumor microenvironment and adjacent regions, contributing to their regulation by revealing their molecular mechanisms. The work aimed to study the quantitative changes of some key components under the influence of *Rumex obtusifolius* (0.25mg/kg) and 5-fluorouracil (5-FU, 10mg/kg) combination related to cancer development such as IL-2, TNF-a, COX-2, MMP-2, and VEGF-a in breast tumors, and lungs in 7,12-Dimethylbenz[a] anthracene-induced breast cancer rat model. The quantitative changes of these components were detected by ELISA analysis. The obtained results indicate that the combination of the plant and the drug has a visible anti-cancer potential, which was expressed by the quantitative decrease of TNF-a, COX-2, MMP-2, and VEGF-a in tumor and lung. Thus, together with the drug, the plant exhibits anti-inflammatory, anti-metastatic, and anti-angiogenic activity, preventing its migration to adjacent tissues. As for IL-2, an increase in the amount was observed in both the

lung and the tumor in contrast to the breast cancer and 5-FU groups, which indicates that the plant itself exhibits an immunostimulatory effect. Quantitative regulation of these components similar to the data of the healthy group in the treated groups was also manifested in the reduction of rat mortality, cessation of histological changes, and reduction of tumor size values. It turns out that the treatment with the combined effect of the plant and the drug effectively reduces the proliferation and invasion of breast cancer cells, becoming the more effective method for cancer treatment compared to chemotherapy.

P-27-090
Enhanced anti-inflammatory and anti-proliferative effects of *Inula helenium* herbal extract and arginase inhibitor in breast cancer *in vivo* study

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The rising prevalence of breast cancer fuels interest in understanding its development mechanisms and seeking new therapies. Nowadays, medicinal plants are being used as potential medicines due to their anti-cancer, antioxidant, and anti-inflammatory properties. In contrast to the negative effects of conventional chemotherapy medicinal plants exhibit minimal side effects. That is why currently phyto-chemotherapy (herb + chemotherapeutic agent) has become an alternative for disease treatment. Based on this, our study aimed to investigate the synergistic anti-inflammatory and anti-proliferative effects of *Inula helenium* herbal extract and arginase enzyme inhibitor nor- NOHA, in an *in vivo* experimental model of breast cancer, evaluating quantitative changes of interleukin-2 (IL-2), tumor necrosis factor-alpha (TNF-α), and cyclooxygenase-2 (COX-2). To obtain an *in vivo* experimental model, 8-week-old female rats were injected with the chemical carcinogen 7,12-dimethylbenz[a]anthracene (DMBA). Quantitative changes of IL2, TNF-α, and COX-2 were observed in rat tumors and lungs, using ELISA analysis. The obtained results indicate that the plant itself has a high anti-cancer effect, which was recorded by the decrease of tumor numbers in rats, and the reduction of TNF-α and COX-2 in the lungs. Moreover, the synergistic effect of the combination of herb and the inhibitor decreases TNF-α and COX-2 levels in the lungs, contrasting with elevated IL-2 levels, indicating an immunostimulant effect. The synergistic anticancer potential of the plant and the inhibitor prevented tumor proliferation and enhanced immune cytotoxicity, exhibiting significant anti-inflammatory effects, thereby mitigating disease exacerbation. Combined administration of Inula helenium and nor-NOHA suggests an interaction between TNF-α and COX-2, mediated by certain regulatory pathways, which may become a new and effective approach to cancer treatment.

P-27-091
Royal jelly-mediated silver nanoparticles show high cytotoxcity on HeLa and A549 cells via inhibition of the VEGFa/PI3K/Akt/MMP-2 pathway

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Cancer poses a significant challenge in the medical field, requiring thorough investigation into its mechanisms and the development of effective treatments. Recently, there has been increasing interest in integrating drugs with metal nanoparticles, aiming to enhance anticancer efficacy. Silver nanoparticles (AgNPs), especially those obtained through environmentally friendly methods known as green synthesis, have garnered attention. Royal jelly (RJ), a substance produced by bees recognized for its antioxidant, anti-inflammatory, and antibacterial properties, is particularly interesting. This study focuses on the green synthesis of AgNPs using royal jelly and its bioactivity against HeLa and A549 cancer cells. AgNP characterization was performed by applying UV-Vis spectroscopy, dynamic light scattering (DLS), and transmission electron microscopy (TEM) in combination with selected area electron diffraction (SAED). The MTT (3[(4,5-dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide) colorimetric method was used to evaluate the cytotoxicity of the investigated substances. The VEGFa/PI3K/Akt/MMP-2 pathway was investigated using ELISA analysis. The results highlight the cytotoxicity induced by AgNPs in HeLa and A549 cells, which is mediated through apoptosis via the PI3K/AKT signaling pathway. We can note that the inhibition of the VEGF/PI3K/Akt pathway and the quantitative increase of MDA (therefore ROS increase) are the basis of RJAgNP cytotoxicity. In addition, these nanoparticles show promise and great potential for positive regulation of anti-angiogenic and anti-metastatic pathways by reducing VEGFa and MMP-2 levels. This study is among the few to elucidate the mechanism of the anticancer effects of nanoparticles synthesized through this method. Overall, our research contributes to the ongoing exploration of cancer biology and offers insights into potential therapeutic approaches by harnessing the capabilities of green-synthesized nanoparticles.

P-27-092
Herb-drug synergy: targeting the PI3K/AKT pathway for enhanced apoptosis in cancer cells

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Cancer remains a prominent cause of mortality globally, underscoring the urgency for innovative treatment modalities. Emerging strategies integrate natural compounds and targeted therapies to disrupt specific signaling pathways crucial for tumor

progression. The PI3K/AKT pathway plays a central role in cell proliferation and survival and presents an attractive therapeutic target in oncology. This study investigates the efficacy of combining herbal extracts (*Inula helenium* L. and *Alchemilla smirnovii* Juz) with the L-arginine pathway enzymes inhibitors Nω-hydroxy-nor-arginine (nor-NOHA) and NG-Nitro-L-arginine Methyl Ester (L-NAME) to target the PI3K/AKT pathway and induce apoptosis in cancer cells. By combining *Inula helenium* and *Alchemilla smirnovi* with nor-NOHA and L-NAME, which indirectly inhibit the PI3K/AKT pathway, enhanced apoptotic effects are expected. This research investigated the anticancer effect of herb-drug combinations on MCF7 and A549 cancer cell lines *in vitro*, and 7,12-dimethylbenz[a]anthracene-induced breast cancer rat model *in vivo*, with a view to the efficacy of our treatment model in suppressing PI3K/AKT signaling, stimulating caspase-3 (CAS-3) activation, and inducing apoptosis. Results indicate reduced levels of phosphorylated and total PI3K and Akt following herb-drug combined treatment, indicating pathway inhibition in cancer cell lines and breast tumors. ELISA assays measure CAS-3 levels and measure chromatin segmentation and degradation by Hoechst 33342, demonstrating increased CAS-3 levels and apoptosis post-combined treatment. Herb-drug combinations induce cell death by stimulating Caspase-3 through negative regulation of the PI3K/Akt pathway. These results emphasize the therapeutic potential of this combined approach as a novel cancer treatment model, offering insights into targeted therapies that exploit the molecular vulnerabilities of cancer cells.

P-27-093
Characterization of futile repair of pyrimidines catalysed by human mismatch-specific thymine-DNA glycosylase and its possible implications in epigenetic modifications

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Human thymine DNA glycosylase (TDG) is one of the key players in the mechanisms of maintaining genome integrity and epigenetic gene regulation through the initiation of base excision repair (BER) and active DNA demethylation pathway to maintain CpG islands in methylation-free state. It has been shown that TDG is able to initiate aberrant mutagenic BER by excising regular T opposite to A* (damaged A). Analysis of tumour samples from individuals exposed to Aristolochic acid (AA) revealed an unusual mutational signature of T→A transversions preferentially in CTG/CAT context, suggesting a possible involvement of TDG-initiated aberrant BER. To study the role of TDG in AA-induced mutations, we reconstituted DNA repair assay *in vitro* using purified TDG protein and radiolabelled DNA duplexes containing dA-AL/T mismatch in different sequence contexts. Unexpectedly, our studies reveal that under the experimental conditions used: prolonged incubation at 37°C, the TDG protein can exhibit DNA glycosylase activity towards regular DNA duplex, particularly to C and T paired with A and G, respectively, with preference in TpG, CpA, CpG and TpA contexts. We refer to this unusual activity as "futile" since removal of regular DNA bases would lead to repeated cycles of repair. We demonstrated that the full-length TDG, but not the truncated one, remains active during long incubation time at 37°C in the presence of equimolar concentration of regular DNA duplex. Interestingly, TDG-catalyzed futile repair is strongly inhibited in the presence of 5-methylcysine (5mC) residue in duplex DNA. Whereas, 5-hydroxymethylcytosine (5hmC) residues can be eliminated by TDG under reaction conditions favourable for futile repair. The TDG-catalyzed excision of 5hmC may play a role in cell differentiation and function as an alternative pathway for active DNA demethylation. In conclusion, our findings provide insight into the role of TDG in the generation of transient DNA strand breaks in neuronal enhancers.

P-27-094
Differential transcriptional regulation role of HMGB2 in adenocarcinoma and neuroendocrine prostate cancer

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Prostate cancer (PCa) is the fourth most common cancer worldwide causing the death of 149.346 people in 2022. Adenocarcinoma is treated by hormonal inhibition therapy, but prostatic small cell neuroendocrine carcinoma (SCNC) does not respond to this treatment. HMGB2 is a human high mobility group box-B (HMGB) protein, overexpressed in prostate cancer and related to the main hallmarks of cancer. Analyzing, in the PC3 line (SCNC model), the expression of 90 genes related to prostate cancer under HMGB2 silencing it was observed that HMGB2 acts mainly as transcriptional activator. The regulatory role of HMGB2 upon SERPINE1, ZWINT, FN1, IGFPB3 and TYMS expression, analyzed in PC3 and LNCaP (adenocarcinoma model) lines, was clearly distinct showing differences between these two types of cancer. This role was analyzed in patient samples finding significant correlation between HMGB2 and ZWINT, FN1, IGFBP3 and TYMS. Two transcriptomic studies of patients with adenocarcinoma (androgen receptor (AR)-positive-neuroendocrine (NE)-negative) or neuroendocrine (AR-negative-NE-positive) PCa showed a positive correlation HMGB2-FN1 in AR-negative-NE-positive, but not in AR-positive-NE-negative indicating a possible clinical significance for differential diagnosis of AR-/NE+ PCa.

P-27-095
Unconventional role of PDL1 in glioblastoma biology

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Glioblastoma, an aggressive brain cancer, frequently expresses PDL1, a co-inhibitory molecule that negatively regulates the immune system. To date, PDL1 represents the unique accepted marker for using immunotherapy to treat tumors. Glioblastoma is however resistant to immunotherapy. The aim of this work was to investigate whether PDL1 affects glioblastoma growth. Moreover, we explored the subcellular localization of PDL1 that could justify an action different from immune modulation, which is exerted by plasma membrane PDL1. We induced silencing and overexpression of PDL1 in glioblastoma cell lines (GB138, D54, U251) and measured proliferation by flow cytometry, using Ki67 as a marker of cell division, and qPCR to assess mRNAs of cyclin B, D, c-Myc and PCNA. Our results show a reduction and increment of these proliferation markers in a similar fashion as PDL1 modulation, suggesting an involvement of PDL1 in the regulation of cell proliferation. We observed a nuclear localization of PDL1 in addition to the canonical membrane localization through a western blot analysis. Nuclear subfractionation showed that a 50 kDa PDL1 band, consistent with glycosylated protein, was localized in the soluble fraction, while 35 kDa PDL1 isoform in the chromatin fraction. Modulation of PDL1 expression through silencing or overexpression affected expression of PDL1 in plasma membrane and in the nuclear soluble fraction, while PDL1 level in chromatin fraction did not change. The chromatin fraction was not recognized by an antibody against the C-terminal suggesting this protein corresponded to a spliced PDL1 isoform. In conclusion, our results support the hypothesis that 50 kDa PDL1 isoform might take part in transcriptional complexes involved in the expression of proliferative genes. The role of the chromatin bound isoform remains unexplored. This study, although preliminary, supports a new role in glioblastoma malignancy mediated by PDL1. *The authors marked with an asterisk equally contributed to the work.

P-27-096
Unraveling the roles of HMGB1 and HMGB2 proteins in epithelial ovarian cancer

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Ovarian cancer is one of the most lethal gynecological malignancies worldwide because it tends to be detected late, when metastasis has taken place. Early diagnosis, when the tumor is still localized in the ovaries, is a clear advantage, since this rate increases to 92%. As a consequence, 5-year survival is achieved by less than 30% of ovarian cancer patients. According to the type of cell in which the tumor originates, ovarian cancer can be classified as stromal, germinal or epithelial, the latter being the most common, accounting for 90% of cases (1). Several studies investigated the association of high mobility group box (HMGB) proteins with cancer due to their importance and the variability of functions inside and outside the cells. HMGB1 and HMGB2 proteins are the most abundant members among HMGB family, and they contribute to a wide variety of hallmarks of cancer and are overexpressed in ovarian tumor cells (2). In the present work, the production of HMGB1 and HMGB2 knockouts in SKOV3 epithelial ovarian cancer cell line were obtained by using CRISPR/Cas9 technology to analyze their functional implications and associated molecular molecular mechanisms. Cell proliferation, cell migration and invasion, qRT-PCR and western blotting methods of several biomarkers, as well as proteome analysis by mass spectrometry, were performed. The results of both knockouts showed decreased invasiveness, that combined with the downregulation of proteins such as EGFR, Vimentin, β-catenin, caveolin-1 or histone-lysine N-methyltransferase EZH2, indicate that both HMGB proteins could be directly implicated in the epithelial–mesenchymal transition (EMT) and metastasis processes. These findings open the possibility of using them as a new promising therapeutic target for ovarian cancer. Previously published in: (1) Reid BM et al. (2017) Cancer Biol Med 14, 9-32. (2) Cámara-Quílez M et al. (2020) Cancers 12, 2435.

P-27-097
Relationship between epithelial-to-mesenchymal transition and lipid raft organisation

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Epithelial-to-mesenchymal transition (EMT) is a complex process that allows epithelial cells to acquire a mesenchymal phenotype, making them motile and invasive, which is crucial for cancer metastasis and treatment resistance. Recent studies have shown that EMT is accompanied by numerous changes in signalling pathways initiated in the plasma membrane (PM). The PM contains a variety of lipids and proteins that can be organised into functional domains such as lipid rafts, which are mainly composed of cholesterol, gangliosides, sphingolipids, and membrane proteins. Lipid rafts regulate cell signalling processes, including those associated with cancer. We hypothesise that changes in lipid raft organisation are associated with EMT in cancer metastasis. To test this hypothesis, we investigated biochemical changes in PM in an EMT-relevant breast cancer cell model: (i) MCF-7 – representing an epithelial phenotype, (ii) – MCF-7 treated with TGFβ-1 – corresponding to an induced mesenchymal phenotype and (iii) MDA-MB-231 – representing a mesenchymal phenotype. Firstly, we assessed cholesterol and GM1 ganglioside levels. Secondly, we examined changes in PM organisation using a fluorescence lifetime imaging microscopy (FLIM) mechanophore probe, which is sensitive to changes in PM tension. Our analysis showed a strong relationship between EMT and changes in PM composition – both cholesterol and GM1 levels were reduced in cells displaying a mesenchymal phenotype. In addition, FLIM analysis

showed a downward trend in PM organisation levels in cells with EMT characteristics. In conclusion, our study shows that PM undergoes biochemical changes during EMT and suggests that organised domains present in PM, such as lipid rafts, may play an important role in EMT, although further research is needed to fully understand this relationship.

P-27-098
A novel protein-protein interaction targeting the permeability transition pore (PTP) protects cancer cells from apoptotic death: molecular bases of the OSCP-IF1 binding process

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ATPase inhibitory factor 1 (IF1) is a mitochondrial protein that binds to ATP synthase in the catalytic domain, inhibiting its hydrolytic activity under anoxic conditions. It is noteworthy that IF1 is overexpressed in many tumours [Previously published in: Cuezva JM et al. (2016) Biochim Biophys Acta 1857, 1167–1182], acting as a pro-oncogenic protein, although its mechanism of action is still unclear. Recently, we found that the lack of IF1 sensitises HeLa cells to the opening of the permeability transition pore (PTP), a mitochondrial channel that promotes cellular apoptosis [Previously published in: Fabbian S et al. (2023) Cell Death & Dis 14 (54)]. Comprehending the molecular mechanisms of PTP regulation may have important implications for human health because the dysregulated activity of PTP is related to several human diseases such as Alzheimer's and cancer. Importantly, ATP synthase has been suggested as the main component of PTP, and its subunit oligomycin sensitivity conferral protein (OSCP) is a key protein for the modulation of the pore [Previously published in: Giorgio V et al. (2019) Br J Pharmacol 176, 4247–4257]. Here, our proximity ligation assay and immunoprecipitation showed that IF1 binds to OSCP in HeLa cells during cellular respiration and this protein-protein interaction protects cancer cells against the PTP-dependent apoptosis. NMR spectroscopy and mass spectrometry (MS) under native conditions were used to characterise the binding process at atomic level. In detail, chemical shift perturbation by NMR was used to map the binding epitopes on both IF1 and OSCP and MS clearly detected the protein-protein complex in solution. Finally, the affinity KD was estimated by the synergic use of NMR and MS. Our results represent the first completely characterised protein-protein interaction underneath the PTP regulation in cancer diseases and help to comprehend the molecular bases of the PTP modulation for further therapeutic applications.

P-27-099
PIN1 regulates YTHDF1 stability to promote breast tumorigenesis via m6a-dependent AURKA mRNA stabilization

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The post-transcriptional processing of N^6-methyladenosine (m^6A)-modified mRNA by YTH domain-containing family protein 1 (YTHDF1) plays a crucial role in the regulation of gene expression. Although YTHDF1 expression is frequently upregulated in breast cancer, the regulatory mechanisms for this remain unclear. In this study, we examined the role of peptidyl-prolyl cis-trans isomerase NIMA-interacting 1 (PIN1) in regulating YTHDF1 stability in breast cancer cells. The WW domain of PIN1 interacted with YTHDF1 in a phosphorylation-dependent manner. Additionally, PIN1 overexpression increased YTHDF1 stability by preventing ubiquitin-dependent proteasomal degradation. Furthermore, using the MS2-tagged RNA pull-down assay, we identified Aurora kinase A (AURKA) mRNA as a bona fide substrate of YTHDF1. PIN1-mediated YTHDF1 stabilization increased the stability of AURKA mRNA in an m^6A-dependent manner. Furthermore, YTHDF1 knockout reduced AURKA protein expression levels, resulting in anticancer effects in breast cancer cells, including decreased cell proliferation, cell cycle arrest at the G0/G1 phase, apoptotic cell death, and decreased spheroid formation. The anticancer effects induced by YTHDF1 knockout were reversed by AURKA overexpression. Similarly, the knockout of PIN1 produced comparable anticancer effects to those observed in YTHDF1-knockout cells, and these effects were reversed upon overexpression of YTHDF1. In conclusion, the findings of our study suggest that increased YTHDF1 stability induced by PIN1 promotes breast tumorigenesis via the stabilization of AURKA mRNA. Targeting the PIN1/YTHDF1 axis may represent a novel therapeutic strategy for breast cancer.

P-27-100
In vitro and *in vivo* investigations of citric acid functionalized magnetic iron oxide nanoparticles for intra-tumoral melanoma treatment

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Due to their unique physical-chemical properties and biodegradable characteristics, magnetic iron oxide nanoparticles (MNPs) have gained interest in a variety of biomedical applications, such as drug delivery, magnetic resonance imaging, biological sensing, and hyperthermia. Here, we report on the complex characterization of citric acid (CA) functionalized MNPs for chemotherapeutic drug delivery into melanoma tumors. We characterized the interaction of MNPs with B16F10 cells using doxorubicin (Dox) as a model drug to enable NPs traceability by fluorescence-based

assays. TEM showed that MNPs had 3 to 21 nm as main size. CA functionalization was confirmed by XPS and Dox loading by a FO-SPR sensor and magnetometry techniques. We estimated a loading degree of about 110 mg of Dox per 1 g of MNPs. FACS analysis has shown that by 24 h all cells treated with Dox-loaded MNPs contained the drug, while a fraction of cells treated with free Dox were resistant to treatment. Imaging flow cytometry has confirmed intranuclear concentration of Dox and initiation of apoptosis. Increased loading of Dox percell was detected in B16F10 treated with the MNP formulation, as compared to free drug; apoptosis was induced in ~50% of cells by 72 h (0.5 μg/mL), similar to the effect of 100nM free Dox. Cell cycling inhibition was shown with increase in applied dose for both MNP-delivered as well as free Dox. Sequential multi-dose treatment of lyophilized fractions and a single-dose injection of fresh formulation were evaluated *in vivo* by monitoring tumor volume in mice. MNP formulations were retained in the B16F10 tumors. Spleen macrophages showed a predominant pro-inflammatory M1 phenotype upon MNP treatment. Tumor evolution was restrained more efficiently by single dose treatment, while multi-dose treatment did not show significant benefit when compared with free drug injection. Noteworthy, MNP-CA-Dox induced cytotoxicity in a Dox-resistant B16F10 cell line, as shown by MTS and ϒ-H2AX detection.

P-27-101
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)ase 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. 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. *The authors marked with an asterisk equally contributed to the work.

P-27-102
IL-17A and Th17 cells contribute to endometriosis by inhibiting apoptosis and NK cell mediated cytotoxicity of endometrial cells via ERK1/2 pathway

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Immune status including the immune cells and cytokine profiles has been implicated in the development of endometriosis. In this study, we analyzed Th17 cells and IL-17A in peritoneal fluid (PF) and endometrial tissues of patients with (n = 10) and without (n = 26) endometriosis. Our study has shown increased Th17 cell population and IL-17A level in PF with endometriosis patients. To determine the roles of IL-17A and Th17 cells in the development of endometriosis, the effect of IL-17A, major cytokine of Th17, on endometrial cells isolated from endometriotic tissues was examined. Recombinant IL-17A promoted survival of endometrial cells accompanied by increased expression of anti-apoptotic genes, including Bcl-2 and MCL1, and the activation of ERK1/2 signaling. In addition, treatment of IL-17A to endometrial cells inhibited NK cell mediated cytotoxicity and induced HLA-G expression on endometrial cells. IL-17A also promoted migration of endometrial cells. Our data suggest that Th17 cells and IL-17A play critical roles in the development of endometriosis by promoting endometrial cell survival and conferring a resistance to NK cell cytotoxicity through the activation of ERK1/2 signaling. *The authors marked with an asterisk equally contributed to the work.

P-27-103
Targeting of MDA-MB-231 breast cancer stem cells through mTOR and Wnt inhibitors

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Emerging evidence suggests the pivotal role of cancer stem cells (CSCs) in breast cancer initiation, progression, and therapy resistance. In this study, we evaluated the impact of mTOR and Wnt inhibitors on breast CSCs. In this concept, CSCs were firstly isolated from the MDA-MB-231 cell line using the MACS method. Briefly, CD24 microbeads (Miltenyi Biotec) were utilized for negative selection, followed by the isolation of CD44+ cells from this population using CD44 microbeads (Miltenyi Biotec). CSCs were incubated for 5 days under mammosphere culture conditions to maintain their stemness in RPMI 1640 medium containing B27, EGF, bFGF, L-glutamine, and penicillin/streptomycin at 37°C with 5% CO2. Flow cytometry analysis (BD Accuri) revealed that the cells expressed 96.9% CD44 and 12.9% CD24 markers. Subsequent analyses were performed following niclosamide and temsirolimus treatment (0-10 μM) to CSCs for 6 hours. According to Annexin-V/7AAD staining, both drugs triggered apoptosis at concentrations of ≥5 μM. Furthermore, western blot analysis was conducted to assess the protein levels of p-mTOR, mTOR, AKT, p-AKT, p70S6KB1, and p-p70S6KB1 following temsirolimus treatment, as well as the protein levels of c-MYC, ß-catenin, and p-GSK3-ß following niclosamide treatment. The

dose-dependent suppression of the Wnt and mTOR pathways in CSCs was demonstrated. Additionally, our RT-qPCR findings indicated that temsirolimus and niclosamide also dose-dependently reduced the expression of p21, SURVIVIN, p53, SOX2, and CXCR4 genes in CSCs. In conclusion, we highlighted that temsirolimus and niclosamide may effectively target CSCs by suppressing survival and stemness pathways. *The authors marked with an asterisk equally contributed to the work.

P-27-104
Evaluation of gankyrin and cysteine dioxygenase type 1 genes promoter methylation changes as a new target for early diagnosis and follow-up of gastric cancer

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Abnormal DNA methylation plays an important role in carcinogenesis by altering the expression of tumor suppressor genes and oncogenes. Reversibility of DNA methylation allows the reactivation of tumor suppressor genes silenced by promoter hypermethylation. Cysteine dioxygenase type 1 (CDO1) and PSMD10 (gankyrin) are genes that affect intracellular carcinogenic pathways, and their expression is mainly regulated by DNA methylation. It is known that precancerous lesions caused by chronic gastritis are risk factors for gastric cancer and no study has been found to investigate the DNA methylation and expression status in the promoter region of gankyrin and CDO1 in comparison with gastric tumors. In our study, we aimed to investigate the potential biomarker feature that can be used in the early stage and follow-up of gastric cancer by determining the methylation and expression levels of gankrin and CDO1 genes and comparing them with clinicopathological data. The expression status of gankyrin and CDO1 determined by immunohistochemically in 40 individuals diagnosed with chronic gastritis and 60 patients diagnosed with gastric cancer and the promoter methylation status was detected by DNA sequencing after bisulfite conversion. The results were compared with clinicopathological data. CDO1 promoter methylation levels in the patient group were significantly higher than those in the control group. It was determined that CDO1 promoter methylation levels increased significantly depending on the clinical stage, tumor size, and lymph node involvement. No significant change was observed in the methylation and expression status of the gankrin gene and clinicopathological comparisons between the two groups. CDO1 promoter methylation status has predictive and prognostic potential for gastric cancer and may be a target for epigenetic therapy of gastric tumors.

P-27-105
4,7-Disubstituted coumarin derivatives as DNA interacting agents and topoisomerase I inhibitors with A549 antiproliferative activity

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Coumarin derivatives display a wide range of pharmacological activities, exhibiting antioxidant, anti-inflammatory, antimicrobial, and anticancer effects. The anticancer properties of certain coumarin derivatives have attracted particular interest and have demonstrated promising results in preclinical studies. These findings warrant further research to determine the precise mechanisms of action and optimize therapeutic efficacy for clinical applications [Song et al. (2020) Arch Pharm (Weinheim) 353, e2000025]. Coumarin derivatives are suitable candidates in the development of novel anticancer therapies as their 7-hydroxy derivatives have been shown to exhibit cytotoxic and antiproliferative activities against multiple human cancer cell lines [Zhou et al. (2022) Sci Rep 12, 21635]. Small molecules often derive this capacity from their interactions with DNA and topoisomerases (Topo). The aim of this study was to identify the interaction mechanism of four 4,7-disubstituted coumarin derivatives, monitor their inhibitory capacity against human Topo I, and investigate their cytotoxic activity against A549 and CCD-18Co normal-like colon fibroblast cell line. Topo I relaxation assays revealed that coumarin derivative C1 is the most potent inhibitor from the series, displaying an inhibitory activity comparable with that of camptothecin. MTT assays revealed that derivative C4 inhibits the metabolic activity of A549 cancer cells more effectively than that of CCD-18Co, while compounds C1-C3 inhibit the metabolic activity of both cell lines. The analysis of the effect of coumarin derivatives on proliferation showed that proliferation of CCD-18Co is influenced by C3, whereas C1, C2, and C4 had no effect on the proliferative activity of this cell line. Conversely, in terms of the effect on tumor cells, C2 exhibited the highest efficacy in inhibiting proliferation. This study was supported by VEGA Grant No. 1/0037/22 and VVGS-2023-2742

P-27-106
The two-faced role of Syndesmos: riboregulation of the DNA damage response

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Breast cancer is the most frequently diagnosed cancer and the second leading cause of death in women. Two main breast cancer-associated genes, BRCA1 and BRCA2, have been identified, which can be exploited for therapy, being BRCA mutations synthetically lethal with PARP inhibitor treatment. Protein Syndesmos (SDOS, aka NUDT16L1 or TIRR) has been identified as a key regulator of 53BP1-mediated DNA damage response (DDR): its overexpression prevents 53BP1 function, leading to

PARP inhibitors resistance in BRCA1-mutated tumors. However, SDOS is also an RNA-binding protein, which associates with polysomes and regulates protein synthesis. Remarkably, SDOS-mediated 53BP1 inhibition is decreased by SDOS-interacting RNAs, clearly indicating that the two SDOS functions are not separated. In support of this hypothesis, our preliminary observation obtained by the OOPs technique shows that global SDOS-RNA binding is reduced by exposure to etoposide. Among the transcripts who's binding to SDOS is reduced by genotoxic stress induced by etoposide, we identified the selective autophagy regulator p62/SQSTM1. Polysome profiling coupled with RT-qPCR showed that SQSTM1 mRNA translation is enhanced upon SDOS KD and following etoposide treatment. Interestingly, the protein SQSTM1 is also an SDOS interactor, suggesting a multiple-level regulation of SQSTM1 activity by SDOS. Indeed, by employing RIP assays, we show that SDOS expression also influences the riboregulation exerted by the small noncoding vault-RNAs on SQSTM1 activity. In fact, binding between the SQSTM1 protein and its riboregulators following etoposide is reduced, and autophagy is increased upon SDOS silencing. These data suggest that SDOS plays a role in the DDR by modulating the RNA metabolism. According to this model, SDOS participates in the DDR at multiple levels by both translation regulation of transcripts encoding stress-responsive proteins, and riboregulation of their activity.

P-27-107
Unveiling the interplay between NMDAR and MET in colorectal cancer pathogenesis

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The N-methyl-D-aspartate receptor (NMDAR) is a glutamate-gated ion channel involved in synaptic transmission. It is encoded by *GRIN* genes and composed by four subunits, including NMDAR2B. Outside the nervous system, NMDAR expression was found in several tissues. Recently, we demonstrated its role in cancer invasion in triple negative breast carcinomas in association with the proto-oncogene MET, the receptor of the natural ligand hepatocyte growth factor (HGF). In this work, we investigated the role of NMDAR/MET interaction in colorectal cancer (CRC). Firstly, RNA sequencing data from 90 CRC patient tissue pairs highlighted, between tumour and adjacent tissues, the alteration of several genes involved in the nervous system and metabolic processes, as well as cancer-related pathways. Interestingly, *MET*, *GRIN2B*, and *GRIN2D* expression was upregulated in tumour tissue from our set and in The Cancer Genome Atlas (TCGA) data. Importantly, through different immunoassays, such as proximity ligation assay, co-immunoprecipitation, and double immunofluorescence analysis, we found that NMDAR2B and MET physically interact in CRC cell lines (e.g. HT115, LS411N). Moreover, the inhibition of NMDAR by two specific inhibitors (MK801 and Ifenprodil) impaired the HGF-driven invasion and migration in CRC cells, as demonstrated through matrigel invasion and wound healing assays. Together, these results highlight the important role of the NMDAR/MET complex in CRC invasive program, suggesting that a combinational therapy may be exploited as a new therapeutic strategy.

P-27-108
In silico analysis of protein-protein interaction network reveals aryl hydrocarbon receptor (AHR) crosstalk in von Hippel-Lindau (VHL)-associated clear cell renal cell carcinoma (ccRCC): implications

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The intricate regulatory mechanisms governing the crosstalk between the Aryl hydrocarbon receptor (AHR) and hypoxia-inducible factor (HIF), both members of the basic helix–loop–helix (bHLH) PER-ARNT-SIM (PAS) transcription factor family, is central to understanding cancer pathogenesis, particularly in clear cell renal cell carcinoma (ccRCC). In this study, we explore the interplay of AHR and HIF pathways, shedding light on potential therapeutic avenues for ccRCC. Dysregulation of HIF due to loss of function mutation of the von Hippel Lindau tumour suppressor (pVHL) leads to constitutive HIF activation in ccRCC. Employing protein-protein interaction network analysis and gene expression profiling, we delineate the impact of pVHL loss on AHR activity and its associated pathways. Our findings reveal distinct expression patterns of AHR interactors following exposure to 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) and in ccRCC. Through Kaplan-Meier survival analysis, we identify AHR interactors significantly associated with poor survival rates in ccRCC patients. Notably, the upregulation of androgen receptor (AR) and retinoblastoma-associated protein (RB1) by TCDD exposure, followed by their downregulation in ccRCC, suggests potential therapeutic targets. Strategic activation of AHR via selective AHR modulators (SAhRM) emerges as a promising approach for treating VHL-mutated ccRCC, thereby reducing reliance on surgical interventions. Our study provides comprehensive insights into the complex interplay between AHR and VHL pathways in ccRCC pathogenesis, offering novel avenues for targeted therapeutic interventions.

P-27-109
Comparative Analysis of punicalin and punicalagin interaction with PDIA3 and PDIA1

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PDIA3 is a pleiotropic protein primarily located in endoplasmic reticulum where it is involved in protein folding, catalyzing the formation, breakage, and rearrangement of disulfide bonds. PDIA3 is implicated in numerous pathologies such as cancer, inflammation, and neurodegeneration. Although punicalagin has been proven to be a highly promising PDIA3 inhibitor and can be used as target protein in glioblastoma (1,2), it does not have a sufficient selectivity for PDIA3 and is a quite large molecule. With the aim to find punicalagin derivates with a simplified structure, we selected punicalin, a punicalagin metabolite that lacks the hexahydroxy-diphenic acid moiety. Previously docking studies suggest that this part of the molecule is not involved in

the binding with PDIA3 (3). For this reason, in this study we compared the ability of punicalin to bind and inhibit PDIA3 and PDIA1. Tryptophan fluorescence quenching and disulfide reductase activity (using both glutathione and insulin as substrates) were evaluated, demonstrating the ability of punicalin to bind and inhibit PDIA3 even with a less extent (IC50 ranging from 11 to 30μM compared to 4-9 μM for punicalagin). On the other hand, punicalin showed a very low inhibition activity towards PDIA1, demonstrating a higher selectivity for PDIA3. Protein thermal shift assays evidenced that both proteins can be destabilized by punicalagin and punicalin, with PDIA3 much more sensitive (up to 8°C of thermal shift). Additionally, punicalin showed a higher change in the thermal shift of PDIA3. This result could explain the presence of PDIA3 aggregates, evidenced by immunofluorescence analysis, that accumulates within treated cell and that are more evident in the presence of punicalin. References: 1 Giamogante et al. (2018) Biochimie doi: 10.1016/j.biochi.2018.01.008. 2 Paglia et al. (2023) Int J Mol Sci doi: 10.3390/ijms241713279. 3 Paglia et al. (2021) Biomedicines doi: 10.3390/biomedicines9111533. *The authors marked with an asterisk equally contributed to the work.

P-27-110
The P2X7 receptor splice-variants as key players for eATP-mediated anti-cancer effects in retinoic acid-induced differentiated human glioma cells

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Extracellular ATP (eATP) stands as a prominent purine element within the tumor microenvironment, acting as the natural ligand for two families of purinergic receptors: ionotropic P2Xs and metabotropic P2Ys. Among them, P2X7 receptor (P2X7R) is the most studied in last years, extensively expressed in both solid and liquid tumors, and plays pivotal roles in cancer and immune cell function. Recently, human P2X7R variants A and B have been identified as key mediators of cancer invasion, displaying distinct regulation patterns under anti-tumoral treatments. Our study focuses on delineating the dynamics of purinergic signaling in two human glioma cell lines, M059K and M059J, subjected to retinoic acid-induced differentiation. This differentiation procedure induced significant alterations in the expression of P2X7 receptor variants at both the mRNA and protein levels, along with a reduction in ecto-nucleotidase activity. Cumulatively, all these changes led to increased cellular homogeneity, decreased proliferation rates, and heightened sensitivity to cytotoxic effects of eATP. Specifically, we observed a 40% and 20% reduction in cell viability in differentiated M059K and M059J cells, respectively, upon exposure to micromolar concentrations of ATP. Additionally, migration capability decreased by up to 60% in the presence of 100 μM ATP, effects mediated by P2X7R activation and reversible with the usage of A740003–P2X7R antagonist. We hypothesize that retinoic acid-induced differentiation, combined with micromolar eATP levels, could serve as an effective anti-cancer strategy by modulating purinergic signaling. Our findings underscore the significance of P2X7R variants and their upregulated expression in creating the biological behavior of glioma cells, suggesting their potential as therapeutic targets.

P-27-111
Anti-CDK4/6 PROTACs and their efficiency in degradation on different cancer cell lines

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Proteolysis targeting chimeras (PROTACs) are bifunctional compounds that consist of 3 components: a ligand binding to the targeted protein, a ligand binding to E3 ubiquitin ligase, and linker between them. PROTACs binding lead to the proteasomal degradation of targeted protein by bringing it to the proximity of the E3 ligase [1]. Due to ability of PROTACs to selectively decrease the level of targeted proteins, their use can be extended to the anticancer treatment. Cyclin dependent kinases 4 and 6 (CDK 4 and 6) are well-known targets in anticancer therapies. Several CDK4/6 inhibitors have been approved by US Food and Drug Administration (FDA) and are being administrated to breast cancer patients [2]. By combining the effectiveness of anti-CDK4 and CDK6 compounds with the properties of PROTACs, it has been demonstrated that they can work more efficiently than FDA-approved therapies [3]. In this study, commercially available CDK4 and CDK6 PROTACs, are tested thoroughly for their activity, using among all western blot technique to assess how PROTACs affect the phosphorylation of the retinoblastoma (Rb) protein a tumor suppressor protein that is highly dysfunctional in many cancers, and the levels of CDK4 and CDK6 in various human and mouse cancer cell lines. The conducted research reveals variations in the activity of these compounds across different cell lines and dose-dependency in both the degradation of targeted CDK4/6 and the phosphorylation levels of Rb protein. Funding sources from the National Science Centre, Poland, grant UMO-2021/43/B/NZ7/03170 *References*: [1] Goel S et al. (2022) Nat Rev Cancer.;22(6):356-372. [2] Spring LM et al. (2020) Lancet.; 395(10226):817-827. [3] Wu X et al. (2021) Nat Cancer.;2(4):429-443.

P-27-112
Covalent small-molecule inhibitors of the PD-1/PD-L1 immune checkpoint

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Among many regulatory pathways, the PD-1/PD-L1 interaction is an important checkpoint that plays a major role in maintaining immune self-tolerance. PD-1 (programmed cell death protein 1) is a receptor present on the surface of T cells and B cells which, upon binding to PD-L1 (programmed cell death protein 1), down-regulates the immune system and suppresses T-cell inflammatory activity. Besides its physiological functions, this mechanism is often also utilized by cancer cells to evade immune surveillance. Upregulation of PD-L1 expression, reported in multiple types of tumor cells, is usually associated with poor clinical outcomes [1]. Hence, targeting the PD-1/PD-L1 checkpoint is one of the approaches in cancer immunotherapy. Our research aims to develop small-molecule compounds with PD-1/PD-L1

inhibitor activity. Our newly discovered compound blocks the PD-1/PD-L1 interaction by binding to and covalently dimerizing PD-L1 protein, which was confirmed by western blot and mass spectrometry analyses. Cytotoxicity was assessed colorimetrically on the Jurkat T cell line. The activity of the compound was verified through a cell-based immune checkpoint blockade (ICB) assay. The blockade of PD-L1 was confirmed by flow cytometry analysis. Our study provides insights into the mechanism of PD-L1 covalent inhibitors that exhibit good therapeutic window *in vitro*. Further experiments, aiming to elucidate the exact mechanism of action and potency of the molecule are underway. The research has been supported by a grant from the Faculty of Chemistry under the Strategic Programme Excellence Initiative at Jagiellonian University and by the National Science Centre, Poland, Grant number UMO-2021/42/E/NZ7/00422. Reference: [1] Wang et al. (2016) *OncoTargets and Therapy*, 9, 5023–5039. *The authors marked with an asterisk equally contributed to the work.

P-27-113
BAG3 promotes metabolic reprogramming of tumor-associated fibroblast and contributes to extracellular matrix remodeling and fibrosis in tumor microenvironment

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A hallmark of pancreatic ductal adenocarcinoma (PDAC) is desmoplastic tumor microenvironment (TME) composed by excessive fibrosis, fibroblasts, and immune cells. Cancer-associated-fibroblasts (CAFs) are the major producers of extensive fibro-inflammatory stroma that undergo constant remodeling during cancer progression. BAG3 is a cytosolic protein that controls several cellular processes such as apoptosis, autophagy, and cytoskeletal-membrane dynamics. BAG3 can be also secreted by PDAC cells, bind to immune and stromal cell in TME thus inducing the release of immunosuppressive and pro-tumor cytokines and chemokines. In order to investigate the role of BAG3 in desmoplastic process, we purified recombinant protein expressed in a *Baculovirus* system, and we evaluated fibroblasts activation by measuring the release of cytokines and chemokines [previously published in: Dufrusine et al. (2022) J Cell Biochem. 123(1): 65–76]. Proteomics profiling of BAG3-activated fibroblasts revealed a significant enrichment of proteins involved in membrane-dynamics process. We therefore performed confocal and western blot analysis to confirm the identified targets. We also report that BAG3-activated fibroblasts have an increased capability in collagen internalization and degradation and an increased intra- and extracellular collagenase activity. To uncover the metabolic alterations that drive changes in extracellular matrix deposition we used Seahorse real-time cell metabolic analysis that has shown that BAG3 can induce the Warburg effect in BAG3-activated fibroblasts associated with changes in extracellular matrix metabolism and cellular autophagy. Therefore, metabolic reprogramming in BAG3-activated fibroblasts appears as an interesting process associated with tumorigenesis and blocking this pathway might be a novel approach for the management of PDAC.

P-27-114
Diacylglycerol kinases as potential molecular targets in acute myeloid leukemia

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Acute myeloid leukemia (AML) is a heterogeneous malignancy of the stem cell precursors of the myeloid lineage, occurring in approximately 1.2% of all cancers and representing one of the most common types of leukemia in adults. Despite the current therapeutical strategies including chemotherapy, radiotherapy and targeted therapies, AML chemoresistance and its high relapse rates underscore the need for the identification of new druggable targets. In this context, the lipid enzymes belonging to diacylglycerol kinases (DGKs) family, being involved in both cell transformation and immunosurveillance processes could be suitable candidates. In this study, we performed a detailed bioinformatic analysis of the various DGKs expression exploring their correlation with AML patients' survival across different databases, including TCGA, BeatAML, and TARGET. We found that DGKA, DGKD and DGKG were overexpressed in AML patients and that high DGKH expression was consistently associated with shorter survival. Moreover, the analysis also examined DGK co-expressed genes using a query on BeatAML database from CBioPortal, revealing isoform-specific functions and some shared ones related to cell signaling, differentiation and vesicular trafficking. In vitro assays on different leukemia cell lines demonstrated the therapeutic efficacy of DGKs targeting, with broad-spectrum inhibitors causing increased cell mortality. Thus, most recently, we are investigating more in deep each DGK isoform contribution to AML phenotype and, so far, our preliminary data suggest DGKZ and DGKA could play a relevant role in this context. Overall, our results suggest that DGK play a relevant role in AML pathogenesis, providing a promising signaling pathway to identify new therapeutic targets. The specific isoforms involved are still debated and may depend on the genetic background of the patient or the disease classification, requiring a personalized medicine approach.

P-27-115
Role of Shp1 in Interleukin-8-induced breast cancer invasiveness

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The Src homology region 2 (SH2) domain-containing phosphatase 1 (Shp1) is a cytosolic tyrosine phosphatase involved in cell cycle control, apoptosis induction, cancer cell migration and invasion. With some exceptions, Shp1-mediated inhibition of

tyrosine kinase signaling is generally associated with antitumor effects in both solid and hematopoietic cancers. Herein, we shown that pharmacological and genetic inhibition of Shp1 in breast cancer cells induces accelerated cell migration and promotes a more invasive phenotype. Furthermore, we found that interleukin-8 (IL8), a chemokine with multiple pro-tumorigenic roles within the tumor microenvironment, directly modulates Shp1 activity. In breast cancer, IL8 elicited its functions through the binding to the two G protein-coupled receptors CXCR1 and CXCR2, and hence the modulation of several intracellular signaling pathways. We show that in breast MCF7 cells, IL8 affects Shp1 activity through the induction of PKC-mediated phospho-S591-dependent inhibition of the phosphatase, resulting in increased phosphorylation levels of the CXCR2 receptor and subsequent control of its degradation. Shp1 inhibition also attenuates IL8-mediated receptor down-regulation and affects the total protein level of CXCR2 receptor. Importantly, our data indicate that Shp1 is crucial for IL8-induced tumor invasiveness in breast cancer cells by regulating CXCR2 turnover. Inhibition of Shp1 activity leads to irreversible down-regulation of the receptor and may aid in controlling CXCR2 function, thereby limiting IL8 signaling within the tumor microenvironment and during excessive inflammation. Thus, in addition to shedding light on the molecular basis of IL8 signaling pathway, our findings suggest that targeting the CXCR2/Shp1 axis could be an attractive strategy for developing alternative treatments for breast cancer.

P-27-116
Bioselective echoviruses as a new therapeutic agent against glioblastoma

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Virotherapy is an effective approach for treatment of advanced cancer, based on direct lysis of tumor cells and activation of system antitumor immunity. We have developed bioselected strains of Echoviruses with expanded tropism and reduced pathogenicity. The bioselection method is based on the natural plasticity of RNA viruses providing increased genetic variety. To obtain a new strain, the original Echovirus 12 (Live enterovirus vaccine 20) has been adapted during more than 20 passages on cells with a deletion of the primary FCGRT receptor. As a result, FCGRT has been abandoned and P1 segment of the viral genome has undergone changes. The nonsynonymous and synonymous amino acid substitutions have occurred, some codons were optimized based on frequency of its usage. To test the new strain, we have chosen models of glioblastomas, incurable tumors containing a large number of tumor stem cells resistant to any therapy. The oncoselective strain showed a high level of onclytic activity compared to the original strain on model and primary tumor cell lines. Among the tested lines (n = 10) original Echovirus 12 efficiently lyse ($lgTCID_{50}$/ml ≥3.5) a limited group of primary and model tumor cell lines (n = 3), while the bioselective strain successfully lysed every tested cell line. The resulting oncoselective strain also showed high efficiency against tumor stem cells, which indicates its promise for use as a therapeutic agent for the treatment of recurrent glioblastoma.

P-27-117
Tumor growth factors sensibilise cells for vaccinia virus infection

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Oncolytic viruses are promising antitumor agents due to their unique abilities to selectively and effectively lyse tumor cells through replication within them. The vaccinia virus (VV) is one of the most compelling oncolytic vectors, demonstrating high specificity for cancer cells, which may be attributed to the action of growth factors secreted by tumors. We assessed the influence of transforming growth factor β (TGFβ), epidermal growth factor (EGF), fibroblast growth factor (FGF), and platelet-derived growth factor (PDGF) on the replication and oncolytic activity of VV. These factors contribute to tumor development and growth regulation, playing an important role in sensibilisation of cancer cells to VV. Pretreatment of normal (pericytes, fibroblasts) and tumor cells (glioma, colorectal and mammary adenocarcinoma) with TGFβ increased the replication efficiency of VV by 5- and 3,5-fold respectively. Type 1 interferons secreted in response to viral infection inhibit translation and prevents virus replication. However, tumor growth factors cancel action of interferons allowing the virus to replicate more effectively and spread specifically within tumor tissue. Pre-treatment of tumor cells with interferon alpha 24 hours before infection led to 30-fold decrease of VV replication, while simultaneous treatment and infection did not show this effect. Based on these findings, a highly tumor specific thymidine kinase deficient strain of VV expressing human interferon alpha (VV-hIFNα) was developed and tested in comparison with RFP expressing one. The oncolytic activity of VV-hIFNα in normal cells was reduced by more than 80 times. Tumor cells exhibited equal sensitivity to both strains – interferon expression did not affect the oncolytic activity of VV.

P-27-118
Significance of survivin expression in neuroblastoma: insights from *in vitro* and tumor tissue studies

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Survivin (SVV) is a 16.5 kDa protein codified by the *BIRC5* gene. It is a potential therapeutic target found to be highly expressed in tumors. Its significance has been little explored in neuroblastoma (NB), the most common solid tumor in children. This work aimed to investigate the associations of SVV expression with chemoresistance and the outcomes of NB. NB immortalized cell lines were maintained, RNA was extracted using Trizol, and cDNA synthesis was conducted. Gene expression was analyzed by RT-qPCR. Cell viability, apoptosis, and migration were assessed after chemotherapeutic agents and the SVV inhibitor YM155 treatment. Data were collected from medical records from 130 patients treated for NB at two pediatric hospitals in Curitiba-PR, Brazil, between 1990 and 2016. FFPE tumor samples were subjected to immunohistochemical staining. The associations of SVV with the study variables were investigated concerning death and progression. Cell lines showed high basal

expression of *BIRC5*, and basal resistance to camptothecin and topotecan was observed. Cells displayed greater sensitivity after treatment with YM155, with 6 μM causing at least a 50% reduction in cell viability. *BIRC5* expression decreased after YM155 treatment, which incremented topotecan-induced apoptosis and reduced cell migration at sub-toxic concentrations. IHC analysis showed that fever at diagnosis associated with low SVV was correlated with a higher risk of death than fever alone. Conversely, high SVV expression reduced the risk of death. A significant risk of death increases when a high LDH level is combined with low SVV. We also noted the more prolonged survival for elevated LDH and high SVV. The immunohistochemical expression of SVV showed potential as a good prognostic factor for patients diagnosed with malignant NB. The higher expression of SVV may define a better response subgroup in this population, as shown for fever and LDH.

P-27-119
With sunitinib resistance renal cancer cells and succinic acid

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Sunitinib (SUN) is the most used first-line chemotherapeutic, especially for metastatic renal cell carcinoma. The toxicity of most chemotherapeutic agents, including SUN, develops in part due to the induction of mitochondrial dysfunction and oxidative stress, furthermore the alteration of the mitophagy process. Current studies pointing out the effect of mitophagy on both cancer development and chemoresistance processes is still controversial and need to be investigated. We also aimed to examine the effects of succinic acid (SUC), which we showed its anticancer activity for different cancers with our previous studies and published the literature, on mitophagy-related proteins BNIP3 and NIX in renal cancer cells that we gained resistant to SUN (ACHN). For this, WST-1 was examined to see the IC50 value of SUN, and increasing doses of SUN were treated to ACHN cells for 9 months. The crystal violet used for monitoring the development of resistance. Thereafter, 50uM dose of SUC was treated to both resistant and sensitive cells, and levels of BNIP3 and NIX proteins were measured by ELISA. BNIP3 level increased 4.6-fold in resistant cells compared to sensitive cells, while NIX level increased 1.08-fold as a result. In addition, we observed that in SUC-treated groups, BNIP3 levels decreased by 90% in sensitive cells and 33% in resistant cells. On the other hand, we found that the effect of SUC treatment on NIX was less than BNIP3, with a 35% decrease in NIX levels in sensitive ones and a 13% decrease in resistant ones. Our results suggest that, unlike the tumor suppressor effect of BNIP3 in pancreatic, gastric, and breast cancer, BNIP3-mediated mitophagy might have a protumorigenic effect in renal cancer. These preliminary findings have elucidated the dual effect of mitophagy on SUN-resistant cells and strengthened the impression that SUC may function via the mitochondrial pathway. This study was supported by Istanbul University Scientific Research Committee (38640).

P-27-120
ESM-1 is involved in breast cancer progression mediated by cancer-associated fibroblasts

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Tumor-stroma interactions play an important role in the pathogenesis of breast cancer (BC). Stromal fibroblasts can be activated by tumor cells to differentiate into cancer-associated fibroblasts (CAFs), which promote tumor progression, metastasis, and invasion by secreting growth and proangiogenic factors [1]. ESM-1 (Endothelial cell-specific molecule 1) is a proteoglycan aberrantly expressed in different pathological conditions and its expression is affected by pro-inflammatory and pro-angiogenic factors. In cancer, ESM-1 can mediate cell proliferation, migration and angiogenesis [2]. Based on this evidence, we aimed to investigate the effects of ESM-1 knockdown in a model of TGF-β -activated CAFs co-cultured with of BC cells to clarify its role in tumor progression. TGF-β treatment induced an increase of ESM-1 mRNA and protein levels in CAFs. To examine whether ESM1 secreted by CAF can induced cell proliferation and migration in BC, we performed co-culture experiments, where CAFs treated with a specific ESM1 siRNA or control siRNA were seeded in upper chamber and MCF-7 (non-aggressive) or MDA-MB231 (aggressive) cells were seeded in lower portion. The results of MTT assay and cell migration assay showed that ESM-1 siRNA treatment reduced cell proliferation and migration in both BC cell lines. Moreover, to better clarify the underling mechanisms, we evaluated the activation of AKT and MAPK/ERK signaling pathway. We observed that the degree of AKT and ERK-1/2 phosphorylation significantly decreased in BC cells co-cultured with CAFs treated with ESM-1 siRNA compared to control cells. Together, these data suggest that ESM-1 produced by CAFs promotes cell migration and proliferation in BC cells, modulating AKT and ERK pathways, indicating a new mechanism by which tumor microenvironment could drive tumor progression. Previously published in: 1. Sahai E et al. (2020) Nat Rev Cancer 20(3), 174–86. 2. Zhang H et al. (2021) Front Oncol 11, 687120.

P-27-121
Preliminary characterization of internalization kinetics of a fully human IgG4 anti-CD99 in AML cell lines compared to PBMC

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CD99 represents a promising therapeutic target in different pathologies, given its overexpression especially in Ewing sarcoma, but also T-ALL (T-cell acute lymphoblastic leukemia) and AML (acute myeloid leukemia). A new fully-human IgG4 anti-CD99 has been recently studied for its ability to selectively induce apoptosis in AML blasts with FLT3-ITD mutation, and cause CD34+ LSC (leukemic stem cells) depletion, but the ways in

which it triggers the pathways of cell death have not yet been totally elucidated due to the lack of information on the behavior of CD99 in the context of AML. Through indirect cytofluorimetric analysis and confocal microscopy, we have demonstrated that the triggering of CD99 by anti-CD99 results in a rapid internalization of the antigen-antibody complex in intracellular space in AML cell lines, both in the presence and absence of the FLT3-ITD mutation, and this involves a reorganization of CD99 in membrane. Given the expression of CD99 also in physiological contexts, especially in monocytes and lymphocytes, the evaluation of the kinetics of anti-CD99 in PBMC from healthy donors allowed to define significant differences in the internalization process compared to those observed in AML blasts. These preliminary characterizations allow to focus on the trafficking of CD99 after its recognition by anti-CD99 as a possible driver for the induction of selective apoptosis in FLT3-ITD AML. These also highlight the need to identify crucial interactors in this mechanism to evaluate possible synergistic treatments that can enhance the anti-leukemic effect mediated by anti-CD99.

P-27-122
A novel coculture system reveals the onset of pro-tumorigenic crosstalk between adipose-derived mesenchymal stem cells (AD-MSCs) and endometrial cancer cells

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Endometrial cancer (EC) is the most prevalent gynecologic malignancy in developed nations, with 40% of new cases attributed to obesity, emphasizing its role as a primary risk factor. However, the complex molecular and metabolic interactions involving adipose-derived mesenchymal stem cells (AD-MSCs), adipocytes, and EC cells remain largely unexplored. In this study, we characterized AD-MSCs obtained from surgical patients and developed a novel co-culture system to investigate the paracrine interaction between AD-MSCs and EC cells. Estrogen-dependent (Ishikawa) and -independent (HEC1A) EC cells were co-cultured with AD-MSCs for 14 days. Notably, co-cultured EC cells exhibited a 1.5-fold increase in cell count compared to controls, highlighting the supportive role of AD-MSCs in EC cell proliferation. Further analyses revealed enhanced 3D growth and migratory potential in co-cultured EC cells. Additionally, co-cultured HEC1A cells showed a three-fold increase in resistance to chemotherapy (specifically, 20-h exposure to 17 nmol/L paclitaxel) over 7 days. Morphological analysis showed a significant accumulation of vesicular structures, lipid droplets, in co-cultured AD-MSCs and EC cells, indicating adipogenic differentiation of AD-MSCs. This was supported by increased expression of lipid metabolism-related genes DGAT2 and CD36 in HEC1A cells, associated with adverse EC prognosis. Furthermore, elevated expression of PGC-1α and increased mitochondrial membrane potential suggested a link between lipid metabolism and mitochondrial function in EC progression. In conclusion, our findings validate the efficacy of our co-culture system and support the role of AD-MSCs in EC progression. Further research is warranted to unravel the molecular pathways involved in this interaction, facilitating the identification of novel therapeutic targets for EC prevention and treatment. *The authors marked with an asterisk equally contributed to the work.

P-27-123
Redox modulation and induction of ferroptosis by dimethyl fumarate in cervical carcinoma

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Cervical cancer (CC) is the fourth most common cause of women death. The papillomavirus (HPV) persistent infection is the leading cause for the development of CC that progresses through multistep transformation. Drug resistance and relapse are frequently observed in CC after conventional chemotherapy and radiotherapy. Therefore, there is a need to find new drugs against CC. Dimethyl fumarate (DMF) is an FDA-approved anti-inflammatory drug and emerging studies suggest that DMF also exert an anti-tumor activity in some cancers. We were interested to ferroptosis, a type of cell death caused by iron-dependent lipid peroxidation. Using cell viability and colony assay, we tested the effectiveness of DMF alone in comparison to other well-known inducers of ferroptosis in SiHa and C4I cells. We performed ferroptosis related-genes expression analysis, lipid peroxidation and malondialdehyde assays demonstrating that DMF induces ferroptosis in a concentration-dependent manner in both cell lines. Next, we investigated if the combination of DMF with sub-cytotoxic ferroptotic-drugs was able to ameliorate ferroptosis. We found that co-treatments of DMF/sulfasalazine (SAS) were associated with enhanced cell death. To elucidate molecular mechanisms underlying these effects we analyzed the NRF2 antioxidant pathway. Real-time PCR and western blot assays showed an induction of NRF2 protein and NRF2-dependent genes, such as SLC7A11 involved in GSH synthesis. In contrast with the observed SLC7A11 increase, we detected a strong reduction of glutathione (GSH) under DMF/SAS treatments. These results indicate that SAS cooperates in GSH depletion favoring ferroptosis. Since DMF has been found to influence NF-kB, STAT3 signaling we also tested IL-6 levels, a NF-kB target, and phosphorylation of STAT3 in DMF/SAS treatments. Our results demonstrate that both these pathways are reduced in presence of SAS, implicating that DMF/SAS exhibited a strong killing-effect than either DMF or SAS alone.

P-27-124
Exploring lipid metabolic dynamics in multiple myeloma: implications for anti-BCMA immunotherapy resistance and ferroptotic cell death

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Decreased cholesterol levels in multiple myeloma (MM), as consequence of increased LDL clearance and lipid handling, have been investigated in the development of drug resistance, leading to relapse. Precise regulation of lipid droplets (LDs) turnover is

essential for PUFA distribution, needed to reduce membrane lipid peroxidation and prevent ferroptosis cell death. Previously, decrease in LDs in OPM2.R cell line higher than in U266.S, was associated to a differential sensitivity to Belantamab Mafodotin (BeMa), a BCMA-targeted therapy for the treatment of patients with relapsed or refractory MM (RR-MM). To validate the clinical relevance of *in vitro* observations, 767 RNA-Seq from NDMM patients enrolled in the CoMMpass study, showed upregulation of ALOX12 associated to inferior progression free survival and overall survival. STRING protein analysis highlighted a dysregulation in lipid metabolism with an increased amount of apoliproteins APO-A1, A2, C2 which hydrolyzes triglycerides to provide free fatty acids for cells. RNAseq under basal conditions revealed upregulation of genes involved in fatty acid uptake (CD36, VLDLR and FABP4), fatty acid biosynthesis (LPCAT3, ALOX, SREBP) and key negative regulators of ferroptotic cell death (GPX4, SLC7A11) in BeMa resistant cytotype, while the sensitive one showed higher levels of ACSL4, key positive regulator responsible for the production of lipids peroxides from PUFA. Since PUFA oxidation is pro-ferroptosis, we evaluated LOOH formation, to confirm BeMA-mediated ferroptosis. Interestingly, palmitic acid supplementation rescued cell viability upon BeMa exposure. In accordance, lipidomics analysis confirmed higher MUFA/PUFA ratio in BeMa resistant cells. Finally, combination of GPX4 inhibitor with BeMa sensitized to ferroptosis. inhibition of ferroptosis may contribute to the onset of BeMa-resistance opening a new scenario about the molecular machinery underlying anti-BCMA immunotherapy. *The authors marked with an asterisk equally contributed to the work.

P-27-125
Clinically relevant CHK1 inhibitors induce the differentiation of myeloid cells by an inflammasome-dependent mechanism

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Acute promyelocytic leukemia (APL) is subtype of acute myeloid leukemia (AML) characterized by the expression of the oncogenic fusion protein PML-RARα. Standard therapy for APL involves the administration of all-trans retinoic acid (ATRA) and arsenic trioxide (ATO). However, the development of resistance to therapy requires the exploration of new treatments inducing leukemic blasts differentiation with low toxicity. APL cells are characterized by the overexpression and overactivation of the DNA damage response. We identified CHK1 kinase as a promising therapeutic target in APL, demonstrating that two specific CHK1 inhibitors, MK-8776 and Prexasertib, induce PML-RARα degradation. Moreover, we demonstrated that CHK1 inhibition affects pathways associated with the inflammatory response, including caspase-1 and IL1B[1]. The aim of this study is to define the crosstalk between CHK1 inhibition and inflammasome activation in regulating PML-RARα stability. We evaluated the effects of the inflammasome activation on CHK1 levels by treating myeloid cells with lipopolysaccharide (LPS). LPS caused the activation of both caspase-1 and NLRP3, and surprisingly induced the downregulation of CHK1. As the role of NLRP3 inflammasome in AML progression remains contested, we investigated the effect of NLRP3 activation on CHK1 levels. We set up a co-culture model that allows the secretome produced by LPS- or PMA-treated THP1 myeloid cells to reach APL cells. The expression levels of CHK1 and PML-RARα were evaluated by immunoblot. Besides, also the effect of NLRP3 or CHK1 silencing on PML-RARα levels and blast differentiation were evaluated. Finally, we demonstrated that CHK1 inhibitors act synergistically with ATO in inducing ATRA-resistant APL cells differentiation. Results obtained will contribute to define the molecular aspects underlying the use of CHK1 inhibitors as anti-leukemic drugs, alone or in combination with ATRA or ATO. Reference: 1 Franza et al., 2023., Biochemical Pharmacology 214:115675.

P-27-126
Screening of NKG2D activating peptides and their functional effects on NK cell activity

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Natural killer (NK) cells are one of the major innate immune cells, and are responsible for controlling transformed cells, such as cancer and virus-infected cells. Viruses can regulate the activity of NK cells in innate immune responses. There is limited understanding of the role of NK receptors and their interaction with virus-induced pathogenesis. Tumors and viruses have devised mechanisms to evade NKG2D recognition by modulating its ligand expression or by secreting soluble forms of its ligands. To understand the interaction between viruses and NK cells, several viral protein-derived peptides capable of binding to the NKG2D receptor were screened by *in silico* analysis. Among them, two peptides bound to NKG2D receptors. These peptides increased NK cytotoxicity toward lung cancer cells, stimulated interferon gamma (IFN-γ) production by NK cells, and likely mediated these responses through the downstream-signaling molecule of NKG2D. The direct modulation of NK cells with peptides has potential clinical application as a therapeutic approach for cancer therapy.

P-27-127
Smart nano-enzymes dynamically activated by ferroptosis and disulfidptosis enable efficient cancer therapy

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Ferroptosis-based chemodynamic therapies use hydroxyl radicals generated by the fenton reaction to kill cancer cells. Glucose oxidase-dependent glucose depletion provides a hydrogen peroxide ubstrate, and oxygen generated by the fenton reaction mitigates tumor hypoxia due to glucose depletion. Cancer cells rely heavily on intracellular glutathione to resist lipid peroxidation induced by ferroptosis, which when combined with glucose starvation causes intracellular cystine accumulation and the reduced form of nicotinamide-adenine dinucleotide phosphate depletion, leading to disulfidptosis. The morphology, elemental composition, and tumour microenvironment responses of various organic/inorganic nanoplatforms were characterised by different analytical methods. There *in vivo* and *in vitro* tumour-targeting efficacy and imaging capability were analysed by magnetic resonance imaging. Confocal microscopy, flow cytometry, and western blotting were used to investigate the therapeutic efficacy and mechanisms of mutually enhanced ferroptosis and disulfidptosis mediated by the nano-enzyme. The smart nano-enzyme relies on

tumor cell membranes for targeted drug delivery and can achieve reactive biodegradation in the presence of glutathione in the tumor microenvironment. It is characterized by lipid peroxide accumulation and aberrant disulfide bond cross-linking between the actin cytoskeleton, inducing tumor cell death via mutually enhanced ferroptosis and disulfidptosis. The smart nano-enzyme also features a superparamagnetic framework that can be used to guide and monitor therapy under T2-weighted magnetic resonance imaging. This rationally designed nano-enzyme is expected to integrate cancer diagnosis, treatment, and monitoring and provide a novel clinical antitumor therapeutic strategy based on ferroptosis, nutrient dependence, and the tumor microenvironment.

P-27-128
FTH1 silencing is required to overcome JQ1 resistance in aggressive non-small cell lung cancer via BRD2 by inducing ferroptosis

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Recent studies reported the presence of ferritin in cell nuclei, specifically, the H ferritin subunit (FTH1) emphasizing its involvement in alternative functions such as DNA protection from oxidative damage and transcriptional regulation. Bromodomain and extra terminal domain (BET) proteins act as epigenome readers for gene transcriptional regulation. Among BET family members, the role of BRD2 in non-small cell lung carcinoma (NSCLC) remains to be elucidated. In this study, we provide the first evidence of the functional interplay between nuclear FTH1 and BRD2. We show that nuclear FTH1 associates with BRD2, but not with BRD4, in a panel of NSCLC cell lines, particularly, in more aggressive types of NSCLC cells. We verified if FTH1 could affect the stability of BRD2 protein in NSCLC cells and observed a reduction of BRD2 protein levels in FTH1-silenced cells, only in more aggressive types of NSCLC cells, without affecting BRD2 mRNA levels. The combination treatment of JQ1, a BET inhibitor, with the FTH1 silencing induces an increase in mortality in JQ1-insensitive cells rather than JQ1-sensitive cells. The potential mechanism by which the combination treatment of JQ1 with the FTH1 silencing induces cell death was explored. The results show that ferroptosis is involved in the anticancer effect of JQ1 upon FTH1 silencing in JQ1-insensitive cells. Of note, the reactive oxygen species levels were increased by iron via the Fenton reaction, leading to ferroptosis. In addition, expression of ferroptosis-associated genes GPX4, SLC7A11, and SLC3A2 was downregulated under JQ1 treatment and FTH1 silencing, indicating that the cotreatment JQ1-FTH1 silencing may regulate ferroptosis by controlling the expression of ferroptosis-associated genes. In summary, for the first time, our data suggests that FTH1 silencing may serve as an effective anti-tumor strategy to enhance the activity of JQ1,acting to overcome the chemotherapy resistance in more aggressive non-small cell lung cancers.

P-27-129
Targeting SLC7A11/xCT counteracts uL3-mediated colorectal cancer drug resistance in 2D cell culture model and *in ovo* CAM xenograft

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Despite advancements in therapeutic strategies, development of drug resistance remains a serious concern for the efficacy of chemotherapy against colorectal cancer (CRC). We have recently demonstrated that low expression of ribosomal protein uL3 positively correlates with chemoresistance and poor prognosis in CRC patients [previously published in: Carotenuto et al. (2023) Mol Pharm 20, 2326-2340]. Here, we report the results of transcriptomic analysis that revealed a notable dysregulation of ferroptosis-related genes in resistant uL3-silenced CRC cells compared to the parental cells. Among them, SLC7A11 is one of the most dysregulated genes; specifically, its expression is inversely correlated with uL3 levels in resistant CRC cells and a large cohort of CRC patients. We demonstrated that uL3 downregulation in CRC cells promoted the upregulation of SLC7A11 at both post-transcriptional and post-translational levels. Erastin, a pharmacological inhibitor of SLC7A11 function, impaired resistant uL3-silenced CRC cell survival by inducing ferroptotic cell death as demonstrated by measurements of intracellular iron, glutathione, and reactive oxygen species levels as well as examination of key ferroptosis-related markers. The antiproliferative and antimetastatic potential of erastin treatment was investigated *in vivo* by grafting treated uL3-silenced CRC cells onto the chorioallantoic membrane (CAM).Overall, our study provides evidence of a novel therapeutic approach for sensitizing drug-resistant CRCs overexpressing SLC7A11 and characterized by low levels of uL3.

P-27-130
Nanotech revolution: fluorescent Nanodiamonds as innovative delivery of MiR-34a replacement in breast and pancreatic cancer

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Nanodiamonds are innovative nanocrystalline carbon particles able to deliver chemically conjugated miRNAs.In oncology the use of miRNA-based therapies may represent an advantage, based on their ability to simultaneously target multiple intracellular oncogenic targets. Here, nanodiamonds (ND) were tested and optimized to deliver miR-34a, a miRNA playing a key role in inhibiting tumor development and progression in many cancers. The physical-chemical properties of Nanodiamonds were investigated by DLS analysis suggesting electrical stability, uniformity of structure and size. Moreover, we evaluated Nanodiamonds cytotoxicity, through LDH assay, on breast and pancreatic cancer cell models and confirmed their excellent biocompatibility. Subsequently, Nanodiamonds were conjugated with miR-34a, using the chemical crosslinker polyethyleneimine; real time PCR analysis revealed a higher level of miR-34a in in both breast and pancreatic cancer cells treated with the different formulations of Nanodiamonds than with commercial transfectant. Investigations focused on the optical-magnetic properties of NDs, confirmed a progressive internalization in cytoplasm of cancer cell lines. A significant and early Nanodiamond-miR-34a uptake was recorded by FACS and fluorescence microscopy analysis in MCF7 and MDA-MB-231 cells. Moreover, Nanodiamond-miR-34a significantly inhibited both cell proliferation and migration conducted by clonogenic and wound healing assay. Finally, a remarkable anti-tumour effect of miR-34a-conjugated nanodiamonds was observed in both heterotopic and orthotopic murine xenograft breast cancer models. In conclusion, this study provides rationale for the development of new therapeutic strategies based on use of miR-34a delivered by Nanodiamonds in order to improve clinical treatment of breast cancer. Previously published in: Abate M et al (2023) Mol Ther Nucleic Acids 127-141. *The authors marked with an asterisk equally contributed to the work.

P-27-131
Vitamin C enhanced erastin-induced ferroptosis in drug-resistant colorectal cancer cells and in CAM-derived xenografts

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Recent studies indicated that the induction of ferroptosis, a type of non-apoptotic and iron-dependent form of cell death, could be a promising therapeutic approach for eradicating drug-resistant colorectal cancer (CRC) cells. Based on our previous findings indicating that the downregulation of human ribosomal protein L3 (uL3) positively correlates with chemoresistance in CRC cells lacking active p53, we investigated the susceptibility of this cell line to erastin, as a ferroptosis inducer, in combination with vitamin C. In this study, we revealed that the low expression state of uL3 renders p53-delete CRC cells more vulnerable to ferroptosis, and the combined treatment of erastin and vitamin C acts synergistically to induce ferroptotic cell death. Specifically, we evaluated intracellular iron, glutathione, reactive oxygen species levels along with the analysis of ferroptosis-related markers. *In vivo* experiments showed that erastin and vitamin C can significantly reduce tumor growth in xenografts derived from uL3-silenced CRC cells in the chorioallantoic membrane (CAM) model. In summary, the combination of erastin and vitamin C exerts a synergistic effect to induce ferroptosis in CRC cells that have low amounts of uL3 and lacking functional p53, thus providing a novel therapeutic strategy for CRC treatment.

P-27-132
Analysis of the complex formation between sorafenib and congo red and the expression of selected MicroRNAs in the molecular model

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Current research shows that the invasion and progression of bladder cancer may be closely related to, among others, the overexpression of the PDGFR with tyrosine kinase activity, the PI3K/AKT/mTOR intracellular signaling pathway, and the overexpression of micro-RNA 145 and micro-RNA 146b. Interestingly, current studies indicate that the overexpression of these micro-RNAs can inhibit the PI3K/AKT pathway. The aim of work was to analyze the complex formation between sorafenib and Congo red (CR), along with assessing the expression of tyrosine kinase receptor genes and micro-RNAs under the influence of sorafenib and its aggregate formed with CR. The analysis of the complex formation between the supramolecular carrier and sorafenib was evaluated using biophysical methods (Previously published in: Lasota M. et al., IJMS 25.1 2023: 269). The evaluation of the expression of selected tyrosine kinase receptor genes

and micro-RNAs under the influence of sorafenib and its aggregate with CR was conducted using the RT PCR method. The conducted analysis of carrier-drug complex formation allowed for the determination of the optimal molar ratios of carrier to drug, which was found to be 5:1. UV/VIS spectroscopy analysis showed a strong absorption band in the 450-550 nm range and confirmed the intercalation of the tested drugs into the ribbon structures of CR. The obtained results showed differences in the expression levels of genes coding for tyrosine kinase receptors (PDGFR and c-KIT) and signaling proteins (AKT, ERK1/2), as well as microRNA-145 and microRNA-146b under the influence of the tested compounds, confirming their anticancer properties. These results shed new light on cancer therapy and underscore the importance of research on supramolecular structures. This research was funded by a grant from the Polish Ministry of Education and Science from state budget resources as part of the "Student scientific groups create innovations" program grant number: SKN/SP/533486/2022. *The authors marked with an asterisk equally contributed to the work.

P-27-133
Induction of extrachromosomal circular DNA by the APOBEC proteins

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Extrachromosomal circular DNAs (eccDNA) are ubiquitous genetic structures found in eukaryotic cells. In approximately half of human cancers, larger eccDNA fragments have been identified. Their presence correlates with aggressive tumor characteristics and unfavourable patient outcomes. The molecular processes contributing to eccDNA-related pathogenesis remain largely elusive. However, eccDNA appears to be associated with genomic instability, as DNA damage can increase eccDNA levels. In cancer, dramatic events such as chromothripsis can lead to the formation and amplification of circular DNAs. The occurrence of kataegis in chromothriptic eccDNAs has hinted at a potential role for the AID/APOBECs. Intriguingly, APOBEC3B-mediated hypermutation has been reported to preferentially occur on large eccDNAs compared to linear chromosomes. Our research aims to determine whether the AID/APOBECs have an impact on the formation of eccDNA. We employed the CRISPR–Cas9 system to model the formation of a proteincoding eccDNA in human cell lines where the circularization event can be assayed by flow cytometry and characterized by Nanopore sequencing platform. Upon examining the eccDNA content in cells expressing the AID/APOBECs, we observed that two APOBEC3 enzymes could induce substantial levels of eccDNA. It appears that APOBEC-mediated deamination is necessary for this process, as catalytically inactive mutants were unable to induce eccDNA formation. Accordingly, mutational analysis revealed that eccDNAs from cells with endogenous expression of APOBEC3B derived mostly from genomic sites enriched for TC/GA dinucleotides, the preferred target site of AID/APOBEC proteins. On the other hand, disruption of the APOBEC3B locus resulted in eccDNA from genomic sites where such enrichment was not present. Our findings suggest that – beyond the mutagenic activity – APOBEC3B could also affect cancer evolution by facilitating the onset of eccDNA.

P-27-134
Effect of long-term hypoglycemic condition on tumor-specific cytotoxic T lymphocyte responses and PD-L1 expression

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The solid tumor microenvironment is unfavorable for the survival and proliferation of cancer cells but promotes more aggressive tumor progression including resistance to anticancer therapies. Research on the impact of glucose deprivation within the tumor microenvironment on anticancer immune responses is limited, particularly studies on whether long-term glucose deprivation regulates PD-L1 expression. In A549 cells, the protein levels of BiP and levels of PD-L1 mRNA and protein gradually increased inversely correlated with glucose concentrations following a minimum deprivation period of four weeks. In contrast, within less than seven days of glucose deprivation, the levels of BiP protein increased, while the levels of PD-L1 protein remained unchanged. Long-term glucose deprivation resulted in an increase of cell surface PD-L1 protein levels, and a decrease of cancer cell death by cytotoxic T lymphocytes (CTL) as well as granzyme B-induced apoptosis. Janus kinase (JAK) and histone deacetylase (HDAC) were involved in PD-L1 expression induced by long-term glucose deprivation. The increase in PD-L1 protein level under the condition was abrogated by inhibition of JAK1/2/3, while increase in PD-L1 mRNA level was unaffected. In contrast, the inhibition of HDAC1/2/6 almost abolished the increase in PD-L1 mRNA and protein levels under glucose-deprived conditions. The suppression of PD-L1 protein expression induced by JAK2 inhibition was reversed by proteasome inhibition, whereas the effect induced by HDAC6 inhibition was nearly unaffected regardless of proteasome inhibition. Taken together, these data suggest that long-term glucose deprivation increases PD-L1 expression, thereby attenuating tumor-specific CTL response, and that it increases PD-L1 expression by inhibiting proteasomal degradation of PD-L1 through JAK2 activation and inducing PD-L1 transcription through HDAC6 activation. This research was supported by the National Research Foundation of Korea (2022R1F1A1071397).

P-34-027
Harnessing plant extracts to overcome antibiotic resistance: leaf extracts of *Ribes nigrum, Ficus carica,* and *Vitis vinifera* as resistance modifiers of *E. coli* strains

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The emergence of bacterial resistance to antibiotics is the increasing global health issue. It continues to escalate due to the rapid development of resistance against newly introduced antibiotics and the decrease in the discovery of new preparations. This research aims to investigate the antibiotic modulatory potential of leaf extracts from *Ribes nigrum, Ficus carica,* and *Vitis vinifera* plants, and their effects on susceptibility of ampicillin and kanamycin-resistant *E. coli* strains toward antibiotics. The study included the extraction of plant leaves using a maceration technique and subsequent metabolomic characterization using LC-Q-Orbitrap HRMS technique. Antibiotic-resistant *E. coli* strains were used to assess the resistance modifying properties of the test extracts. Broth microdilution assay was applied to determine the minimal inhibitory concentrations of antibiotics and plant extracts, both individually and in combination. Additionally, measurements of H^+-fluxes across bacterial membranes were conducted to assess possible mechanisms behind the resistance-modifying effects of the extracts. The obtained data revealed that while the extracts exhibited no direct antibacterial activity, *R. nigrum* and *F. carica* demonstrated significant modulatory effects on antibiotics, enhancing the efficacy of ampicillin and kanamycin. The study also found that all plant extracts enhanced H^+-fluxes and promoted ATPase activity in kanamycin-resistant *E. coli* strain, suggesting the potential role of these plant preparations in altering bacterial membrane integrity as resistance modulation. The findings show that leaf extracts from *R. nigrum, F. carica,* and *V. vinifera* may be considered as the natural modulators of antibiotic resistance. These extracts can increase the efficacy of antibiotics against resistant bacterial strains, thereby offering a promising avenue for developing new strategies to combat antibiotic resistance.

Bioinformatics and AI for Precision Medicine

P-28-001
Pancreatic ductal adenocarcinoma metastatic biomarker discovery using machine learning approach

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Pancreatic ductal adenocarcinoma (PDAC) is a deadly, metastasised disease that is hard to find and treat early. This is because imaging and early PDAC biomarker detection are not effective. While many studies have searched for early diagnostic indicators, metastatic biomarkers remain elusive. Although cancer omics studies exist, none have integrated data to create a composite marker. Machine learning (ML) is commonly used in cancer biomarker discovery. Using machine learning to find biomarkers for PDAC metastases is shown in this study, showing the benefits of using useful data. We pooled primary tumour RNAseq data from five public repositories and split the data into training and validation sets. In the training set, we used LASSO for variable selection, followed by Boruta or VarselRF in 100 models per fold during 10-fold cross-validation. Genes found in at least 80% of models and five-fold were suitable for multivariate modelling. We constructed and validated a random forest model using selected genes from the train set and evaluated on the validation data. The biological context was explored through enrichment and pathway analyses using QIAGEN Ingenuity Pathway Analysis(IPA) and GeneMANIA. Our study comprehensively analysed transcriptomics data from diverse sources, proposing a composite biomarker candidate with strong predictive performance. These genes were consistently selected using various variable selection methods and data splits. The biological context analysis revealed associations with cancer progression and metastasis. While we adhered to best practices in machine learning, some limitations remain, particularly in data integration. This study establishes a robust framework for identifying a composite biomarker for PDAC metastasis. By reusing data from prior studies, we highlight the sustainability of our research, and the preliminary findings suggest a viable path for validation and clinical applications.

P-28-002
Atomistic description of the OCTN1 recognition mechanism via *in silico* methods

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The organic cation transporter novel 1 (OCTN1), or SLC22A4, is a pivotal element in the transport of organic cations and zwitterions, influencing diverse physiological and pathological processes. Despite its importance, the transported substrates of OCTN1 have been a subject of ongoing debate. This study addresses this gap by presenting a chimeric 3D model of OCTN1, integrating the AlphaFold-generated large extracellular loop 1 (EL1) onto a homology model derived from OCT3. Molecular dynamics simulations unveil domain-specific mobility, emphasizing the impact of EL1 and intracellular loop 4 (IL4) on overall stability. Molecular docking simulations identify cytarabine and verapamil as top-scoring ligands, aligning with their known inhibitory effects on OCTN1. Substrate categorization based on MM/GBSA analysis reveals a correlation between molecular weight and binding affinity to the extracellular recognition site. Key recognition residues, such as Tyr211, Glu381, and Arg469, are identified through interaction analysis. Acetylcholine (Ach) exhibits low interaction energy, supporting its one-directional transport hypothesis towards the extracellular side. The study delves into the role of sodium, suggesting Glu381's involvement in sodium binding. Molecular dynamics simulations at varying Na^+ concentrations indicate increased sodium occupancy around Glu381, supporting experimental data linking Na^+ concentration to molecule transport. In summary, this research provides valuable insights into OCTN1's 3D structure, substrate preferences, and sodium's role. These findings enhance our comprehension of OCTN1 in physiological and pathological processes, with potential implications for drug development and disease management. *The authors marked with an asterisk equally contributed to the work.

P-28-003
A population pharmacokinetic model to predict the individual dose of tacrolimus prescribed during the first three months post-transplantation for Tunisian transplant recipients

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Tacrolimus is one of the most prescribed immunosuppressive drugs for kidney transplant recipients. However, this drug has a narrow therapeutic index and high intra- and inter-patient pharmacokinetic (PK) variability [1]. It is, therefore, necessary to personalize the treatment to avoid any ineffectiveness and toxicity. Our objective was to optimize the dose of tacrolimus, prescribed during the first 3 months after kidney transplantation, by developing a population pharmacokinetic model (final model) for the Tunisian population. A starting dose model previously developed [2] to be used directly after transplantation for the same population, was used to develop the final model. A total of 1901 whole-blood pre-dose concentrations (C0) of tacrolimus from 196 adult kidney transplant recipients were collected for the analysis. Genotyping for CYP3A5*3, CYP3A4*22, ABCB1-C3435T, ABCB1-G2677T, ABCB1-C1236T, and POR*28 SNPs was performed. The pharmacokinetic analysis was performed using nonlinear mixed-effects modeling (NONMEM). We found that CYP3A5*1 carriers and homozygotes had higher tacrolimus CL/F. Higher body mass index (BMI) and lower hematocrit resulted in increased tacrolimus CL/F. Thus, our model incorporated CYP3A5*1, BMI and hematocrit: DOSE (mg) = 365 ng.h/ml* 23.5/h * (1 + 0,08 e-0,08*Time) * (1.0, if CYP3A5*3/*3) or (1.28, if CYP3A5*3/*1 or CYP3A5*1/*1) * (BMI/22.48)0.35 *(hematocrit / 32)-0.81/1000. We found that the proportion of patients on target was 53.06% in the patients having received a tacrolimus dose based on body weight, while 76.02% of patients were estimated to have had tacrolimus C0 within target if they would have taken a model-based dose of tacrolimus. Using the dose calculated by this model can potentially increase the percentage of patients on target. 1. Previously published in: Staatz CE et al. (2004) Clin. Pharmacokinet 43, 623-653. 2. Previously published in: Abderahmene A et al. (2024). Ther. Drug. Monit 46, 57–66.

P-28-004

Abstract withdrawn.

P-28-005

Lactobacillus rhamnosus GG against bisphenol A: focus on Claudin-1, Claudin-2, and Aquaporin via SDS-PAGE and bioinformatics

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Bisphenol A (BPA) is an environmental endocrine disruptor commonly used in plastic production and is known to mimic estrogen. This ability impacts not only the reproductive system but also various tissues throughout the body. This study aimed to investigate the effects of *Lactobacillus rhamnosus* GG (LGG) on bisphenol A (BPA)-induced intestinal damage with a particular focus on the alterations in key proteins, namely Claudin-1, Claudin-2, and Aquaporin. The primary goal of this study was to utilize SDS-PAGE and bioinformatic analysis to identify dynamic changes in the proteins Claudin-1, Claudin-2, and Aquaporin. The rats were divided into three groups: control, BPA, and BPA + LGG. The 50 mg/kg BPA was administered intraperitoneally for 6 weeks to the BPA-related groups. LGG was administered in a dose of 1 x 109 CFU/day orally for 6 weeks to the BPA + LGG group. After six weeks, the small intestine tissues were obtained. Subsequent intestinal protein expression profiles were analyzed using SDS polyacrylamide gel electrophoresis (SDS-PAGE). Bioinformatic tools (Autodock Vina, Avogadro, Schrödinger Maestro, BIOVINA) was used to characterize changes in Claudin-1, Claudin-2, and Aquaporin proteins. The *in silico* analyses provided evidence that BPA can bind to Claudin1-2 and Aquaporin-1 proteins. It was also determined *in silico* that the major proteins of LGG, Msp1 (LGG_00324) and Msp2 (LGG_00031), may have some roles in mitigating BPA-induced changes in Claudin1-2 and Aquaporin-1 proteins. In conclusion, by combining biochemical and bioinformatic approaches, molecular mechanistic information regarding the therapeutic potential of probiotic treatments in BPA-induced intestinal damage was provided.

P-28-006

Supporting machine learning model in the treatment of chronic pain

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Conventional therapy options for chronic pain are still insufficient and patients most frequently request alternative medical treatments, such as medical cannabis. Although clinical evidence supports the use of cannabis for pain, very little is known about the efficacy, dosage, administration methods, or side effects of widely used and accessible cannabis products. A possible solution could be given by pharmacogenetics, with the identification of several polymorphic genes that may play a role in the pharmacodynamics and pharmacokinetics of cannabis. Based on these findings, data from patients treated with cannabis and genotyped for several candidate polymorphic genes (single-nucleotide polymorphism: SNP) were collected, integrated, and analyzed through a machine learning (ML) model to demonstrate that the reduction in pain intensity is closely related to gene polymorphisms. Starting from the patient's data collected, the method supports the therapeutic process, avoiding ineffective results or the occurrence of side effects. This approach will possibly contribute to clarifying the pharmacokinetics and pharmacodynamics of cannabis, increasing the clinical community's trust in its therapeutic application, and supporting their choices with a tool that can provide the importance of each component. Our findings suggest that ML prediction has the potential to positively influence clinical pharmacogenomics and facilitate the translation of a patient's genomic profile into useful therapeutic knowledge.

P-28-007

E-pRSA: protein language models improve the prediction of residue solvent accessibility from sequence

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Knowledge of residue solvent accessibility in a protein is important for different applications, including the identification of interacting functional surfaces and the characterisation of residues undergoing variations. Relative solvent accessibility (RSA) values can be directly computed from the 3-dimensional structure. When resolved structures are unavailable, machine learning-based tools can provide estimates starting from the protein sequence. Protein language models (PLMs) allow the development of faster and more accurate tools than canonical encodings such as multiple sequence alignments (MSAs). After being trained on huge datasets, PLMs can generate a representation of the protein sequence that casts relevant evolutionary, structural, and contextual information. Here we present E-pRSA, a tool to accurately estimate the RSA values for residues of a protein sequence in the absence of the structure. E-pRSA is trained on 6,552 proteins and benchmarked on a blind test set comprising 165 proteins. The method adopts two state-of-the-art PLM-based embeddings, namely ProtT5 and ESM2, to encode the input protein. A cascading convolutional neural network then processes a window context to compute a putative RSA value for each position. E-pRSA achieves a 0.80 Pearson correlation coefficient on the blind test set, outperforming our previous method DeepREx, which exploits evolutionary information contained in MSAs. Moreover, the method outperforms two recently released methods based on PLM encoding, NetSurfP-2.0 and SPOT-1D-LM. E-pRSA is freely available at https://e-prsa.biocomp.unibo.it/main/, where users can submit single sequence and/or batch prediction jobs. The web server additionally integrates predictions made by ISPRED-SEQ, a tool for predicting residues involved in protein-protein interactions. The combined visualization allows users to quickly analyse and characterize proteins of interest that lack a resolved 3D structure.

P-28-008
Machine learning for salivary proteome investigation and disease activity exploration in relapsing-remitting multiple sclerosis patients

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Proteomics coupled to machine learning (ML) represents a cutting-edge strategy to explore mechanisms underlying diseases development. The complex mosaic of multiple sclerosis (MS) represents an intriguing scenario for the application of omics approaches searching for new biomarkers [Previously published in: Lorefice L et al. (2023) Front Genet 14, 1076421]. The salivary proteome, as mirror of several systemic processes [Previously published in: Manconi B et al. (2018) J Proteom 187, 212–222], has been analyzed searching for proteomic profiles associated with disease activity in subjects with relapsing-remitting (RR) MS. The present study included RR-MS patients without pharmacological therapy at the moment of saliva sampling (T0), further classified into T0_Active (T0A, n = 30), and T0_not Active (T0nA, n = 36) based on the identification of clinical relapses and new/enlarged lesions; the same patients were recruited after 6 months of therapy (T6A, n = 16; T6nA, n = 24). A group of 31 sex-/age-matched healthy controls (HCs) were also involved. Protein label-free quantitation was obtained from whole saliva by a bottom-up pipeline based on nano-HPLC-high-resolution-MS/MS analyses, followed by ML and enrichment analysis. Protein abundances (1305 proteins) were analyzed by Random Forest, which evidenced a high classification accuracy (96%) among T0, T6 and HCs. In addition, Mann-Whitney non-parametric statistical analysis showed groups of proteins with abundances significantly varied ($pV < 0.05$, FDR < 5%) between comparison pairs. Among that, levels of 59 proteins varied in T0A respect to T6A, showing involvement ($pV < 0.01$) in pathways related to activity of BRAF mutants, regulation of insulin-like growth factor, bacterial infections and protein folding in endoplasmic reticulum. 75 proteins, involved in interleukin-12 signaling and platelet degranulation, showed varied levels between T0nA and T6nA. Further investigations are needed to relate results with disease activity.

P-28-009
Accelerating GPCR discovery through AI and big data

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G protein-coupled receptors (GPCRs) are among the most studied classes of proteins and are members of the largest and most diverse group of receptors. They are responsible for approximately two-thirds of hormones and neurotransmitter signalling. This fact has led to enormous interest in GPCRs as drug targets, with one-third of all approved drugs by the US FDA targeting GPCRs. We leveraged the wealth of experimental data available on GPCRs in order to develop two novel AI algorithms to rapidly and accurately screen for small molecule hits and rationally optimise GPCR stability. These methods were validated using independent experimental data, outperforming previous approaches. Initially, we explored the druggability of 36 GPCRs of great medical interest. By applying a ligand-based strategy, we developed an AI predictor of molecule affinity for each of these receptors. This approach enables fast and reliable screening for potential hits or cross-reactivity in drug development pipelines. Across blind tests, our approach achieved Pearson's, of up to 0.89 on 10-fold cross-validation, and a Pearson's correlation above 0.74 on blind tests [published: Velloso JPL et al. (2021) Bio Adv], https://biosig.lab.uq.edu.au/pdcsm_gpcr. We then focused on the development of a tool to predict the effects of mutations on the stability of a GPCR, a rate limiting step in many experimental assays. During the validation, it achieved Pearson's correlation coefficients of 0.74 and 0.46 on 10-fold cross-validation and blind test sets, respectively. The user can then choose the more stabilising mutations to produce a more stable structure, which facilitates the process of structure discovery, https://biosig.lab.uq.edu.au/gpcr_tm. These projects highlighted some of the fundamental molecular insights that support complexities in GPCR pharmacology. These insights, together with the applicability of our tools, have significant potential to improve human health and reduce the time and cost of research.

P-28-010
RKIP and YY1 cross-talks in pan-cancer

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Recent research highlights the intriguing interaction between PEBP1 (RKIP) and YY1, two molecules with unique molecular functions that appear to significantly influence each other. Their reciprocal regulatory mechanisms suggest a critical role in cancer progression and drug resistance. Our study employed bioinformatics analyses across various cancers, utilizing data from the TCGA for mRNA expression, UCSC Xena for CNV and methylation, and the Synapse project for SNV data. We meticulously normalized and calculated methylation levels to investigate the differential expression of YY1 and RKIP and their correlation with patient survival, cancer stage, and pathway activity. Further, we explored the relationship between these genes and immune infiltration, mutations, methylation, and drug sensitivity

using pharmacogenomic data from sources like NCI-DTP NCI-60, Sanger GDSC, and Broad CCLE/CTRP. This included analyzing the IC50 of 265 small molecules across 860 cell lines in relation to YY1 and PEBP1 mRNA levels. Our findings reveal that elevated YY1 mRNA levels are linked to varied survival outcomes and show that YY1 and RKIP influence key cancer pathways including apoptosis, the cell cycle, and DNA damage repair. We also found significant correlations between their expression/methylation and immune infiltration, as well as genomic alterations in several cancers. Interestingly, we identified associations between YY1/PEBP1 and indicators of immunosuppression, such as responses to immune checkpoint blockade and T-cell dysfunction/exclusion, highlighting their potential role in modulating tumor immunity and the microenvironment. Importantly, our research indicates that YY1 and RKIP's interactions affect the efficacy of anticancer drugs, suggesting their potential as targets for novel therapeutic strategies. Overall, our study emphasizes the importance of YY1 and PEBP1's interplay in cancer biology, offering insights that could lead to improved cancer treatments.

P-28-011
The development of machine learning models for predicting the obesity risk from MARK-AGE data

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Obesity is one of the most important cardiovascular risk factors worldwide, having a significant impact on patients' health and quality of life. However, machine learning algorithms which are able to accurately predict overweight and obesity risk based on both important clinical parameters [body mass index (BMI) and waist-to-hip ratio (WTHR)] are yet to be implemented. Therefore, the aim of the current study was to develop and validate such algorithms, based on specific predictors obtained from the MARK-AGE database. The analyzed MARK-AGE data consisted in clinical and biochemical parameters obtained from patients aged 40-75 years within the RASIG population. Based on specific predictive variables [metabolic (including the susceptibility of LDL particles to oxidation, inflammatory, epigenetic, and clinical markers) as well as relevant lifestyle factors (such as nutrition, fitness levels and education and marital status)] several machine learning algorithms were developed, with the aim of estimating an individual's overweight and obesity risk, as defined through the BMI and WTHR recommended thresholds. The average optimum ROC AUC scores obtained through a repeated cross-validation process based on the data from 1089 patients yielded values ranging from 0.75 for the WTHR prediction models (Support Vector Machines) to 0.81–0.82 for the BMI prediction models (XGBoost), showing good values considering the relatively small dataset and the complex nature of obesity. By optimizing the developed algorithms and validating them on a larger number of patients, out study could provide clinically relevant tools for modelling the impact of specific lifestyle interventions and relevant biomarker levels on the risk of weight gain and obesity.

P-28-012
Molecular dynamics simulations of the interaction between antibacterial small molecules and the *Staphylococcus aureus* lipid bilayer

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Bacterial resistance to antimicrobial drugs is a critical public health concern, as various organisms evolve into multidrug-resistant strains, leading to increased mortality and morbidity.(1) Innovative antibacterial therapeutics are urgently needed. This study employed molecular dynamics (MD) simulations to explore the mechanism of action of antibacterial small molecules targeting the membrane of *S. aureus*. Using the CHARMM-GUI Membrane Builder tool,(2) we constructed the lipid bilayer *S. aureus* membrane, and MD simulations were conducted with AMBER18 using the CHARMM36m38 force field. Two independent MD replicas of 500 ns were performed for each membrane/small molecule complex, investigating the interaction with two molecules with opposite bioactivity profiles but high chemical similarity. MD analysis of bioactive compound 1 showed tight binding to the *S. aureus* membrane model. Ligand mass density distribution revealed full integration of compound 1 into the membrane from 70 ns onwards, persisting until the simulation's end. In contrast, inactive compound 2 displayed less stable interactions with the phospholipid bilayer, residing outside the membrane. These findings suggest that the *S. aureus* membrane likely serves as the primary site of action for compound 1, aligning with experimental observations. Furthermore, slight alterations in chemical structure could significantly affect interaction with the membrane and the bioactivity profile of related small molecules. MD simulations offer valuable insights into small molecule interaction with the *S. aureus* membrane, with potential applications in the lead optimization of antibacterial compounds.(3) References 1) Mancuso, Giuseppe et al. (2021) Pathogens vol. 10,10 1310. 2) Li, Yupeng et al. (2021) Methods Mol Biol vol. 2302 (2021): 237-251. 3) Princiotto S, Casciaro B, G Temprano A, et al. (2024) Bioorg Chem. 145:107227. *The authors marked with an asterisk equally contributed to the work.

P-28-013
In silico investigation of the dual role of autophagy

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Autophagy is an evolutionarily conserved dynamic catabolic process in eukaryotic cells, where double-membrane vesicles containing cytoplasmic components are formed and degraded in specialized vesicles. It primarily influences cell survival by maintaining the cell's energy balance and degrading damaged macromolecules and organelles. However, due to it being tightly connected to apoptosis, autophagy can also promote cell death. Consequently, the modulation of autophagy plays dual roles in tumor cells, both promoting and suppressing tumor proliferation.

To better understand this nature of autophagy we made a chemical reaction kinetics model of autophagy and apoptosis. The initial model was made by Bing Liu [1]. It is a mass action kinetic model containing 94 species, 119 reactions and 129 parameters. They only used one data set for the parameter estimation and initial conditions for the species were not provided. Therefore, the model cannot be used for general simulations. To further develop the model realistic initial value conditions were given for each species. Then the model was optimized with the Optima + + software package. A new parameter set was estimated with our group's previously published data to estimate the parameters and validate the model [2,3]. The improved model is able to track the concentration changes in time of several regulatory proteins with reasonable accuracy, making the model usable for *in silico* investigation of autophagy related diseases. Supported by the ÚNKP-23-3-II new National Excellence Program of the Ministry for Culture and Innovation from the sources of the National Research, Development and Innovation Fund. References: [1] Liu, B. et al. (2017) Sci Rep 7(1), 17605. [2] Holczer, M et al. (2020) Sci Rep10(1), 17803. [3] Márton, M. et al. (2017) Int J Mol Sci 18(1), 58.

P-28-014
Prediction of intensive care requirement in preeclamptic pregnancy using artificial neural network based on biochemical parameters

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Preeclampsia is a multisystem progressive disease characterized by new-onset hypertension and proteinuria, sometimes accompanied by marked end-organ dysfunction with or without proteinuria, typically occurring after the 20th week of pregnancy or postpartum. Severe preeclampsia and HELLP syndrome (hemolysis, elevated liver enzymes, low platelets) are life threatening, increasing the maternal and fetal morbidity and mortality. intensive care unit (ICU) care is recommended when two or more organ systems are failing and there is need for ventilator support. With the development of the artificial intelligence world, many models have been created using a variety of data types, including demographic and clinical data etc. The Deep Neural Network model was developed by using Keras with Tensorflow based on python. This study, based on the artificial neural network (ANN) method, aims to predict the risk of pregnant women entering intensive care after birth, allowing clinicians to improve close follow-up and extended management of preeclamptic patients and take precautions at an early stage. The database for ANN includes demographic clinical, laboratory characteristics of 367 patients. Biochemical markers (hemoglobin, hematocrite, platelet count, total protein, albumin, total bilirubin, indirect bilirubin, alanine transaminase (ALT), aspartate transaminase (AST), urea, creatinine, urinary protein excretion (ESBACH)) used in the current ANN method selected from this database. According to this model, the second group of maternal biochemical parameters; including AST, ALT, indirect bilirubin, and platelet count, had similar accuracy (89% and 91%, respectively) when compared with other parameters (hemoglobin, hematocrit, etc.). However, the accuracy of the combination of these two groups was 97,7. This study revealed the accuracy of the combination of these biochemical parameters in predicting the intensive care needs of preeclamptic pregnant women. *The authors marked with an asterisk equally contributed to the work.

P-28-015
A bioinformatic and *in vitro* combined approach to identify new diacylglycerol kinase inhibitors for cancer treatment

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Diacylglycerol kinases (DGKs) are a family of enzymes which catalyse the phosphorylation of diacylglycerol in phosphatidic acid, regulating several cellular pathways related to these two bioactive lipids. Nevertheless, abnormalities in DGK activity have been detected in different pathologies as cancer, where they are involved not only in cell's resistance to apoptosis and the uncontrolled proliferation, but also in the immune suppression. Focusing on the acute myeloid leukaemia (AML) case indeed, after a deep bioinformatic analysis and further *in vitro* study, we showed that the isoforms DGKα, DGKζ and DGKη are particularly relevant in this pathology, and may represent an attractive therapeutic target not only for AML, but potentially for other cancers and/or diseases in which DGKs are involved. Since nowadays only few molecules have been tested as DGK inhibitors, and the majority hits principally the α isoform, we focused on the research of new putative DGKη and/or DGKζ inhibitors exploring the ZINC20 database (containing over 750 million commercially available compounds) after the creation of DGKη and ζ models and the validation of a structure-based virtual screen protocol. Finally, we aim to confirm their inhibitory activity against these isoforms through *in vitro* kinase assays. Partially previously published in: Gravina T. et al. (2023) Biomedicines, 11, 1877.

P-28-016
AI-predicted drugs as possible therapeutic solutions for familial hypomagnesemia with hypercalciuria and nephrocalcinosis: cell models and read-outs characterization

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Familial hypomagnesemia with hypercalciuria and nephrocalcinosis (FHHNC) is an ultra-rare autosomal recessive tubulopathy caused by loss-of-function mutations in *CLDN16* or *CLDN19*. In

Spain, 90% of patients carry the *CLDN19* c.59G>A (p.G20D) mutation, 60% of them in homozygosis. FHHNC is characterized by severe urinary Mg^{2+}/Ca^{2+} wasting and progression to kidney failure. Currently, there are no specific treatments available, highlighting the urgent need for therapeutic targets and prognostic biomarkers. Aiming to obtain therapeutic solutions for this disease, our lab has employed artificial intelligence (AI) algorithms to predict possible repositioning drugs combining clinical information and differential urinary miRNAs profiles among patients with different disease progression and controls. To validate the efficacy of these AI-predicted drugs, we have generated and characterized FHHNC cell models stably expressing either CLDN19 WT or the p.G20D mutation. Our results confirmed that CLDN19 p.G20D mutation causes an intracellular retention of the protein, impairing its trafficking, processing and release to the extracellular medium (as observed in CLDN19 WT form). Besides, we found that ATF6, a marker of UPR, and *CLDN16* levels are altered in cells carrying the p.G20D form. Altogether, we used these features as reliable read-outs that clearly distinguish between control or FHHNC cell lines. Next, we proceeded to test AI-predicted repositioning drugs in our FHNNC cell models. We used three different combinations and three single proposed drugs (110 μM) and their effect was first analyzed by the presence of CLDN19 in the conditioned medium. Our future research will involve verifying the most promising drugs testing other FHHNC related parameters. In conclusion, our findings revealed useful read-outs for screening multiple pharmacological possibilities in FHHNC cell models, which we expect could be used to develop therapeutic solutions for the disease.

P-28-017
Investigating the avermectin family compounds against tubulin protein: a molecular docking study

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The Avermectins belong to the 16-membered macrocyclic lactone family, which includes ivermectin, abamectin, moxidectin, milbemycin oxime, doramectin, selamectin, and eprinomectin. The recent findings regarding the anticancer activity of avermectins have provided additional impetus to studies on their application for treatment. These compounds are predicted to have anticancer effects on several cell lines. The objective of this study is to utilize *in silico* approaches to investigate the avermectin family compound's therapeutic potential against the tubulin protein. Molecular docking analyses were performed using tubulin protein (PDB: 1TUB) on the CB-Dock 2 webserver and visualized using PyMol software. The attached ligands were removed using Notepad++, and SWISSMODE was employed to build a template for missing amino acid residues. Interestingly, the results revealed that all the avermectin family compounds displayed higher binding scores compared to the reference anticancer drug, taxol. The compound ivermectin B1a, followed by doramectin, eprinomectin, and selamectin, exhibited the highest scores of -8.8, -8.7, -8.7, and -8.7 Kcal/mol, respectively. The present work provides scientific evidence that these avermectin compounds could be considered potential candidates for the development of anticancer drugs targeting tubulin protein in the future. *The authors marked with an asterisk equally contributed to the work.

P-28-018
EEG signals in mental fatigue detection: a comparing study of machine learning technics vs deep learning

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Mental fatigue is a complex disorganization that affects the human efficiency in work and daily activities such as driving, exercising, etc. To discern and detect that fatigue, encephalography (EEG) is routinely used. Many studies have been deploying conventional approaches to support neurologists in mental fatigue detection episodes e.g. sleepy vs normal. The aim of this work is the use of the EEG's to mainly detect the mental fatigue manifested as drowsiness, difficulty in concentrating, decreased alertness, disordered thinking, slow reaction, reduced work efficiency. The machine learning approach and deep learning method was used. The data is organized as follow: EEG data of 10 normal people and other 10 people who are deprived from sleep, the recording time is 7 min in each session, and the experiment includes three sessions for each person, none of the volunteers have a mental history and none of them are on medication. The main of this study is to compare the different methods for the analysis of EEG signals for the detection of fatigue, using machine learning and deep learning. *The authors marked with an asterisk equally contributed to the work.

P-28-019
Robosample: integrated robot mechanics with Gibbs sampling for enhanced biomolecular simulation

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Simulating large biomolecular systems is computationally demanding due to the large number of atoms and the complex interactions between them. We previously developed Robosample, a software [1] that implements Hamiltonian Monte Carlo coupled with Gibbs Sampling (GCHMC) [2] method using 15 types of robotic joints able to simulate diverse chemical bond dynamics. The program is built on top of three packages: OpenMM for hardware accelerated molecular mechanics, Simbody and Molmodel for robot mechanics. In this version, we refactored Robosample such that 1) linear algebra computations would be fully coded in Lapack; 2) all dependencies would compile into one executable, allowing for better compiler optimizations; 3) the code would be exposed through a Python interface. However, the most significant enhancement was 4) the externalization of Cartesian type-joints to OpenMM which improved the speed of execution ~40 fold. Tangent/cotangent transition on top of the conformational space was achieved through a new procedure that implements the square root of the mass metric tensor inverse. Additionally, we implemented 5) automated parameter selection for optimal acceptance rate. This enhanced version of Robosample, featuring a user-friendly Python interface, externalization of Cartesian type-joint dynamics to OpenMM, and automated parameter selection, significantly improves simulation

speed and efficiency for large biomolecular systems. References: [1] Spiridon L et al., Biochim Biophys Acta General Subjects 1864(8): 129616, (2020). [2] Spiridon L, Minh DDL. J Chem Theor Comput 13(10): 4649-4659, (2017). *The authors marked with an asterisk equally contributed to the work.

P-28-020
Integration of pyDock and AI-based approaches for structural modeling of antibody-antigen complexes

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AI-based methodologies, like AlphaFold (AF) [1], have dramatically changed the field of protein structure prediction, providing accurate structural models for the large majority of annotated proteins. Similarly, AlphaFold-Multimer has improved the structural modeling of protein-protein complexes, in comparison with previous docking strategies. However, as shown in the most recent CASP and CAPRI experiments, this software has limited predictive capabilities in difficult complexes, like antibody-antigen interactions. To address this limitation, we have explored the capabilities of AF-Multimer to generate accurate structural models for antibody-antigen complexes on a set of 67 cases. For each case, we have generated a large ensemble of conformational models by using different versions of AF-Multimer, activating dropout and increasing the number of recycled models, aiming to capture the variability and complexity inherent in antibody-antigen interactions. Then we have scored these models by combining AF model confidence metrics with energy-based pyDock [2] scoring function, in order to systematically enhance the success of model selection. The goal is to delineate the strengths and limitations of both approaches, providing insights into their comparative effectiveness in accurately modeling protein interactions. In addition to AF model confidence and pyDock scoring function, we have used near 100 descriptors from CCharPPI [2], combined through a variety of machine learning algorithms, including Gradient-Boosted Trees and K-means. This study illustrates the transformative potential of integrating diverse computational methodologies and machine learning techniques to address remaining challenges in predicting the structure of difficult protein-protein complexes. *References*: 1. Jumper J, et al (2021) Nature 596(7873), 583-589. 2. Cheng TM-K, Blundell TL, Fernandez-Recio J (2007) Proteins 68, 503-515. 3. Moal IH et al. (2014) Bioinformatics 31, 123-125.

P-28-021

Abstract withdrawn.

P-28-022
Computational approaches to estimate aminoacidic mutation effects on proteins involved in biological pathways for precision medicine

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The high variability in treatment efficacy among patients needs a personalized approach for improving disease diagnosis and therapy effectiveness[1]. This variability can be related to aminoacidic mutations; even if a mutation is not completely deleterious for protein activity, it can modify its affinity toward ligands, like a drug, leading to a different therapy responsiveness. Moreover, the mutations can affect the binding of mutated proteins to other proteins, impacting the entire pathway. The evaluation of mutations' impact is expensive and time-consuming, especially for considering the protein's entire pathway. Thus, conducting *in silico* these investigations can be an effective strategy. Here, a computational workflow (named ProMI) to automatically predict protein-ligand binding affinity changes upon mutation is presented. Then, the computed values integrated into a more systemic pipeline, are suitable for investigating the mutation impact on the entire protein signaling pathway. ProMI, validated against a benchmark of proteins with available wet lab affinity data, can successfully compute free energy changes through Molecular Dynamics Simulation, without requiring the mutated experimental model thanks to AlphaFold2[2]. The estimated mutation effect on the single protein-ligand interaction is used to evaluate the impact on the protein pathway by simulating mathematical models, like in BioModels[3], considering mutations as perturbated parameters. A set of equations describes how the biological system responds to perturbation and the employment of patient-specific parameters can provide data for a personalized therapeutical or diagnostic approach. (This work is partially funded by PNRR ECS00000017 "THE - Tuscany Health Ecosystem" - Spoke 6 "Precision medicine & personalized healthcare") Refences: 1. Björnsson B et al (2019) Genome Med. 12, 4. 2. J. Jumper et al (2021) Nature 596, 583-589. 3. Malik-Sheriff RS et al (2020) Nucleic Acids Res. 48, D407-D415.

P-28-023
PathLay: a novel graphical server for -omics integration and interpretation

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Over the recent years -omics studies have provided valuable information for depicting the global properties of biological systems and how they respond to stimuli. Differently from traditional approaches, -omics offer the opportunity to capture coordinated alterations that usually take place at the system level. Although hard to be explored, the system may be properly

addressed at the level of biological pathways with tools able to properly integrate them. Here we present PathLay, a freely accessible platform for multi-omics data interaction and integration directly on graphical pathway maps. Users may associate up to 6 different -omics to an experiment: transcripts, proteins, microRNAs, metabolites, methylation status and the accessibility of chromatin with a built-in feature that can recognize transcription factors from transcripts or proteins and assign them to their targets. PathLay will take care of associating every input data to appropriate nodes in graphical maps derived from KEGG and WikiPathways and represent such integrated information as intuitive interactive symbols. The PathLay user intuitively plays with such symbols using a battery of interactive methods that interact with each other and with the map viewer, which in turn displays relevant information in real-time. Such methods allow displaying specific players or groups of them, or players that are expressed with a specific trend or that see their actions localized in specific cellular compartments. PathLay allows rapid refocusing of the experimental perspective with biological lenses, allowing to intuitively follow paths within and through maps without being distracted by technicalities, with great benefit for data interpretation. *The authors marked with an asterisk equally contributed to the work.

P-28-024
Acceleration of biomolecular simulations by time-lagged t-SNE

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t-Distributed stochastic network embedding (t-SNE) is a popular method for visualization of high-dimensional data in genomics, proteomics, flow cytometry, machine learning, and other fields. It has also been applied on trajectories from biomolecular simulations to map complex structures in 2D space. We developed a variant of t-SNE called time-lagged t-SNE. Unlike the standard t-SNE that clusters together similar structures, time-lagged t-SNE clusters structures based on kinetics of their interconversions. Here, we present a series of trajectories analyzed by time-lagged t-SNE demonstrating its advantages. A disadvantage of t-SNE and time-lagged t-SNE is that these methods cannot calculate t-SNE coordinates for new out-of-sample structures. We addressed it by combining t-SNE and time-lagged t-SNE with an artificial neural network. The resulting parametric time-lagged t-SNE can be used to accelerate miniprotein folding simulations by metadynamics. The application on Trp-cage miniprotein folding will be presented. The work was supported by Czech Science Foundation (22-29667S).

P-28-025
In silico investigation of natural polyphenols that may inhibit ERK2

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Signal-regulated kinase 2 (ERK2) is one of the main kinases in the mitogen-activated protein kinase (MAPK) cascade that regulates cellular processes, such as proliferation, differentiation, survival, adhesion, migration and apoptosis [1,2]. Recent data have suggested, that ERK2 may be regulated by the glucose-regulated protein 78 [3]. GRP78 is a protein that serves as a chaperone within the endoplasmic reticulum (ER). Cellular stress conditions such as hypoxia, chemotherapy, radiation therapy, and drug resistance induce the expression of cell surface GRP78. Moreover, GRP78 may stimulate an anti-apoptotic receptor such as Cripto-1, which can suppress pro-apoptotic signals [4, 5]. The ERK2 binding site is located at the ATP-binding pocket, which consists of two lobes connected by a hinge. The ATP-binding pocket is composed of several key residues (Lys52, Glu69, Asp166, Lys114, and Asp208) that interact with ATP. Some examples of natural compounds like curcumin, resveratrol, apigenin are polyphenols that have been suggested to regulate the ERK signaling pathway. The aim of this study was evaluation of these compounds' affinity towards ERK2 protein. The 3D structure of ERK2 (PDB ID:2Y9Q) was acquired; natural compound structures were downloaded and virtually analyzed by Chimera. The binding energy was calculated using Autodock and compared with Sorafenib, commercial ERK2 inhibitor. Obtained results indicate that among these polyphenols curcumin has the highest affinity towards ERK2 [-4.62kcal/mol] and along with apigenin may be suggested as inhibitors for ERK2, which plays a significant role in cell survival. This project was financially supported by the NCN grant in Poland 2023/49/B/ST4/03288 References: 1. Kumar D, Hassan I, Disc to Therap, 2022, 129-178 2. Jafari S, et al. RSC Adv, 2021, 11, 11048-56. 3. Zhou X, et al. Oncology Reports 2016, 36, 2723-2730. 4. Shani G, et al. Mol Cell Biol. 2008, 28, 666-77. 5. Wang M, Wey S, Zhang Y, Ye R, Lee AS. Antiox Redox Sig. 2009, 11, 2307-16.

P-28-026
PACT – prediction of amyloid cross-interaction by threading

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Amyloid proteins are often associated with diseases, including Alzheimer's, Parkinson's and others. However, there is a wide class of functional amyloids that are involved in physiological functions, e.g., formation of microbial biofilms or storage of hormones. Recent studies showed that an amyloid fibril could affect the aggregation of another protein, even from a different species. The interactions between functional bacterial amyloids and human amyloidogenic proteins may result in amplification or attenuation of the aggregation process e.g., affecting the development of neurodegenerative diseases. We present PACT (Prediction of Amyloid Cross-interaction by Threading) – the computational method for prediction of potential amyloid cross-interactions, which may occur between human and bacterial proteins [1]. PACT was developed and first evaluated mostly on data collected in the AmyloGraph database [2] of interacting amyloids and achieved high values of Area Under ROC (AUC = 0.88) and F1 (0.82). Then, we applied the method to study interactions of proteins not used in our in-reference datasets and experimentally confirmed the novel results. We experimentally tested several of the novel results regarding interacting fragments. The study showed that PACT correctly predicted the appearance of most cross-interactions and indicated specific regions of proteins which played a central role in the interactions. The tool is available as

a web server at https://pact.e-science.pl/pact/. References: [1] Wojciechowski JW et al. Sci Rep. 2023; 13(1):22268. [2] Burdukiewicz M, et al. Nucleic Acids Res. 2023; 51(D1):D352-D357.

P-28-027
TANGO2-Deficiency Disorder: multiomics data integration toward a personalized network approach

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TANGO2 Deficiency Disorder (TDD) is a rare inherited condition characterized by a spectrum of clinical features, including metabolic crises, encephalopathy, cardiac arrhythmia, and neurodevelopmental delay. The variability in its manifestations underscores its heterogeneous nature, which extends even within families, suggesting potential genetic modifiers at play. Although TANGO2 role in mitochondrial function and lipid metabolism is emerging, its physiological functions remain unclear. Here, we report on two TDD-siblings, one exhibiting severe symptoms, while the other remained asymptomatic despite carrying the same loss-of-function compound heterozygous *TANGO2* variants. Leveraging genomics and transcriptomics data, we employed a network-based approach to elucidate altered cellular pathways and potential modifiers influencing TDD severity. We constructed a TDD protein-protein interaction (PPI) network utilizing TDD-associated genes from the Human Phenotype Ontology database. Using both univariate and multivariate approaches, we identified differentially expressed genes that we used to generate a functional network. By integrating pathogenicity prediction scores from genomics data with PPI-functional networks, we identified 30 variants of unknown significance in the severe sibling and 24 in the asymptomatic one. Notably, several genes, with distinct variants in the siblings, are also implicated in lipid homeostasis, similar to TANGO2. Our strategy, integrating multi-omics data into a network-based framework, illuminates molecular mechanisms in TDD and aids in discovering genetic modifiers. This approach holds promise for personalized interventions and targeted therapies not only for TDD but also for related rare disorders.

G-protein coupled receptors

P-29-001
GPR27 modulates hepatitis B virus internalization

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The hepatitis B virus (HBV) is responsible for 800,000 annual fatalities among 296 million individuals suffering from chronic infections globally. Currently, there is no available curative treatment for these cases. Earlier research has suggested a close interdependence between the HBV life-cycle and the host cell metabolism. Exploring the molecular mechanisms of these interactions could unveil new targets for antiviral interventions and therapeutic approaches. G protein-coupled receptors (GPCRs) make up the largest superfamily of human membrane receptors, playing a crucial role in signal transductions through the activation of intracellular G proteins. GPCRs regulate diverse physiological processes and stand out as a significant group of pharmacological targets. Recent findings indicate that GPR27, a member of this receptor family, is well-expressed in hepatic cells. Our hypothesis is that GPR27 could regulate host cell pathways that are crucial to the HBV viral life-cycle. To investigate this possibility, GPR27 expression was modulated in hepatoma cell lines permissive for HBV replication (HepG2.2.2.15) and infection (HepG2-NTCP) and further confirmed by qRT-PCR and immunofluorescence assays. Analysis of HBV entry and replication in these cellular models suggests that GPR27 plays a role in HBV internalization. Preliminary results indicate a strong GPR27 co-localization with the HBV receptor, NTCP. Further investigation will address the molecular mechanisms underlying modulation of HBV entry by GPR27, possibly involving regulation of NTCP receptor trafficking and/or activity.

P-29-002
Lipidized PrRP analogs targeting GPR10 and NPFFR2 receptors: a therapeutic approach for obesity

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Obesity, a growing global health concern with limited treatment options, prompts exploration into novel therapeutic approaches. Prolactin-releasing peptide (PrRP) is a neuropeptide that reduces food intake when administered centrally but loses this ability after peripheral administration. In recent studies, we demonstrated that palmitoylated PrRP analog (palm^{11}-PrRP31), bound to receptor GPR10 and neuropeptide FF receptor type 2 (NPFFR2), effectively decreased food intake, body weight and improved metabolic parameters in mouse models of diet-induced obesity after peripheral administration. This study investigates the effect of palm^{11}-PrRP31 on mice with deletion of either GPR10, NPFFR2, or both receptors treated with a high-fat diet. A comparative analysis between wild-type (WT) and knockout (KO) mice involved subcutaneous administration of saline or palm^{11}-PrRP31. Interestingly, the intervention with palm^{11}-PrRP31 resulted in significantly decreased body weight and food intake in WT and GPR10 KO mice. Decreased body weight and slightly reduced food intake was observed in NPFFR2 KO mice. Only double knock-out (dKO) mice did not reduce body weight or food intake. Palm^{11}-PrRP31 significantly improved glucose tolerance in WT mice, with no observed effect in KO models. Additionally, it led to a significantly decreased respiratory quotient (RQ) as a measure of substrate oxidation in WT mice, but not in dKO mice. Nevertheless, further investigation to elucidate the mechanism of action is needed. Our findings suggest the essential role of dual GPR10 and NPFFR2 receptor agonism for the anti-obesity and antidiabetic effect of palm^{11}-PrRP31 analog

as a promising candidate for obesity treatment. Previously published in: Strnadová et al. (2023) Neuropeptides 98, 102319 Karnošová et al. (2023) Clinical Science 137, 847-862.

P-29-003
Studies on the metabolic role of serotonin 2C receptors expressed by TRH neurons

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The thyrotropin-releasing hormone (TRH) neurons are neurohormone-expressing neurons that regulate metabolism. Since it was previously shown that TRH neurons express serotonin 2C receptor (Htr2c) that controls many aspects of metabolism, we aimed to identify the metabolic role of Htr2c expressed by TRH neurons. We used male Trh-ires-cre::$Htr2c^{flox/Y}$ and $Htr2c^{flox/Y}$ mouse models fed high fat diet. We measured body weight, food intake, energy expenditure, and performed glucose tolerance test and insulin tolerance test. We also recorded the electrical activity of TRH neurons using patch-clamp technique. We did not observe any significant difference in body weight, food intake, and energy expenditure between the Trh-ires-cre::$Htr2c^{flox/Y}$ and $Htr2c^{flox/Y}$ mice. However, fasting glucose level was significantly lower in Trh-ires-cre::$Htr2c^{flox/Y}$ mice compared to $Htr2c^{flox/Y}$ mice. We also found that CP809101, an Htr2c agonist, does not affect TRH neurons within the paraventricular nucleus of the hypothalamus (PVH), but inhibits TRH neurons within the dorsomedial nucleus of the hypothalamus (DMH). We found that Htr2c expressed by TRH neurons regulate fasting glucose levels without affecting body weight, food intake, and energy expenditure. We also provide evidence that the observed phenotypes may not be due to the activity of PVH TRH neurons, but Htr2c may work on DMH TRH neurons to regulate glucose homeostasis.

P-29-004
GPR27, a tale of an atypical GPCR signaling

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G protein-coupled receptors (GPCRs) are membrane proteins essential in regulating cellular and physiological processes. They are the most successful drug targets, with 30% of the FDA-approved drugs targeting them. In humans, 160 of these receptors still have unknown functions, and are recognized as orphan GPCRs. The Super-conserved Receptors Expressed in Brain family includes three orphan receptors, known as GPR27, GPR85 and GPR173. GPR27 was linked with increased insulin production, revealing a possible role in type II diabetes [Previously published in: Ku GM et al. (2012) PLoS Genet 8(1), e1002449]. Moreover, hypermethylated CpG sites were found in the GPR27 gene, suggesting a possible role in cancer [Previously published in: Lando M et al. (2015) Epigenetics 10 (10), 970-980]. Here we show that when heterologously expressed in HEK293T cells, human GPR27 exerts profound cellular effects that resemble a constitutively active state of the receptor in a G-protein independent manner. By using a more integrative cellular analysis of GPR27-dependent modulation of transcriptional factors, we identified a family of metabolites (designated XNm) that specifically inhibits serum response element (SRE) activity. However, this effect was insensitive to G-protein inhibitors and independent of beta-arrestin, as revealed by a beta-arrestin recruitment assay. Interestingly, subsequent experiments demonstrated that stimulation of cells expressing human GPR27 with XNm strongly inhibited epidermal growth factor (EGF)-induced SRE activation, in a concentration and receptor-dependent fashion. Furthermore, knockdown of GPR27 in INS1E cells by siRNA strategy resulted in elevated intracellular calcium signaling and augmented glucose-stimulated insulin secretion. In conclusion, GPR27 exhibits a signaling behavior atypical for the GPCR family. A better understanding of its signaling is important in discovering the role of this novel molecular target in diabetes and even cancer.

P-29-005
Inhibition of TRPM8 function by prostacyclin receptor agonists requires coupling to Gq/11 proteins

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The transient receptor potential cation channel subfamily M member 8 (TRPM8) plays a crucial role in the detection of cold stimuli and exhibits a strong anti-inflammatory effects, when activated by low temperatures or specific agonists like menthol and icilin [Previously published in: Liu, B. et al. (2013) Pain 154 (10), 2169-2177]. In contrast, prostacyclin (PGI2) exacerbates pain and inflammation through the activation of the G-protein coupled receptor, the prostacyclin receptor (IP-R) [Previously published in: Murata, T et al. (1997) Nature 388(6643), 678-682]. Given the pivotal functions of both TRPM8 and IP receptors in the modulation of inflammatory pain, and considering their overlapping expression patterns, we conducted an analysis to explore the functional interaction between human TRPM8 and IP receptors. To study a possible functional interaction between IP-R and TRPM8 we heterologously expressed them in HEK293T cells. Upon prestimulation of IP-R with PGI2 and analogs, icilin-induced TRPM8 activation was dose dependently and non-competitively inhibited, indicating a significant functional interaction between IP-R and TRPM8. Surprisingly the inhibition of TRPM8 by IP-R is mediated through unusual coupling of IP-R to Gq/11 proteins whereas the well-described Gs-coupling of IP-R did not affect TRPM8 functionality. Further studies in DRG neurons isolated from mice, showed that the activation of IP-R resulted in a significant inhibition of TRPM8 as observed in heterologous coexpression of IP-R and TRPM8.

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These results contribute to a better understanding of mechanism involved in pain and inflammation, meditated by TRPM8 and IP-R [Published in: Trif, C. et al. (2023) British Journal of Pharmacology].

Neurobiochemistry

P-30-001
Acute brain edema associated with the elevated levels of oxidative stress mediators and TNF-alpha in rats: biochemical and immunohistochemical assessment

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A high-altitude environment is known as an extreme condition characterized by low barometric pressure and oxygen deficiency that can affect the nervous system in distinctive ways. High-altitude cerebral edema is one of the consequences of neurological disorders that harm human health. Maladaptation in high altitude conditions confronted by low barometric pressure and oxygen supply scarcity to the tissues stimulates biochemical and histological alterations in the brain damaging neurons, DNA, lipids, and other biomolecules. It is reported that MDA (malondialdehyde) and NO (nitric oxide) are related to hypoxic brain injury, and are also biomarkers of oxidative stress. In the current study, we have investigated the levels of NO and MDA along with TNF-alpha expression in the brain following acute hypobaric hypoxia (7500m, SO_2 = 7%, 64-58mm Hg, with a duration of 24 hours) which was modeled via the special decompression chamber. Immunohistochemical analysis was used to evaluate the expression of TNF-alpha after hypoxic exposure. Acute hypoxic injury juxtaposed with the predominance of TNF-alpha positive cells indicating the presence of neuroinflammation in the brain, mainly in the cerebral cortex, hippocampus, and striatum. Additionally, the level of MDA and NO activity was increased ($p < 0.05$) after acute hypoxia, pointing out the active lipid peroxidation and redox imbalance in the brain. The study shows the effects of oxygen deprivation on rat brain edema development through biochemical and immunohistochemical studies and the effects of the disease processes on the cellular and tissue levels. The study outcomes may provide valuable insights into the mechanisms underlying the brain's response to acute hypoxia and may inform future research in this area. The study was performed in the frames of projects 21AA-1F041 and 22YSSPD-003 supported by the Science Committee of MESCS RA and the 'Young Scientists Support Program' of NAS RA.

P-30-002
The role of perinuclear mAKAP signalosome in the regulation of NFAT function in primary rat hippocampal neurons

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Muscle A-kinase anchoring protein (mAKAP) is a scaffold protein localized to the nuclear envelope of neurons and striated myocytes, but the function of neuronal mAKAP has not been well-characterized. mAKAP binds a large number of enzymes involved in cell signaling, including calcium-dependent phosphatase calcineurin (CaN) which is an upstream activator of nuclear factor of activated T-cells (NFAT). To verify the importance of mAKAP-mediated CaN scaffolding, we have constructed mCherry-fused anchoring disrupting peptide corresponding to 1286-1345 of mAKAP. The expression of the peptide below the saturation level, disrupted KCl-stimulated axonal elongation of hippocampal neurons compared to mCherry-only labelled neurons without affecting the survival. The staining of βIII-tubulin and neurofilament L showed changes in the organization of neuronal cytoskeleton suggesting a putative underlying mechanism. Silencing of mAKAP with shRNA resulted in similar effect to mCherry-1286-1354, pointing out mAKAP importance for CaN-dependent axonal outgrowth. Interestingly, neither mAKAP shRNA nor mCherry-1286-1354 were able to affect neuronal growth in the absence of depolarizing stimulus. This observation corresponded to enhanced CaN binding to mAKAP following neuronal depolarization and subsequent promotion of NFATc4-mAKAP association. Testing for the site of NFATc interaction, we found NFATc binding site location in N-terminal domain of mAKAP (amino acids 1-196). However, in contrast to mCherry-1286-1354, NFAT competing peptide (mCherry-1-196) did not affect the axonal length of depolarized neurons indicating a mechanism independent from NFATc. Our data strongly suggests that mAKAP located in the perinuclear space may serve as a nodal point for the control of CaN action in the outgrowth of hippocampal neurons. Supported by National Science Centre (Narodowe Centrum Nauki) grant number 2019/33/B/NZ4/00587.

P-30-003
Trifloxystrobin-induced apoptosis through DELE1-HRI-ISR pathway in SH-SY5Y cells

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The effects of some strobilurin fungicides on the mitochondria is well established. However, the mechanisms of neurotoxicity associated with exposure to Trifloxystrobin (TFX), an inhibitor of complex III of respiratory chain and one of the major strobilurins widely used has not been fully assessed. To address this knowledge gap, we investigated molecular mechanisms of neurotoxicity of TFX in SH-SY5Y neuroblastoma cell line. Our results showed that TFX is cytotoxic with an IC50 of approximately 100 μM after 24 hours of treatment on neuronal cells. A

mitochondrial stress test revealed that treatment of SH-SY5Y cells with TFX, generate a decreased ATP level, increased mitochondrial superoxide anion and mitochondrial transmembrane potential drop. In molecular terms, our findings indicate that TFX activate significantly integrated stress response (ISR) via DELE1-HRI-ISR pathway in cells transfected with scramble siRNA or HRI siRNA and treated with ½ IC50 or IC50 of TFX for 24 h as demonstrated by western blot analysis. In addition, in case of mitochondrial dysfunction, DELE1 mitochondrial import is arrested, thereby stabilizing DELE1 on the mitochondrial surface, activate the HRI-mediated integrated stress response (ISR). Activating HRI-kinase lead to eIF2α phosphorylation. P-eIF2α stimulate downstream effectors of this pathway including activating transcription factor 4 (ATF4) and pro-apoptotic transcription factor C-EBP homology protein (CHOP). Studying the possible induction of apoptosis, our findings showed that TFX is able to dissolve the cytoskeleton, increasing the amount of apoptosis by double staining annexin V/PI using flow cytometry and activating caspase 3, after treatment of neuronal cells for 24 hours. According to these results, TFX-induced neurotoxicity in SH-SY5Y cells may be caused by mitochondrial dysfunction and ISR activation. This suggests that the ISR mitochondrial pathway is most likely responsible for the apoptosis caused by TFX exposure.

P-30-004
Regionally distinct alterations in the proportion of enteric glial cells in myenteric and submucous ganglia of type 1 diabetic rats

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The enteric nervous system (ENS) consists of a network of neurons and glial cells. In most mammals, the ENS comprise two large ganglionated plexuses: the myenteric and submucous plexuses. Glial cells are responsible for the organization and function of the ENS, can promote neuronal survival, and have neuromodulatory functions as well [Cabarrocas J et al. (2003) Glia 41, 81-93]. It is known that glial cells and their neurotrophic factors are involved in diabetes [Liu W et al. (2010) Auto Neurosci 154, 79-83]. Therefore, we aimed to investigate the quantitative changes of enteric glial cells in acute and chronic phase of type 1 diabetes. Adult male Wistar rats were divided into three groups: control, streptozotocin-induced diabetic and insulin-treated diabetic rats. After 1 and 10 weeks of hyperglycemia, myenteric and submucous whole-mount preparations originated from different gut segments processed for double-labeling fluorescent immunohistochemistry. HuC/D was used as a pan-neuronal marker, while Sox10 was applied to label glial cells. Diabetic alterations in the proportion of enteric glial cells were strictly dependent on duration of hyperglycemia, enteric plexuses and investigated gut segments. In the acute phase of hyperglycemia, this proportion was affected only in the colon where the elevated glia/neuron ratio also remained after 10 weeks in the myenteric ganglia. In the chronic experiment, the myenteric glial cell proportion was markedly decreased in the duodenum, while it did not change significantly in the ileum. In the submucous ganglia of diabetics, the proportion of glial cells was less than in the myenteric ones and it was significantly increased in all gut segments. The insulin treatment had also segment-specific protective effects in both plexuses. These findings reveal that type 1 diabetes has time-dependent, plexus- and gut segment-specific effects on the enteric glial cell proportion.

P-30-005
Adressing microglial dysfunction in Alzheimer's disease: a role for endocannabinoid signaling in immunosenescence

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The pathogenesis of Alzheimer's disease (AD) remains poorly understood, yet the progressive dysfunctional accumulation of neurotoxic amyloid β (Aβ) peptides remains the main explanation for it. In recent years, chronic inflammation has been identified as an additional player in AD onset and progression. Prolonged and continuous activation of microglia – the main immune cells of the brain – can damage neurons by causing immune cells to acquire an exacerbated pro-inflammatory state. This dysfunctional phenotype can aggravate AD by releasing pro-inflammatory cytokines as well as other inflammatory mediators. Among these, endocannabinoids (eCBs) are bioactive lipids that increase or decrease distinct immune functions. Specifically, microglia express an array of receptors and metabolic enzymes (collectively referred to as the "eCB system") that control the immune functions of eCBs. In particular, N-arachidonoylethanolamine (AEA) and 2-arachidonoylglycerol (2-AG) – the two most active eCBs – are increasingly recognized for their essential roles in regulating microglial activity, under both normal and AD-associated conditions. We have recently discovered that continuous exposure to Aβ during embryonic development significantly alters the signaling of 2-AG in primary microglia (AD-like microglia). This alteration leads to an increase in the expression of cannabinoid receptor 2, which is typically associated with an activated proinflammatory phenotype. Consistently, when exposed to an inflammatory stimulus, AD-like microglia showed an exacerbated production of nitric oxide. Therefore, we further investigated the immunological and physiological aspects of Aβ-exposed microglia following prolonged and continuous inflammatory stimulation, and found significant changes in oxidative stress, as well as in senescence and dystrophic markers. References: Scipioni et al. (2023) Int J Mol Sci 3;24(7):6684; Scipioni et al. (2022) Cells 6;11(7):1237.

P-30-006
Inhibition of Aβ42 oligomers relevant in Alzheimer's disease by a chaperone multimer

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Protein aggregation is central in Alzheimer´s disease (AD) in which aggregation of Aβ and tau occurs. While extracellular space is the primary site for Aβ aggregation, tau is promptly secreted to nearby cells. Therefore, extracellular proteostasis is key to define molecular events in AD brain. Our recent work established proteins of the S100 family as anti-aggregation chaperones. S100 proteins are Ca-binding proteins with intra/extracellular roles which are neuronally abundant and secreted as alarmins. We showed that S100 proteins co-localize with aggregates in AD mice brains, and that S100B inhibits the aggregation and toxicity of Aβ42 and tau [Previously published in: Cristóvão 2018 Sci Adv; Moreira 2021 Nat Comm]. S100B occurs predominantly as homodimers but also exists as tetramers [Previously published in: Hagmeyer 2019 Front Neurosci 13:640], whose chaperone activity we here investigated. Using ThT-monitored Aβ42 aggregation kinetics, we discovered that tetrameric S100B, unlike the dimer, effectively inhibits Aβ42 aggregation even in the absence of Ca^{2+} binding, at sub/equimolar ratios. Structural analysis revealed a secondary Ca^{2+}-independent binding site formed through tetramerization, which facilitates the binding of monomeric and fibrillar Aβ42, as suggested by molecular docking calculations, CD and electron microscopy. Additionally, our investigation explored the impact of S100 multimers on the generation of neurotoxic Aβ42 oligomers (AβO). Mechanistic analysis revealed that dimeric and tetrameric S100B preferentially inhibit Aβ42surface-catalysed nucleation, reducing AβO formation by 90%. Overall, our findings highlight S100B multimers as versatile inhibitors of Aβ42 oligomerization and aggregation. Ongoing research on brain-related S100 proteins reveals a network of chaperones with multiple client interactions, mitigating protein aggregation and toxicity. Funded by EU-TWIN2PIPSA/GA101079147 and FCT-Portugal BD/06393/2021 (AJF)/UID/MULTI/04046/2020 (BioISI).

P-30-007
Pharmacophore-based screening, molecular docking, and dynamic simulation of fungal metabolites as inhibitors of multi-targets in neurodegenerative disorders

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Neurodegenerative disorders, such as Alzheimer's disease (AD), negatively affect the economic and psychological system. For AD, there is still a lack of disease-altering treatments and promising cures due to its complex pathophysiology. In this study, we computationally screened the natural database of fungal metabolites against three known therapeutic target proteins of AD. Initially, a pharmacophore-based, drug-likeness category was employed for screening, and it filtered the 14 (A-N) best hits out of 17,544 fungal metabolites. We found that compounds B, F, and L were immuno-toxic, whereas E, H, I, and J had a higher LD50 dose (5000 mg/kg). Among the examined metabolites, the Bisacremine-C (compound I) was found to be the most active molecule against GSK-3β (ΔG: -8.7 ± 0.2 Kcal/mol, Ki: 2.4 × 106 M^{-1}), NMDA (ΔG: -9.5 ± 0.1 Kcal/mol, Ki: 9.2 × 106 M^{-1}), and BACE-1 (ΔG: -9.1 ± 0.2 Kcal/mol, Ki: 4.7 × 106 M^{-1}). It showed a 25-fold higher affinity with GSK-3β, 6.3-fold higher affinity with NMDA, and 9.04-fold higher affinity with BACE-1 than their native ligands, respectively. Molecular dynamic simulation parameters, such as RMSD, RMSF, Rg, and SASA, all confirmed that the overall structures of the targeted enzymes did not change significantly after binding with Bisacremine-C, and the ligand remained inside the binding cavity in a stable conformation for most of the simulation time. The most significant hydrophobic contacts for the GSK-3β-Bisacremine-C complex are with ILE62, VAL70, ALA83, and LEU188, whereas GLN185 is significant for H-bonds. In terms of hydrophobic contacts, TYR184 and PHE246 are the most important, while SER180 is vital for H-bonds in NMDA-Bisacremine-C. THR232 is the most crucial for H-bonds in BACE-1-Bisacremine-C and ILE110-produced hydrophobic contacts. This study laid a foundation for further experimental validation and clinical trials regarding the biopotency of Bisacremine-C.

P-30-008
Magnesium transport through the blood–brain barrier: a visualisation of subcellular transport systems in human brain microvascular endothelial cells

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Magnesium (Mg) is classified as an essential macroelement crucial for maintaining cellular functions. Intricate Mg homeostasis is orchestrated through controlled activities of transport systems located on cell and organelle membranes. The growing evidence underscores the importance of Mg deficiency in neurodegenerative processes. The blood–brain barrier (BBB) serves as a selective interface between the central nervous system and peripheral tissues. However, the precise mechanisms governing the passage of Mg cations through BBB cells and the regulatory aspects of this transport remain elusive. We hypothesise that, considering Mg's pivotal role in neuronal physiology, Mg permeation across the BBB is a tightly regulated process, akin to transport systems observed in intestinal epithelial cells and the renal tubular system. Our objective is to employ immunocytochemistry to identify the transport systems within the endothelial cells of the capillaries that form the BBB and ascertain their subcellular localisation. In our investigation, we visualised transport systems in human brain microvascular endothelial cell (HBMEC) cultures. Cells cultured on coverslips were fixed with 4% formaldehyde and incubated with primary antibodies targeting established transport systems, including TRPM7 and MRS2 channels, SLC41A1 and SLC41A3 transporters, Claudin-16, and the homeostatic factors CNNM2 and MAGT1. Secondary antibodies

conjugated to fluorophores were used. and signals were detected microscopically through Zeiss LSM 880 system. Our findings reveal the expression of transport systems and homeostatic factors in HBMEC cell cultures that actively contribute to the regulation of both cellular and systemic Mg homeostasis. Consequently, this cell culture model holds promise for studying Mg homeostasis regulation at the BBB permeation level.

P-30-009
Expression of genes encoding for transport systems involved in the transfer of magnesium to the central nervous system

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Magnesium (Mg) stands as a pivotal biogenic macronutrient acting as a cofactor for hundreds of enzymes, participating in energy metabolism, and functioning as a calcium cation antagonist. These roles are imperative for the proper operation of nerve cells, and subclinical Mg deficiency has been linked to an increased risk of various neurodegenerative diseases. The blood–brain barrier (BBB) separates the central nervous system from the rest of the body, regulating the passage of substances. The transport systems involved in the transfer of Mg cations to the central nervous system through cells of the BBB have not yet been described in detail. However, we postulate that Mg cation transfer is contingent on specific transport systems, warranting further investigation. This study aims to scrutinise the expression of genes that encode known transporters (SLC41A1, SLC41A3, TRPM7, MRS2, Claudin-16) and homeostatic factors (MAGT1 and CNNM2) involved in Mg cation transport across epithelial barriers in the intestine and renal tubular system. Our analysis encompasses transcriptional and translational levels, utilising material derived from the HBMEC cell line, the primary endothelial cell line derived from brain capillary endothelial cells. Cells were lysed and RNA and proteins were isolated from the lysates. The mRNA was transcribed into cDNA and subsequently amplified in a real-time PCR reaction on a ViiA 7 system (Applied Bioystems, USA) with primers specifically designed for each gene analysed. Protein samples were separated by PAGE and subsequently transferred to a nitrocellulose membrane by western blotting. The target proteins were labelled with antibodies. The signal was detected by the ChemiDoc XRS+ system (Bio-Rad, USA). We found that genes encoding important determinants of Mg homeostasis are expressed at the transcriptional and translational level in HBMEC cells.

P-30-010
Brain tissue factor activity and nitric oxide levels were modulated by the exposure of bisphenol A and *Lactobacillus rhamnosus* GG

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Nitric oxide (NO) acts as a reversible neurotransmitter in synapses, regulates blood flow in the brain, and plays crucial roles in intracellular communication in neurons. Tissue factor (TF) is the main physiological initiator of coagulation. It is highly expressed as a transmembrane protein in various cell types of the central nervous system. Therefore, this study aimed to determine the effect of *Lactobacillus rhamnosus* on bisphenol A-induced brain damage in terms of NO and TF. Bisphenol A (BPA) exhibits estrogenic, toxic, carcinogenic, and mutagenic effects, potentially hindering brain development in newborns and leading to infertility and learning disorders. *Lactobacillus rhamnosus* (LGG), one of the most widely used probiotic species, has positive effects on health. In this study, rats were divided into three groups: control, BPA, and BPA + LGG. The BPA and BPA + LGG groups were administered intraperitoneally at a dose of 50 mg/kg for 6 weeks. The BPA + LGG group received oral LGG at a dose of 1 x 10^9 colony-forming units per day for 6 weeks. Nitric oxide level and tissue factor activity were determined in the homogenized brain tissue samples. The results of this study demonstrate a significant decrease in TF activity and NO levels in the BPA group. LGG administration to the BPA group increased brain TF activity and NO levels. The results indicate that LGG alleviates the BPA-induced modulations in TF activity and NO levels. In conclusion, these findings imply that LGG may have an important role in mitigating BPA-induced changes in TF activity and NO levels in the brain, highlighting its therapeutic potential in ameliorating the neurobiological effects caused by BPA exposure. *The authors marked with an asterisk equally contributed to the work.

P-30-011
Anti-dementia effects of mansorin A and mansonone G in an okadaic acid-induced zebrafish model of Alzheimer's disease

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Dementia is an intricate clinical syndrome characterized by progressive cognitive decline and encompasses four distinct types, with Alzheimer's disease (AD) standing out as the most prevalent. The pathogenesis of dementia is not fully understood, hence the lack of a definitive cure. Urgency looms as the number of AD cases escalates annually, demanding innovative anti-dementia treatments. Traditional medicine proves that plant extracts from *Mansonia gagei* possess antioxidant effects on the central nervous system. The hypothesis is that, given that there is an intimate link between oxidative stress and AD pathology, mansorin A and mansonone G from *M. gagei* might improve AD-like pathology in animal models of AD. To test the hypothesis, a zebrafish model of AD was induced using okadaic acid 10 nm, manifesting cognitive decline, anxiety-like state, and high levels of oxidative stress. Then, mansorin A (MA) and mansonone G (MG) were chronically administered by immersion to the animal model in concentrations of 1, 3, and 6 μg/L. Following, a battery of behavioral tests was conducted to verify the promnesic (Novel Object Recognition test and Y-maze) and anxiolytic (Novel Tank and Novel Object Approach tests) effects of MA and MG in zebrafish, and biochemical analysis was performed to assess the oxidative stress and cholinergic status in the brain of the animals. The results of the study confirm the hypothesis. MA and MG improved the short-term recognition and spatial memory and anxiety-like status in the animal model. MA and MG significantly reduced the oxidative stress induced by the okadaic acid by increasing the activity of superoxide dismutase, glutathione

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peroxidase, catalase, and reduced glutathione, and decreasing malondialdehyde levels and the acetylcholinesterase activity in the brain. In conclusion, the promnesic, anxiolytic, antioxidant, and anti-acetylcholinesterase effects pose MA and MG as great therapeutic solutions in the management of dementia.

P-30-012
Impact of carbosilane dendrimers and their complexes with siRNA directed against Alzheimer's disease on in vitro blood–brain barrier model and blood flow

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In recent years, scientists have made great strides in better understanding the mechanisms behind the development of Alzheimer's disease. Alzheimer's disease's most substantial risk factor is the occurrence of the Apolipoprotein E4 gene. Therefore, one way to effectively slow the disease progression is through cellular mechanisms that enable the selective silencing of specific genes. Since naked nucleic acid implementations are largely inefficient because of their limited resilience to enzymatic activity, intrinsic stability, and inability to penetrate lipid bilayers, applying an effective delivery vector is necessary. A promising tool for the transport of siRNAs are positively charged dendrimers. These nanoparticles can be thoroughly complex with siRNAs through electrostatic interactions. Positively charged complexes have increased enzymatic resistance and enhanced cellular uptake, thereby increasing their transport into cells. The aim of this study was to investigate the carbosilane dendrimer and its complexes with siRNA on the blood flow, brain microvascular endothelial cell line (HBEC-5i) and *in vitro* Blood blood–brain barrier (BBB) model. The hemorheological impact of the tested system was measured utilizing a rheometer simulating blood flow conditions in different blood vessel types. Internalization efficiency was assessed using confocal microscopy. The quality of cell adhesion and integrity in the BBB *in vitro* model was evaluated by real-time cellular impedance analysis. Both the dendrimer and dendriplex significantly disturb blood flow but without physiological relevance. It was observed that the studied dendrimer efficiently internalizes to cells and significantly induces co-internalization of siRNA. The tested nano-carriers and their siRNA complexes can effectively reduce barrier integrity. Research financed by the National Science Center under the M-ERA.NET2 program, funded under agreement No. 685451 under the European Union's research.

P-30-013
Understanding the metabolic role of trehalose in inducing autophagy

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Trehalose is a non-reducing disaccharide made of two glucose molecules linked by an alpha-1,1-glycosidic bond. In the last years, trehalose applications increased exponentially, and now it is widely used in various fields, leading to an increased "involuntary" intake. Different works indicated that trehalose reaches the central nervous system, where it is probably metabolized by neurons. Here, it has demonstrated to decrease aggregate depositions in several neurodegenerative disorders thanks to its ability to induce m-TOR-independent autophagy. However, the molecular mechanism behind the autophagy triggering is still unclear. In this study, we propose to clarify the molecular pathways underpinning trehalose-induced autophagy. Rat hippocampal neurons were treated with trehalose, and with glucose as control. At the same time, similar analyses were performed *in vivo*, in WT mice chronically treated with 2% trehalose dissolved in drinking water and relative controls. Neuronal cell lysates, plasma and hippocampus homogenates have been analyzed with untargeted LC/MS-based lipidomics and metabolomics approaches. Our preliminary results show an augmented concentration of short-chain carnitines and a down-regulation of metabolites involved in energetic metabolism and aminoacidic metabolism in trehalose-treated cells. We also highlighted increased levels of lysophosphocholines and lysophosphatidylethanolamines in cells treated with trehalose, suggesting that the mobilization of membrane lipids happened, probably due to phosphatases activity. Moreover, investigations of the hippocampal and plasma samples indicate that trehalose induces significant metabolic changes in different body districts. Overall, our preliminary results show that the trehalose perturbation cause important metabolic variations both *in vitro* and *in vivo* that could be linked to its effect on central nervous system. *The authors marked with an asterisk equally contributed to the work.

P-30-014
PER2:Luciferase bioluminescence rhythms in murine ocular tissues are affected by neurobasal A medium preincubation, mouse background but not sex

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Our understanding of ocular circadian clocks has been profoundly advanced by the development of real-time recording of bioluminescence of PER2::LUC knock-in mouse explants. However, the effect of animal sex, mouse strain and culturing

conditions on ocular clocks remains unknown. Here, we studied the role these variables play on PER2::LUC bioluminescence rhythms of ocular tissues: retinas, corneas and posterior eye cups (PEC). We also tested the hypothesis that the sclera contains a circadian oscillator by using scraped PEC as a proxy. Retinas, corneas, intact and scraped PECs were obtained from PER2::LUC knock-in mice. PER2:LUC bioluminescence rhythms in ocular tissues were measured using a Lumicycle®. We used male and female PER2::LUC mice maintained on either a pigmented C57BL/6J or albino RjOrl:SWISS background. We compared PER2:LUC bioluminescence rhythms between ocular tissues and found all studied tissues oscillated, including the scraped PEC oscillated which was previously not known to oscillate. The rhythms in scraped PECs had lower amplitudes, longer periods and distinct acrophases compared to other ocular tissues. Ocular tissues of RjOrl:SWISS mice oscillated with higher amplitudes compared to the ones of C57BL/6J, with corneal rhythms being most affected by mouse strain. A 24 h preincubation with Neurobasal A medium enhanced rhythms of ocular tissues, whereas sex differences did not affect these rhythms. In conclusion, we discovered a novel oscillator in the sclera. PER2:LUC bioluminescence rhythms in murine ocular tissues are affected by Neurobasal A medium preincubation, mouse background but not sex.

P-30-015
Lipopolysaccharide (LPS) induces cellular antioxidant machinery upregulation in BV-2 microglial cells

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Lipopolysaccharide (LPS) is one of the most potent stimuli for proinflammatory microglial activation and has been widely used to mimic the neuroinflammatory state of neurodegenerative diseases in both *in vitro* and *in vivo* models. LPS is also related to oxidative stress since it induces reactive oxygen species (ROS) production. Furthermore, there is evidence that many anti-inflammatory compounds exert their beneficial effects through the upregulation of antioxidant enzymes in LPS-induced neuroinflammation models. However, the role of LPS itself in regulating the cellular antioxidant machinery has never been explored. This work aims to evaluate the ability of LPS to modulate the antioxidant response in the BV-2 microglial cell line. Cells were treated with LPS at different concentrations (50, 100, 500, and 1000 ng/ml) for 24 h. The dichlorofluorescin diacetate (DCF-DA) assay confirmed the induction of ROS production by LPS. Interestingly, LPS treatment increased the gene expression of the nuclear factor erythroid 2-related factor 2 (Nrf2) and of different antioxidant enzymes like heme oxygenase 1 (HO-1), NADPH oxidoreductase 1 (NQO1), glutathione reductase (GR), glutathione S-transferase (GST), glutathione peroxidase 1 (GPx-1), peroxiredoxin-1 (Prdx1), and superoxide dismutase 2 (SOD2) in a concentration-dependent manner ranging from 50 to 500 ng/ml. However, between the 500 and 1000 ng/ml concentrations, the expression of all the analyzed genes declines. This is probably due to the elevated toxicity of this dose, which exceeds the cell's ability to react to the insult. The protein levels of these enzymes, analyzed by immunoblotting, exhibited a consistent pattern with the qPCR findings. In conclusion, our data indicate that exposure to LPS stimulates an adaptive response in BV-2 microglial cells by enhancing the antioxidant machinery in an attempt to provide protection. This work was supported by MUIR-PRIN 2022 (Prot. 20222W7P7S) to Cristina Angeloni.

P-30-016
Alterations of the endocannabinoid system and microglia reactivity in the retina precede the onset of β-amyloid plaques in the brain of AD-like mice

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Alzheimer's disease (AD) develops extra-cerebral manifestations in the retina. Here, we explored for the first time the possible alterations of the endocannabinoid (eCB) system (ECS) and the onset of gliosis in the retina of AD-like mice. 12-month-old Tg2576 (TG) mice over-expressing the amyloid precursor protein (APP) were used. Via immunohistochemistry, we demonstrated the absence of β-amyloid plaques in TG brains. Then, retinal gliosis was investigated through immunofluorescence on cryosections, showing a significant increase of IBA1 (+) microglia cells in TG *versus* wild type (WT), and an increase in GFAP staining without retinal degeneration. Analysis of the ECS (receptors/metabolic enzymes) through western blot revealed the up-regulation of cannabinoid receptor 2 (CB_2) in TG (1.5 fold over WT), consistently with fluorescence intensity analysis of anti-CB_2 immunostained cryosections. Instead, no statistically significant differences were found for the other enzymes and receptors of the ECS; however, linear regression analysis showed a significant correlation between CB_2 and fatty acid amide hydrolase (FAAH), diacylglycerol lipase α/β (DAGLα/β), and APP. Finally, liquid chromatography-mass spectrometry (LC–MS) revealed a significant reduction of the eCB 2-arachidonoylglycerol (2-AG) in TG retinas (~0,34 ng/mg) compared to WT (~1,70 ng/mg), while a trend towards increase was found for the eCB anandamide (AEA) (WT:~0,15 ng/mg; TG:~0,24 ng/mg). Overall, our data indicate that the ECS may play a role in AD-associated retinal inflammation, resembling the AD brain, with a central role of CB_2 and 2-AG. Of note, gliosis and ECS dysregulation in the retina precede the onset of β-amyloid plaques in the brain. This investigation was supported by the MUR under the competitive grant 07_PRIN_20224CPSYL (CUP: E53D23009710006) to S.O. and M.M., and by the University of L'Aquila under the intramural competitive grant "Progetti di Ateneo 2023" to A.T.

P-30-017
Reduction of neuroinflammation through administration of URB597 in an animal model of Alzheimer's disease

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Arachidonoylethanolamide (AEA) is an endocannabinoid with recognized neuroprotective properties. Here, we investigated the potential anti-inflammatory effects of URB597, an inhibitor of fatty acid amide hydrolase (FAAH) responsible for AEA degradation, in an animal model of Alzheimer's disease (AD). 6-month-old AD-like Tg2576 (TG) and wild-type (WT) mice were treated intraperitoneally with lipopolysaccharide (LPS) to exacerbate neuroinflammation, and intranasally with URB597 for 3 months. Confocal microscopy was used to identify anti-IBA-1 immunopositive microglial cells. A significant increase in the number of IBA-1(+) cells was found after LPS treatment in the hippocampus of both TG and WT, and was significantly reduced by URB597 in both genotypes compared to LPS alone. Moreover, morphometric analysis was conducted on 360 individual microglial cells in the CA1 and dentate gyrus (DG) of the hippocampus, in order to measure major hallmarks of microglial activation: cell area, area of the soma, number and area of the branches. We found intriguing morphometric changes in LPS-treated groups in the CA1 and DG of both WT and TG, compared to vehicle-treated mice. Indeed, we found a reduction of the cell and branches areas, without alterations in the number of branches and area of the soma, suggesting that the morphometric changes induced by LPS predominantly involved a retraction of the branches. Importantly, URB597 restored the area values of cells and branches in the CA1 of WT and in the DG of TG, indicating a regionalization of the effect. Finally, anti-β-amyloid immunostaining revealed the absence of plaques under all experimental conditions. Overall, our data demonstrate that: (i) URB597 dampens LPS-induced microglia reactivity in both WT and AD-like mice; (ii) neuroinflammation precedes the onset of β-amyloid plaques in Tg2576 mice. Funded by the Italian Ministry of University and Research (MUR) under the competitive project PRIN 2022 (n. 20224CPSYL) to S.O. and M.M.

P-30-018
Beyond symptom management: FAAH inhibition as a path to altering Alzheimer's disease course

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The relevance of the endocannabinoid (eCB) signalling in Alzheimer's disease (AD) is supported by distinct changes in eCB system elements (such as the cannabinoid receptor 1, CB1, and its agonist N-arachidonoylethanolamine, AEA) in human patients, and by their critical engagement in the anti-inflammatory, antioxidative, immunomodulatory and neuroprotective actions in several preclinical models of this disorder. To further explore the anti-AD therapeutic potential of eCB system modulation, the effects of pharmacologic and genetic inhibition of the major AEA-hydrolysing enzyme fatty acid amide hydrolase (FAAH) were evaluated in the Tg2576 mouse model of AD. Enhancing AEA signalling was shown to elicit beneficial effects on the symptoms of AD mice. Chronic inhibition of FAAH promoted neuroprotective mechanisms, by simultaneously attenuating neuroinflammation and neurocognitive decline. Inactivation of FAAH was also found to robustly suppress the production and accumulation of β-amyloid, associated with reduced expression of β-site amyloid precursor protein cleaving enzyme 1 (BACE1), primarily via a CB1-dependent epigenetic mechanism. Taken together, our findings contribute to improving mechanistic understanding of the role of FAAH in AD pathogenesis and provide proof-of-concept that targeting eCB signalling may become an alternative and effective therapeutic strategy against this major neurodegenerative disorder. This investigation was supported by the Italian Ministry of University and Research (MUR) under the competitive PRIN 2022 grant (n. 20224CPSYL) to S.O. and M.M.

P-30-019
Effects of aminosterols and berberine on the structural and dynamical properties of biological membranes against the action of misfolded protein oligomers

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Natural aminosterols and berberine are promising drug candidates against neurodegenerative diseases, and one aminosterol has successfully ended a phase 2 clinical trial against Parkinson's disease. One protective mechanism of aminosterols occurs via their binding to biological membranes and displacement of amyloidogenic proteins and their cytotoxic oligomers. We compared three aminosterols, including one recently isolated from mammals, that differ in their chemical formulas and properties. Fluorescence binding assays, ζ potential measurements, breakthrough force measurements and FRET revealed that the three aminosterols exert differential binding affinities, a partial neutralisation of the negative charge, mechanical reinforcements of the bilayer, and redistribution of key lipids of LUVs membranes. Moreover, they displayed different potencies in dose-response curves in protecting the plasma membrane of cultured SH-SY5Y cells from the destabilisation caused by Aβ oligomers. A global fitting analysis enabled an analytical equation to describe quantitatively the protective effects of aminosterols as a function of their concentration and relevant membrane effects and to attribute the protective effects to well-defined chemical moieties. These results link quantitatively the chemical structures of aminosterols with their protective effects on biological membranes and provide hints to anticipate the effects of other similar small molecules (Errico et al. (2023) J Med Chem 66, 9519-9536). The same approach was used to study the plant alkaloid berberine, which shares chemical aspects with aminosterols and is known to possess antioxidant properties. Fluorescence quenching assays using lipophilic probes embedded in LUVs that localise in different regions of the lipid bilayers revealed that berberine can interact with LUVs membranes and decrease the affinity of toxic oligomers for the membrane.

P-30-020
Structural insight into the unfolding intermediate state of the TDP-43 N-terminal domain

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TAR DNA-binding protein 43 (TDP-43) forms intraneuronal cytoplasmic inclusions associated with amyotrophic lateral sclerosis (ALS), ubiquitin-positive frontotemporal lobar degeneration (FTLD-U) and limbic-predominant age-related TDP-43 encephalopathy (LATE). Its N-terminal domain (NTD) is responsible for the dimerization/oligomerization of the full-length protein, which can favor TDP-43 liquid-liquid phase separation and inclusion formation. While it was found to undergo the formation of a transient intermediate during its folding pathway, the NTD unfolding process is still poorly characterized. We aimed to investigate NTD unfolding kinetics at high urea concentrations using a battery of biophysical methods to evaluate the presence of an unfolding intermediate state. Exploiting far-UV circular dichroism (far-UV CD), NTD in urea before the major unfolding step showed some variations in its CD signal when compared to the initial native state. Differences were also observed in their intrinsic fluorescence when analyzed using a spectrofluorometer and the stopped-flow technique, suggesting that the NTD may undergo the formation of an intermediate state during the unfolding process. The unfolding intermediate was observed even at protein concentrations lower than the equilibrium dissociation constant between dimer and monomer, indicating that the NTD structural variations do not depend on its oligomeric state. In addition, to assess whether the formation of the intermediate affects its dimeric state, NTD unfolding kinetics were investigated using dynamic light scattering and it was found to maintain the native partially folded dimeric state during the unfolding process. The results show that dimeric NTD can adopt different conformational ensembles in its unfolding process, a structural feature that could potentially enable this domain to regulate the assembly state of TDP-43.

P-30-021
Deciphering myelin protein zero mutations in Charcot-Marie-Tooth development: a biophysical approach to study homomeric and heteromeric interactions of extracellular domain

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Myelin protein zero (MPZ) is an integral membrane protein of Schwann cells whose function is the promotion of adhesion

between myelin wraps across the intraperiod line in peripheral nervous system (PNS). Mutations in the gene encoding MPZ are responsible for 5% of cases of Charcot-Marie-Tooth (CMT) disease, a set of hereditary demyelinating peripheral neuropathies. To date, more than 200 MPZ mutations, mainly missense mutations or deletions have been reported, most of them being localized within the extracellular (EC) globular domain. The EC plays a pivotal role in maintaining the compactness of the myelin sheath, through homotypic interactions with other MPZ molecules and heterotypic interactions with PMP22, a tetraspan protein of PNS myelin. However, the structural changes by which mutant MPZ destabilise myelin structure are not well understood. The present study aims to obtain a biophysical characterization of the wild-type EC domain and compare the results with those obtained on a set of disease-involved mutants, to highlight the structural changes that lead to CMT and map the hot-spots for possible misfolding events. Here, we present a circular dichroism analysis in the far- UV of histidine-tagged EC domains to assess the secondary structure of the different variants. Wild-type and mutant EC domains are being investigated by means thermal and chemical denaturation to study conformational stability. We also present data that elucidate the tendency of the EC domain to form tetrameric structures and how individual mutations affect adhesion properties with a binding experiment using the wild-type EC domain labelled with the fluorescent probe BODIPY TMR. We also tested the difference in heterotypic interaction with PMP22 with a binding experiment using the second loop of PMP22 labelled with the Alexa Fluor™ 594 probe. The outcome of this research will provide some clues to explore the etiopathogenesis of CMT concerning particularly the failure of myelin packing.

P-30-022
Modelling the hyperexcitability preceding Alzheimer's disease by treating neuronal cells with sub-threshold concentrations of amyloid-β oligomers and glutamate

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Alzheimer's disease (AD) is the most common form of dementia, characterized by the aberrant deposition of amyloid-β (Aβ) peptide fibrils in the brain resulting in cognitive impairment. Aβ oligomers can induce the activation of NMDA receptors and elicit neuronal pre-synaptic and astrocytic release of glutamate, the main neurotransmitter and NMDA receptor agonist, leading to Ca^{2+} ions influx and neuronal hyperexcitability, which is considered as a potential very early indicator of the stage preceding even pre-clinical AD. We focused on this very early stage of AD, mimicking it by treating SH-SY5Y neuroblastoma cells with sub-threshold Aβ oligomer and glutamate concentrations. Using fluorescence confocal scanning microscopy Ca^{2+} ion entry was not observed, but using Ca^{2+} pump inhibitors its influx became significant, indicating an active influx effectively faced by the pumps. Additionally, the Aβ/glutamate treatment did not cause cell loss and downregulation of membrane NMDA receptors, but determined ROS production. Mitochondria were not involved in ROS increase, as using the mitoSOX probe we observed that mitochondrial superoxide ion is not produced and an Oroboros analysis did not show an increased consumption of oxygen in mitochondria. The same analysis revealed a rise of the residual oxygen consumption (ROX) index, which is the mitochondria-independent consumption of oxygen. We observed that the Aβ/glutamate treatment in the presence of the pan-NADPH oxidase (NOX) inhibitor APX115 determined the suppression of ROS production, indicating that NOX is the main protein involved in ROS generation. These results suggest the use of our experimental approach as a model system to study the stage preceding pre-clinical AD and how neuronal cells may behave in response to hyperexcitability.

P-30-023
Structural and functional characterization of aggregates from purified TDP-43: a novel approach for understanding TDP-43 aggregate effects in cells

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TAR DNA-binding protein 43 (TDP-43) is a nuclear ubiquitously expressed protein that plays a critical role in RNA metabolism, transcription, and splicing. In healthy cells, TDP-43 is dynamically shuttled between the nucleus and the cytoplasm, but in pathological conditions, such as amyotrophic lateral sclerosis (ALS) and in some forms of frontotemporal dementia (FTD), TDP-43 accumulates in the cytoplasm of neurons leading to the formation of proteinaceous inclusions. In this study, we purified human full-length (FL) TDP-43 from bacteria using a combination of affinity and size-exclusion chromatography according to a well-defined protocol. Pure FL TDP-43 has been converted in aggregates of various forms, which have then been delivered into the cytosol of murine motoneuron-like (NSC-34) cells, by using a protein transfection kit. Then, we used confocal scanning microscopy and anti-human TDP-43 specific antibodies to visualize the internalization of the aggregates and their evolution over time. We also assessed the TDP-43-related neurotoxicity using the MTT reduction assay and the evaluating intracellular ROS production. In order to characterize the structural properties of the aggregates before cellular internalization, we used an array of well-established biophysical methods, including dynamic light scattering (DLS), circular dichroism (CD), thioflavin T (ThT) assay, and ANS staining. Our results showed that the internalized aggregates cause cell dysfunction. We also demonstrated that these aggregates were composed of both the exogenously and endogenously expressed proteins, suggesting that the former can recruit the endogenous protein into the aggregates. Moreover, our biophysical characterization showed that TDP-43 aggregates possess a disordered structure. In conclusion, this preliminary study provides a new valuable tool for studying the effect of TDP-43 aggregates in cells with implications for the development of therapies for neurodegenerative diseases.

P-30-024
A cyclin-dependent kinase 2-associated protein (CDK2AP1) plays as adaptor protein that links kinesin-1

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The intracellular transport of various cargos contributes for the functioning of the many cell type. This transport is mediated by microtubule-dependent motor protein such as kinesins and cytoplasmic dynein. Kinesin moves along the microtubule to the positive end of the microtubule. Kinesin-1 was first identified as a kinesin superfamily proteins (KIFs) that functions in the intracellular transport of various cargos, including organelles, neurotransmitter receptors, and mRNA-protein complexes, through interactions between the carboxyl terminal domain of KIF5s and the cargo. It interacts with many other cargos, but adapter proteins that mediate between kinesin-1 and the cargo have not yet been fully identified. To identify the binding proteins for kinesin-1, we performed yeast two-hybrid screening and found a specific interaction with cyclin-dependent kinase 2 associated protein (CDK2AP1), originally identified in malignant hamster oral keratinocytes. CDK2AP1 bound to the C-terminal region of KIF5 and did not directly interact with KIF3A (the motor of kinesin-2), and kinesin light chain 1 (KLC1). The carboxyl terminal region of CDK2AP1 is essential for its interaction with KIF5. KIF5 interacted with GST-CDK2AP1, but not with GST alone. When co-expressed in HEK-293T cells, CDK2AP1 and kinesin-1 co-immunoprecipitated and co-localized at the same region in cells. These results suggest that KIF5-CDK2AP1 interaction serves as an adapter protein connecting kinesin-1 and cargo when kinesin-1 transports cargo. We will further discuss to the kinesin-1, CDK2AP1, and cargo transport.

P-30-025
Diabetes-related alterations in the expression of NFκB and its negative regulator in the myenteric ganglia and neighbouring muscular environment of rat duodenum

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Type 1 diabetes has gut region-specific effects on the expression of pro-inflammatory cytokines in myenteric ganglia and their intestinal milieu. These cytokines have potent impact on nuclear factor kappa-light-chain-enhancer of activated B cell (NFκB) signalling pathway involved in the regulation of inflammatory and immune responses and neuronal survival. Our goal was to evaluate the effects of chronic hyperglycaemia and immediate insulin treatment on the expression of NFκB (p65 component) and its negative regulator, nuclear factor-2 erythroid related factor-2 (Nrf2) in myenteric ganglia and their muscular environment of rat duodenum. Ten weeks after the onset of hyperglycaemia, duodenal samples of streptozotocin-induced diabetic, insulin-treated diabetic and control rats were processed for fluorescent immunohistochemistry, immunogold electron microscopy and enzyme-linked immunosorbent assay. The proportion of NFκB p65-immunoreactive (IR) myenteric neurons was 2% in the duodenal ganglia of controls, while it decreased significantly (less than 1%) in the diabetic rats. Moreover, the ratio of NFκB p65-labelling gold particles' density between the neuronal perykarya and nuclei was also decreased in diabetics relative to controls. The density of Nrf2 gold labels significantly increased in the myenteric ganglia of diabetic duodenum. In the intestinal smooth muscle, both the NFκB p65 and Nrf2 density were enhanced significantly in diabetic rats. NFκB p65 and Nrf2 tissue levels were changed in the opposite way in smooth muscle/myenteric plexus homogenates. Insulin treatment prevented all hyperglycaemia-related alterations in NFκB p65 and Nrf2 expression. These findings support that diabetes-related NFκB p65 and Nrf2 expression changed in an opposite way in myenteric neurons. In addition, the ganglionic muscular environment is also affected in diabetic damage of NFκB signalling, which may contribute to diabetic motility disturbances.

P-30-026
Small non-coding RNAs in osteoarthritis: a focus on microRNAs and tRNA-derived fragments

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Osteoarthritis (OA), a widespread chronic joint disorder affecting the elderly population, is characterized by abnormal amyloid protein accumulation. Although it is well known that the dysregulation of protein homeostasis can occur in OA, there is no evidence regarding its crosstalk with RNA biology. Thus, the urgent need to decipher the role of this network in OA pathogenesis prompted us to investigate two classes of small non-coding RNAs (ncRNAs): microRNAs (miRNAs) and tRNA-derived small RNAs (tsRNAs). The latter belong to a novel class of ncRNAs provided through tRNA cleavage by specific nucleases as a result of several stress conditions. Primary chondrocytes derived from OA patients were treated for 6 hours with lipopolysaccharide (LPS), a pro-inflammatory stimulus known to impair proteostasis, thus favouring amyloid deposition. Subsequently, NGS analysis of RNA samples and differential expression (DE) analysis were performed. tsRNA bioinformatic analysis revealed a distinct profile after LPS treatment: a significant upregulation of tsRNAs primarily belonging to the category of 3'-tRNA-derived fragments (tRFs) originated from $tRNA^{Arg}$ and $tRNA^{Asp}$. Meanwhile it also showed a significant downregulation of tsRNAs belonging to the class of i-tRFs deriving from $tRNA^{Val}$, $tRNA^{Ser}$, and $tRNA^{Arg}$. As concerns miRNA, expression analysis allowed the identification of miR-146a-5p, miR-129-5p, and miR-155-5p as significantly upregulated, while miR-27a-5p and miR-1-3p appeared significantly downregulated. Gene

Ontology Enrichment analysis of their potential targets performed by MultiMiR and clusterProfiler indicated a significant enrichment of cellular compartments and biological processes directly associated with proteostasis maintenance and OA pathogenesis. In conclusion, even though tsRNA role in OA has to be further investigated, our approach led us to unravel the impact of inflammation on RNA metabolism and its involvement in modulating molecular pathways underlying OA.

P-30-027
The anti-diabetic effects of the new mixture on rats with streptozotocin induced diabetes model

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Diabetes mellitus is a endocrine diseases characterized with lack/resistance of insulin, leading to hyperglycemia. The neurotransmitter GABA exhibits anti-inflammatory properties, stimulates the growth and replication of pancreatic β-cells. In this research we have studied antidiabetic (therapeutic) effects of the GABA-supporting new mixture containing glutamine, β-alanine, asparagine and ethanolamine-O-sulfate on streptozotocin (STZ) induced rats. We monitored the glycemic status and enzymatic activity of glutaminase (GLS) responsible for GABA synthesis in serum, pancreas and brain of STZ induced rats. In our experiments blood glucose level of the control rats' group was 4.43 ± 0.6 mM/L, while the same of STZ induced group of rats was 23.27 ± 0.64 mM/L ($p < 0.0001$) (about 5 times higher). The part of diabetic rats was injected with above mentioned mixture. On the 5th day of the injection the blood glucose level decreased to 13.17 ± 1.64 mM/L, about 2 times lower compared to the diabetic animals ($p = 0.0007$). GLS activity in the serum, pancreas and brain of all groups was determined. In the serum samples of the control group it was equal to 0.026 ± 0.009 μmol/min/μg of protein, for STZ induced rats 0.077 ± 0.017 μmol/min/μg of protein $p < 0.0001$), for treated group 0.046 ± 0.014 μmol/min/μg of protein; $p = 0.0001$. In the pancreatic homogenate GLS activity was 0.15 ± 0.04, 0.22 ± 0.06, 0.17 ± 0.04 μmol/min/μg of protein, $p < 0.0001$ respectively. GLS activity in the brain homogenate samples was 0.18 ± 0.06, 0.28 ± 0.09, 0.23 ± 0.06 μmol/min/μg of protein, $p < 0.0001$, respectively. The lowering of glucose level and GLS activity suggests the possible theurapteic effect of the new mixture as a new alternative approach for diabetes mellitus treatment.

P-30-028
Exploring the neuroprotective activity of a lignanamides-rich extract against dimethyl sulfoxide (DMSO)-induced stress

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Dimethyl sulfoxide (DMSO) is a widely used polar organic solvent with various biological applications[1]. In neuroscience, DMSO is extensively used as a vehicle for drug therapy, allowing the administration of various pharmacological compounds. Conflicting findings have been reported on the effects of DMSO on the nervous system. Phenylamides and lignanamides (LnHS) have been found to exhibit various biological effects including anti-inflammatory activities. Nevertheless, one of the most crucial features of lignanamides is their ability to preserve the neuronal health, making them promising neuroprotective candidates. Considering this background, we evaluated the impact of DMSO and/or LnHS on viability of SH-SY5Y and U-87 cells used as *in vitro* models of neurons and glia[2]. Preliminary results indicated that DMSO reduces the viability of both neuronal and glial cells, whereas the administration of LnHS has no impact on the viability of SH-SY5Y cells but significantly diminishes that of U-87 cells. Consequently, we focused on SH-SY5Y cells and investigated whether LnHS could mitigate DMSO-induced toxicity concerning proliferation, oxidative stress, and inflammatory response. When co-administered with DMSO, LnHS partially alleviates the inhibitory effects of DMSO on cell viability. Moreover, the co-administration of LnHS influences the expression of SIRT3 and SOD2 enzymes, reduces nitrite release and ROS generation, while increasing IL-8 mRNA levels. In summary, our preliminary findings suggest that LnHS could be a promising compound that mitigates *in vitro* the cytotoxic effects induced by DMSO, thereby enhancing the survival of SH-SY5Y cells. However, additional research is necessary to elucidate the molecular mechanisms that underlie the biological activities of DMSO and LnHS. Referencs: [1]Tunçer, S. et al. (2018). Sci Rep 8(1):14828. [2]Mallardo, M. et al. (2024). Front. Cell Dev. Biol 12:1374626. *The authors marked with an asterisk equally contributed to the work.

P-30-029
An *in vitro* model of glioma development and potential therapy

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Gliomas are complex and heterogeneous brain tumors arising from glial cells. Despite the efforts made to define the pathology, at the molecular level, and to set up new approaches in order to reach infiltrating cells, gliomas are still fatal. The main objective of this study is to acquire a better knowledge of the cellular and molecular processes that accompany transformation of astrocyte lines. Therefore, starting from primary astrocytes, we have selected three astrocyte cell lines, A-GS1, A-VV5 and A-FC6, which exhibit a gradually more transformed phenotype, and we analyzed them at the cytogenetic, epigenetic, and proteomic level. We found that the most modified astrocytes (A-FC6 clone) have epigenetic and chromosomal alterations typical of cancer, such as an isochromosome (i8q), and that the other two clones (A-GS1 and A-VV5) have intermediate properties. Morphological differences among astrocytes and cell lines have been also found, even if GFAP is expressed in all the clones. Clone A-FC6 has duplication of the long arm of chromosome 8, which leads to the doubling of the genes located there: among these, some encode proteins involved in matrix remodeling, such as MMP3, that

indeed has been found by western blot, mostly in EVs deriving from the astrocyte most transformed clone [1]. Therefore, unstable cells such as clone A-FC6, equipped with a double dose of these genes, can represent a cell population with a high degree of malignant and metastatic properties. Furthermore, we are analyzing the possibility that extracellular vesicles (EVs) from this clone could be useful for the evaluation of alternative roads of diagnosis from blood, such the liquid biopsy. Moreover, EVs could be studied as a new instrument for targeted therapy. Reference: [1] Schiera, G et al. Genes 2023, 14, 990.

P-30-030
Novel dodecylaminoquinuclidines as potent inhibitors of components of the cholinergic system

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Drugs targeting neurotransmission have remained pivotal in the treatment of organophosphate poisoning and numerous neurological diseases and conditions. Despite the awareness that neurotransmission signaling effects within the nervous systems involve multiple components (like enzymes and receptors), their crosstalk is frequently disregarded during the evaluation of a drug's potential for action. In this study, novel dodecylaminoquinuclidines (QAs) were designed and tested as inhibitors of several enzymes linked to either acetylcholine or serotonin/dopamine neurotransmission system: acetylcholinesterase (AChE), butyrylcholinesterase (BChE), neuronal nicotinic receptors (nAChR) and monoamine oxidase A (MAO-A). All quaternized compounds inhibited both cholinesterases in 0.7–9 μM range but with a binding preference for AChE over BChE. Furthermore, QAs inhibited also neuronal nAChR activation in the presence of both acetylcholine (IC_{50} range 0.035–0.2 μM) or nicotine (IC_{50} range 0.01–1μM). The most pronounced antagonism was determined for QAs with para-nitro-benzyl or benzyl moiety on the QA-core. On the other hand, QAs did not inhibit MAO-A in concentrations up to 100 μM. In addition, QAs were screened for cytotoxicity on neuronal SH-SY5Y cell model and displayed toxic effects, within the 24 h exposure time. The highest toxicity with IC_{50} was observed for para-methyl-benzyl moiety on the QA-core. Taken together, these compounds showed a potential to act on the cholinergic neurotransmission but not on the selected component of the serotonin/dopamine neurotransmission system. However, the cytotoxic effect should be further evaluated to understand their limitations in development as new drugs. Funded by the European Union – Next Generation EU (Class: 643-02/23-01/00016, Reg. no. 533-03-23-0006) and performed using the facilities and equipment funded within the European Regional Development Fund project KK.01.1.1.02.0007.

P-30-031
Hemorphin regulates calcineurin activity in experimental Parkinson's disease

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Ca^{2+}-dependent mechanisms of neuronal damage were well demonstrated in a rotenone-induced PD model. Our recent *in vivo* studies showed that rotenone administration causes significant increase of Ca^{2+}/calmodulin-dependent protein phosphatase calcineurin activity in the rat brain (76%) and spinal cord (75%) compared to control. This indicates calcineurin involvement in neurodegeneration machinery at PD and possibilities of its regulation for neuroprotection. Taking into account the role of calcineurin in inflammation, regulation of calcineurin activity might be a prospective tool to modify neuroinflammatory responses that accompany and aggravate PD pathogenesis. Our studies showed that hemorphins, a family of endogenous nonclassical opioid peptides derived from hemoglobin (Hb), diminish inflammation through regulation of calcineurin enzymatic activity. The aim of this study was to evaluate the potential neuroregulatory and/or neuroprotective role of hemorphins in the brain and spinal cord exploring rotenone-induced experimental PD in albino rats. The results showed that synthetic LVV-hemorphin-3 (LVVYPW, LVV-H3; 1 mg/kg) given in post treatment regimen significantly decreased calcineurin activity by 69% in the brain and 97% ($p < 0.05$) in the spinal cord of rotenone-injected rats compared to control. Nevertheless, in pretreatment regimen LVV-H3 was less effective, causing not significant reduction of calcineurin activity in the brain (32%) and spinal cord (18%) of rotenone-injected rats compared to control. The results of these new studies demonstrate potential neuroregulatory efficacy of hemorphins in calcineurin-mediated neurodegenerative processes occurring in PD. Further evaluation of neuroprotective and anti-inflammatory capacity of hemorphins will contribute to the field of PD neuropharmacology. Partially supported by Yervant Terzian Armenian National Science and Education Fund (based in New York, USA) 2022 award #NS-biochem-2635.

P-30-032
Some modulators of Na,K-ATPase activity

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The effect of noradrenaline on the activity of Na,K-ATPase from synaptic membrane fractions of rat brain was studied *in vitro*. High noradrenaline concentrations (above 0.1mM) inhibit Na,K-ATPase activity; however, the detailed intracellular mechanism of noradrenaline's action remains unknown. Our study has shown that this inhibition mechanism is not Ca^{2+}-dependent. To investigate the details of noradrenaline inhibition, we studied its effect on the alpha1 and alpha2/3 subunits of Na,K-ATPase separately, using varying concentrations of the specific Na,K-ATPase inhibitor ouabain. The study demonstrated that the alpha2/3 subunit is sensitive to noradrenaline, while alpha1 subunit activity is not changed. Noradrenaline shifts the enzyme system from an MgATP-dependent cycle to an Mg^{2+}-dependent cycle.

P-30-033
New and old challenges in reversing organophosphorus toxicity

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Organophosphate (OP) compounds such as nerve agents (NA) inhibit the physiological function of acetylcholinesterase (AChE) promoting hypercholinergic activity, which induces seizures leading to brain damage and neuroinflammation. OP-induced overstimulation of nicotinic and muscarinic membrane receptors can in severe cases lead to hypoxia, vasodepression, and respiratory arrest, followed by death. Butyrylcholinesterase (BChE) serves as a backup for AChE and the protection of synaptic AChE from man-made and naturally occurring poisons. Strong nucleophiles such as oximes can reduce health effects after OP exposure by restoring enzyme activity. However, both inhibition and reactivation are fine-tuning chemical processes that depend on the structure of all of the reactants. The oximes approved for therapy have potency in restoring AChE activity inhibited by sarin and VX, but there is no universality in the reactivation of inhibition by different NA or duality in the efficacy to reactivate AChE and BChE. Novel challenges in restoring cholinesterase activity were introduced with the A-series of NA. In this study, we evaluated the inhibition of both cholinesterases' activity with known and newly scheduled A-series and tested standard and novel oximes to investigate their reactivation potency and universality. Our findings show that A-230 and A-234 are the most potent inhibitors of AChE and BChE among the A-series, respectively, and in the range of G-series cyclosarin and soman. A-series challenged the oxime reactivation potency, but we demonstrated that OP toxicity can be reversed by pyridinium or zwitter-ionic oximes. Even though no universally superior antidote was found, this work will accelerate progress in improving protection against A-series of OP and developing therapy for treating poisoning casualties. Funded by the Croatian Science Foundation (IP-2022-10-6685) and the European Union – Next Generation EU (Class: 643-02/23-01/00016, Reg. no. 533-03-23-0006).

P-30-034
Vitamin D3 down-regulates glucocorticoid-induced activation of the canonical NF-κB pathway and low-grade inflammation in the rat brain

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Synthetic glucocorticoids (GC) may have neurotoxic effects. Since vitamin D_3 (VD_3) is an important neurosteroid, the aim was to investigate its action on the canonical NF-κB pathway in relation to inflammation in GC-induced neurotoxicity. Female Wistar rats received GC prednisolone (5 mg/kg b.w.) with/without VD_3 (1000 IU/kg b.w., 30 days). Total and phosphoNF-κB p65, IκB, and inflammatory markers in the brain were determined by qRT-PCR/western blotting. IHC staining was used to quantify neurocytes ($NG2^+$), micro($Iba1^+$)- and macroglial ($GFAP^+$) cells in the cortex/hippocampus. 25-Hydroxyvitamin D_3 ($25OHD_3$) was measured by ELISA. Prednisolone induced 1.25-fold increase in NF-κB protein level, while IκB mRNA decreased (8.3-fold) vs. control. NF-κB activation, that plays a crucial role in brain inflammation, was confirmed by NF-κB p65 specific phosphorylation at Ser311, Ser536, and Thr435 (1.78-, 2.45- and 1.21-fold elevation, respectively). In parallel, GC caused an increase in TNFα (1.2-fold), IL-1β (1.55-fold) and iNOS (1.6-fold) levels vs. control, suggesting GC-induced low-grade inflammation in the brain. We found decreased number of $NG2^+$ cells in hippocampus and $Iba1^+$-microglial cells in cortex, as well as an increased amount of astrocytes after GC administration. This was associated with a depletion of $25OHD_3$ pool in serum, cerebrospinal fluid and brain tissue. VD_3 treatment led to a partial normalization of $25OHD_3$ circulating pool that resulted in down-regulation of TNFα and NF-κB p65 up to control values, and partial reduction of IL-1β (1.12-fold) vs. prednisolone. VD_3 elevated IκB mRNA level and reduced NF-κB p65 phosphorylation at Ser311 (3-fold) and Ser536 (1.11-fold), without affecting Thr435 phosphorylation compared to prednisolone group. Normalization of astrocytes number in the cortex after VD_3 supplementation was observed. In summary, restoration of VD_3 status may reverse GC-induced brain low-grade inflammation and inhibit NF-κB activation and astrogliosis.

P-30-035
Protective effect of exercise on METH-induced hippocampal cell damage in C57BL/6 mice

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Currently, it is estimated that over 35 million people around the world have a problem with methamphetamine (METH) abuse. METH is known to impair the dopaminergic system by increasing the release of both dopamine (DA) and serotonin (5HT) from nerve endings in the central nervous system. The above actions strongly affect glial cells, neurons and astrocytes located in the hippocampus, causing a reduction in hippocampal volume and activating inflammation in people long-term exposed to METH. Moreover, studies indicate that METH inhibits the proliferation, differentiation, maturation and survival of neural progenitor cells (NPC) in the dentate gyrus (DG). One of the ways to fight METH addiction is physical exercise. Voluntary running impairs the acquisition and maintenance of METH-related behaviors. Additionally, running selectively improves hippocampal plasticity and learning ability. Therefore, in our research, we hypothesized that physical exercises slow down neurodegenerative changes induced by METH in C57BL/6 mice. Mice were injected with METH three times per day for 5 days in ascending doses (starting with 0.2 mg/kg to the final dose of 2.4 mg/kg) using a stepwise increase of 0.2 mg/kg with each injection. Then, the mice were exposed for one more day to a high-dose METH binge based on three successive injections of 4.0 mg/kg METH at 4 h intervals. Inflammation activation was verified in mice using the Bio-Plex Pro Mouse Cytokine 23-plex Assay. Neurogenesis disturbances in the DG were verified using immunofluorescence methods. We have observed the changes in the markers levels either of immature (DCX, doublecortin) or mature neurons

(NeuN, neuron-specific nuclear protein). Moreover, differences in the proliferation of NPCs in the DG were demonstrated. Thus, physical exercise slows the neurodegenerative changes in METH-exposed mice. The research leading to these results has received funding from the National Science Centre, Poland, grant no. 2019/33/B/NZ4/02721.

P-30-036
Sex-specific variations in hippocampal FAAH expression and response to PEA in Alzheimer's model mice Tg2576

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Alzheimer's disease (AD) presents distinct molecular pathologies that differ by sex, affecting manifestation and progression. The enzyme fatty acid amide hydrolase (FAAH), responsible for degrading endocannabinoids like palmitoylethanolamide (PEA), is one such molecular target under scrutiny for its role in AD, considering that its differential expression and activity are regulated by sex hormones such as progesterone. This research investigates the sex-based differences in FAAH expression in the hippocampus of both wild-type and Tg2576 transgenic AD model mice and examines the differential response to chronic PEA treatment, a neuroprotective nutraceutical catabolized by FAAH. We conducted a detailed study involving gene expression profiling, protein quantification, enzymatic activity, and LC-MS quantification of endocannabinoids. The study further assessed the therapeutic impact of PEA through cognitive performance using the Novel Object Recognition (NOR) test and explored hippocampal markers of neuroinflammation, neurodegeneration, and oxidative stress. Our findings reveal a marked reduction in FAAH expression in male mice compared to females across age and genotype. Moreover, after 6 months of PEA administration, male Tg2576 mice at 12 months of age demonstrated a more significant therapeutic benefit from PEA treatment. This was substantiated by cognitive tests and biological markers assessments in the hippocampus. The study highlights the critical role of sex in AD pathology and suggests that targeting the endocannabinoid system with nutraceutical treatments like PEA could be refined by tailoring approaches to sex-specific differences. This opens new avenues for personalized therapeutic strategies in Alzheimer's disease management. *References*: Maccarrone M et al. (2004) Eur J Biochem. 271, 4666-76; Sabatucci A et al. (2019) Cannabis Cannabinoid Res. 13, 42-50; Jain S et al. (2022) Cell Biochem Funct. 40, 106-117.

P-30-037
Micro RNA-146, HMGB1 and IL-17 profile and cognitive symptoms in chronic migraine

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Chronic migraine (CM) is a complex neurological disorder primarily affects women, and characterized by reduced the quality of life and productivity due to chronic headaches, sensory hypersensitivity and comorbidities. The role of inflammation and innate immune system such as HMGB1 has been shown. micro RNAs in migraine pathogenesis is one of the issues that has attracted attention recently (1). miR-146, has negative regulatory properties in the TLR-4/NFkb pathway (2). IL-17 is a pro-inflammatory cytokine that can be triggered by HMGB1 and related to inflammation and cognition. In our study, we aimed to investigate the relationship between these parameters in CM. This study enrolled 35 women. Serum samples were collected from CM patients (n = 20) and age matched non-headache healthy controls (n = 15). Clinical features and migraine related cognitive symptoms scale (MigScog) were recorded. Serum HMGB1 and IL-17 were evaluated by ELISA method. Serum miR-146 levels were detected by quantitative Real-time PCR (RT-qPCR). Statistical analysis of the data was performed by the SSPS 25.0. Results: Serum HMGB1 ($p = 0.012$) levels were significantly higher in CM patients than control while IL-17 ($p = 0.737$) and miR-146 ($p = 0.560$) were comparable in 2 groups. There was no significant correlation between HMGB1 and IL-17($r = 0.167$) in addition HMGB1 and miR146 ($r = 0.073$). MigScog ($p = 0.001$) was significantly higher in CM patients, and it was positively correlated with HMGB1 ($r = -0.403$). We demonstrated elevated serum HMGB1 levels in CM patients. Serum HMGB1 levels showed correlation with migraine related disability but not with serum levels of miR-146 and IL-17. We suggest that HMGB1 may be a potential biomarker and therapeutic target for chronic migraine (3-4). The study was supported by TÜBİTAK-1004 (23AG014), the Gazi University ADEP project (TGA-2022-7857) and the TÜBA.

P-30-038
Trisomy21 and aberrant BACH1/Nrf-2 axis: implications for neurodegeneration

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Several studies support the implication of oxidative stress (OS) in phenotypic changes in Down syndrome (DS) subjects. Mapping of Human Chromosome 21 (HSA21) has shown the involvement of several genes, such as SOD-1, BACH1, APP, CBR, and S100B, in reactive oxygen species (ROS) overproduction in DS subjects and in animal models. We focused our attention on

BACH1, a transcription repressor that competes with the Keap1Nrf–2–ARE complex and negatively regulates the Nrf-2-mediated antioxidant response. For this reason, we studied the role of BACH1 in the brain and its implication in the failure of antioxidant response in DS. In this scenario, we investigated the BACH1/Nrf-2 dysregulation in human DS cells and animal models of the disorder: for human studies we analyzed lymphoblastoid cell lines (LCLs), whereas for animal studies we isolated hippocampal astrocytes and neurons from Ts2cje mice. Our results revealed that overexpression of BACH1 alters the BACH1/Nrf-2 ratio in the nucleus and impaired the transcriptional activation of antioxidant response genes, ultimately leading to the accumulation of oxidative damage. Overall, our study supports the hypothesis that BACH–1 triplication in DS subjects plays a critical role in the alteration of redox homeostasis; therapeutic strategies able to restore BACH1/Nrf2 axis are currently under investigation in our laboratory. In this context, we propose to pharmacologically target BACH1/Nrf-2 axis as a strategy to promote neuroprotective effects for DS population, ameliorating intellectual disability and possibly age-associated neurodegeneration. So, we test two anti-oxidant compounds (CAPE and VP961), proposing the modulation of the altered Nrf-2 pathway in DS. This two molecules are able to disrupt the Keap1-Nrf-2 complex interacting with Keap1, in this way Nrf-2 can translocate into the nucleus, takes the place of BACH1 and activates the transcription of anti-oxidant genes response. *The authors marked with an asterisk equally contributed to the work.

P-30-039
Exploring the role of propionic acid: link between gut microbiome and neurodevelopmental disorders

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Elevated levels of propionic acid (PPA) can disrupt the gut microbiome and the gut-brain axis, potentially leading to the development of neurodevelopmental disorders like autism spectrum disorders (ASDs). In this study, we examined *Escherichia coli* K12 and *E. coli* strains isolated from the gut of healthy rats and rats exhibiting autism-like behavior. The growth of strains was assessed by specific growth rate (μ) using spectrophotometric method in the presence and absence of PPA (11.7 mM and 33.4 mM). The results showed that μ of *E. coli* K12 was 1.03 h^{-1}, but decreased by ~40% and ~75% with the addition of 11.7 mM and 33.4 mM of PPA, respectively. High concentrations of PPA (33.4 mM) reduced the μ in all gut-isolated strains. Strains isolated from the cecum of healthy rat CCEC30 and from the PPA injected rat PPACEC30 exhibited similar reductions in growth rate (~30%) while initial μ of these strains was 0.90 h^{-1}. In strain isolated from the healthy rat's colon CCOL30 the μ was 0.8 h^{-1}. This rate decreased by 39% when 33.4 mM of PPA was added. For the strain isolated from the PPA injected rat's colon PPACOL30 μ was 0.60 h^{-1}, while it was decreased by ~87% in the presence of 33.4 mM of PPA. In strains isolated from the small intestine, μ was 0.80 h^{-1}. CS30 strain (healthy rat) μ was decreased by ~45%, while the PPAS30 strain (PPA injected rat) showed ~30% decrease. The effects of 11.7 mM of PPA were insignificant, except for the PPACEC30 strain, where μ increased by ~20%. This could be a mechanism developed by these bacteria in response to PPA injection. Supposedly, while the acid reaches the cecum, it may alter the internal pH. Thus, gut bacteria may have developed or activated mechanisms to become resistant to acidity. These findings underscore the significant impact of PPA on *E. coli* growth properties, which could influence intestinal microbial community balance and thus potentially contribute to ASDs development. *The authors marked with an asterisk equally contributed to the work.

P-30-040
Gold nanoparticles loaded with afzelin boost afzelin's neuroprotective effects on cognition and memory functions in Alzheimer's disease mice models

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Gold nanoparticles (AuNP) are valuable tools in pharmacological and biological research, offering unique properties for drug delivery in the treatment of neurodegenerative diseases. In this investigation, we explored the potential of gold nanoparticles loaded with afzelin (AuNP–Afz) to enhance the neuroprotective effects of afzelin, demonstrating a remarkable 10-fold increase compared to individual afzelin treatments. To validate these findings, we developed AuNP using a modified Turkevich method to optimize the conjugation of AuNP and afzelin. The distinctive characteristics of AuNP–Afz were confirmed through various techniques, including TEM, DLS, and UV-visible spectroscopy. Subsequently, AuNP–Afz was centrally administered into the third ventricle of mice brains for one month, with doses of 10 or 100 ng of afzelin, three times a week. To induce cognitive deficits, scopolamine (1.0 mg/kg) was intraperitoneally injected before behavioral experiments. The central administration of AuNP–Afz successfully restored cognition and memory behaviors in mice subjected to scopolamine, accompanied by the recovery of cholinergic dysfunction and modulation of BDNF, pCREB, and pAkt signaling pathways. Intriguingly, lower doses of AuNP–Afz (10 ng of afzelin) exhibited superior neuroprotective effects compared to equivalent levels of afzelin alone (100 ng) or higher doses of AuNP–Afz (100 ng of afzelin). Further validation through immunofluorescence of doublecortin (DCX) revealed that the low-dose AuNP–Afz treatment (10 ng of afzelin) rescued maturing neuronal cells in the brain. In conclusion, central administration of AuNP–Afz amplified afzelin's neuroprotective effects by enhancing neuronal activities through BDNF signaling and upregulating DCX expression. This study highlights the potential of AuNP–Afz as a promising therapeutic approach for mitigating cognitive impairments associated with neurodegenerative diseases, offering a new avenue for future research and drug development.

P-30-041
Effects of long-term oral administration of palmitoylethanolamide on subjects with mild cognitive impairment

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Palmitoylethanolamide (PEA) is an endogenous bioactive lipid with anti-inflammatory and neuroprotective properties. It is marketed as a nutraceutical for managing chronic pain and inflammation in humans due to its favorable safety profile. This study investigated the cognitive and anti-neuroinflammatory effects of a 12-month oral administration of PEA on individuals with mild cognitive impairment (MCI). Baseline and post-intervention assessments of cognitive performance were conducted, as well as evaluations of key markers of peripheral and central inflammation. Following PEA treatment, the entire cohort showed significant improvements in cognitive tests, including a single cognitive task, the Repeatable Battery for the Assessment of Neuropsychological Status (RBANS) total scale, and the RBANS visual-constructive ability scale, followed by a non-significant decline after 6 months of integration interruption. Interestingly, MCI subjects who received PEA treatment showed a significant improvement in visual skills. In contrast, the MCI placebo group showed a gradual decrease in performance throughout the assessment period. Furthermore, chronic PEA intake led to a significant reduction in the nitrosylation of plasma proteins in both healthy and MCI subjects, indicating a systemic antioxidant effect. This evaluation suggests a potential therapeutic role for PEA in mitigating cognitive decline and neuroinflammation associated with MCI, substantiated by molecular markers highlighting its antioxidant properties. *References*: Bossa M et al. (2023) Brain Sci. 13 (8):113; Kim N et al. (2024) Nutrients. 8;16(4):489; Raso GM et al. (2014) Pharmacol. Res. 86, 32-41.

P-30-042
Nicotinic acetylcholine receptors (nAChR) as targets of quinuclidine-based anticholinesterase drugs

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We screened the potential of quinuclidine-based compounds to interact with nicotinic acetylcholine receptors (nAChRs), important ligand-gated ion channels. The quinuclidine compounds were designed as inhibitors of the acetylcholinesterase (AChE; essential in neurotransmission) in the synapses. We hypothesized that compounds developed to bind to AChE could also act on nAChRs due to the shared substrate, the neurotransmitter acetylcholine (ACh). The interaction with the nAChRs was studied on SH-SY5Y cells as a well-accepted model, following changes in the intracellular Ca^{2+} release upon nAChRs stimulation by agonists ACh or nicotine. As results indicate, 24 out of 38 compounds inhibited nAChR signaling with both agonists. The highest IC_{50} of 0.02 μM was observed for the compound with a C14 alkyl chain attached to the quinuclidine core. This compound was also a potent inhibitor of AChE which affirms the hypothesis of its multitarget action. Also, we developed two QSAR regression models for the estimation of quinuclidine nAChR inhibition potential in the presence of ACh or nicotine. The models included only two descriptors, with MLOGP2 (octanol-water partition coefficient) present in both models, and the second descriptor being either nCs (number of total secondary C(sp3) atoms) in the case of nicotine or nH (number of hydrogen atoms) in the case of acetylcholine. Both models showed very good statistics r = 0.968, S.E. = 0.27 and S.E.cv = 0.30 (N = 29) and r = 0.968, S.E. = 0.32 and S.E.cv = 0.36 (N = 31) for pIC_{50} (nicotine) and pIC_{30} (ACh), respectively. Overall, the results indicated the potential of tested compounds to be further studied as nAChR modulators and the possibility of predicting their action using generated models. Research funded by the European Union – NextGenerationEU (Class: 643-02/23-01/00016, Reg. no. 533-03-23-0006) and performed using the facilities and equipment funded within the European Regional Development Fund project KK.01.1.1.02.0007.

P-30-043
Mitochondrial and endoplasmic reticulum unfolded protein response of SH-SY5Y cells induced by CDDO methyl ester

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Neurodegenerative disorders, including Alzheimer's, Parkinson's, Huntington's, and prion-related diseases, share a commonality as misfolded protein diseases, exhibiting comparable characteristics such as protein aggregation and neuronal dysfunction. The accumulation of misfolded proteins within mitochondria or

endoplasmic reticulum (ER) triggers organelle-specific unfolded protein responses (UPR) aimed at restoring cellular homeostasis. Prolonged cellular stress, where homeostasis restoration fails, leads to cell death. It is known that mitochondria and ER have very close contact sites called mitochondrial-associated endoplasmic reticulum membranes (MAMs), which they use for communication and for the exchange of Ca^{2+} ions, proteins, and lipids. Therefore, dysfunction of ER is often associated with mitochondrial dysfunction and vice versa. For experimental studies, we used neuroblastoma cell line SH-SY5Y, frequently used in neurobiology as an *in vitro* model for the study of neurotoxicity in dopaminergic neurons. Cells were treated with CDDO methyl ester (CDDO-Me), a synthetic triterpenoid explored in cancer treatment research, that works as inhibitor of mitochondrial protease LonP1. Utilizing the MTT assay, we observed a significant decrease in relative cell viability following 24-h exposure to 2.5 and 5 μM CDDO-Me. Western blot analysis targeting UPR markers, HRD1 (ER) and HSP60 (mitochondrial), did not reveal significant changes. Nevertheless, significantly elevated levels of HSP70 and HSP27 recognized as apoptosis inhibitors, were observed in cells treated with 2.5 and 5 μM CDDO-Me. Our preliminary findings suggest that CDDO-Me does not activate classical mitochondrial or ER UPR pathways. The observed decline in cell viability and upregulation of HSP70 and HSP27 hint at the induction of an alternative pathway, warranting further exploration in the ongoing investigation. *The authors marked with an asterisk equally contributed to the work.

P-30-044
Evaluation of CB1 agonistic activity and immobility behavior of synthetic cannabinoid receptor agonists

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Synthetic cannabinoid receptor agonists (SCRA) are one of the largest group of new psychoactive substances (NPS). SCRA has structural diversity which may be related to different pharmacological and toxicological activities. In this study, total 16 SCRA was chosen for the CB1 activity test, which include indole or indazole core, ester or carboxamide linker, naphthyl, adamantly, cumyl, or valine head, and other variable moiety. We evaluated CB1 agonism of SCRA in *in vitro* calcium assay at first. Calcium ion mobility was measured by using fluorescent calcium indicator in CB1 and Gα15 expressed cells. We calculated EC50 and ECmax of CB1 agonist activity of SCRA. It is known that SCRA induces immobility behavior on an elevated wire ring in animals, which is one of cardinal signs of CB1 activation. *In vivo* immobility behavior induced by SCRA was studied by catalepsy test in mice. In last, we observed a significant correlation between EC50 of calcium assay in vitro and the amount of time in catalepsy study *in vivo*. We will also discuss structure-activity relationships in the presentation.

P-30-045
Exploring the biophysical and biological properties of TDP-43 protein in extracellular vesicles

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Amyotrophic lateral sclerosis (ALS) and frontotemporal lobar degeneration (FTLD) are two neurodegenerative disorders sharing a common histopathology within neurons and glia. This consists in the mislocalisation of the TAR DNA-binding protein 43 (TDP-43) from the nucleus, where it normally resides and plays its function, to the cytosol, where it forms toxic inclusions. In all TDP-43 proteinopathies, including also Alzheimer's disease (AD) and Parkinson's disease (PD), TDP-43 undergoes a series of post-translational modifications, resulting in abnormal fragmentation, hyper phosphorylation and aggregation. TDP-43 aggregates can trigger template-dependent aggregation and amplification of new aggregate. Considering that cells release TDP-43 into extracellular vesicles (EVs), lipid bilayer-delimited particles which can move through biological fluids and release their content into other cells, EVs play a fundamental role in the spreading of TDP-43 proteinopathies. We aim to characterize the structure/composition of TDP-43 species transported into EVs in physiological and pathological condition and evaluate their toxicity in recipient cells. EVs have been isolated from motoneuron-like NSC-34 cells untreated or treated with inhibitors of the protein quality control (PQC) system to mimic TDP-43 disease and a battery of well-established biophysical methods have been adopted. Far-UV circular dichroism (CD) showed a similar secondary structure between the two groups of EVs, whereas the intrinsic tryptophan fluorescence data demonstrated that the inhibition of PQC system promotes protein aggregation. Super resolution STED microscopy was used to monitor the morphology of EVs and TDP-43 localization and MTT test to evaluate EVs toxicity. Our results suggest that PQC system plays a key role in the extracellular disposal of TDP-43 species as well as in the progression of TDP-43 proteinopathies. This study was supported by MUR (PRIN2022, project 2022KSJZF5 to R.C.).

P-30-046
Metabolism evaluation of neural and glial cells towards cadmium exposure

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The widespread toxic pollutant cadmium is released into the environment mainly by anthropogenic activities at a rate of 30,000 tons/year. Human exposure can occur through occupational exposure, inhalation of polluted air, cigarette smoking or ingestion of contaminated food and water. It mainly enters the human body through the respiratory and the gastrointestinal tract, accumulating in liver and kidneys with an estimated half-life of 25-30 years. Brain is also a target of cadmium toxicity since this metal may enter the central nervous system through the olfactory nerves or by increasing blood brain barrier

permeability. Once inside the body, cadmium can interfere with essential bio-elements, altering their homeostasis and biological functions, and weaken the antioxidant enzymatic and non-enzymatic defence systems, resulting in the damage of key macromolecules, intracellular organelles, and cellular membranes. Since cadmium exposure has been related to impaired functions of the nervous system, but the exact mechanism of its neurotoxicity is yet to be elucidated, our work aims to investigate cadmium toxicity towards not only neuronal cells, but glial ones too, focusing on cellular metabolism, mitochondrial functionality, and antioxidant defense mechanisms. On neuronal cells cadmium exacerbates the Warburg effect, causing an increase in glycolysis and in glycolytic ATP production, paralleled by a decrease in ATP production by oxidative phosphorylation, due to an impairment of mitochondrial respiration. Moreover, following cadmium administration, mitochondria increased their dependency on glutamine, as a substrate for lipid biosynthesis. On the other hand, on microglia cadmium induces the release of proinflammatory cytokines, without markedly switching to M1 phenotype, and the alterations of cell morphology and metabolism leading to a mitochondrial impairment, but not to a Warburg effect.

P-30-047
Targeting the insoluble inclusions of TDP-43 by trodusquemine as a potential therapeutic strategy for ALS and FTLD

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Mislocalization of the TAR DNA-binding protein 43 (TDP-43) from the nucleus to the cytoplasm with the formation of aberrant inclusions is a common feature of neurodegenerative conditions such as amyotrophic lateral sclerosis (ALS) and frontotemporal lobar degeneration (FTLD). The natural aminosterol trodusquemine (TRO) have been reported to modulate the aggregation and to suppress the toxicity of α-synuclein (αS) protein and amyloid-β peptide (Aβ42), thus representing putative drug candidates against Parkinson's disease and Alzheimer's disease, and possibly other neurodegenerative conditions. In this study we evaluated the effect of TRO in modulating TDP-43 phase separation through the fluorescence recovery after photobleaching (FRAP) technique; we also assessed its ability to prevent TDP-43 aggregation and neurotoxicity both in neuronal cells and in a *Caenorhabditis elegans* model, taking advantage of Raman spectroscopy *in situ* and the super resolution STED microscopy. We showed that TRO binds with high affinity to purified full-length (FL) TDP-43, promoting its phase separation. We also found that TRO dramatically affects TDP-43 accumulation and pathology in NSC-34 cells, promoting a nuclear redistribution of the protein, following the overexpression of human FL TDP-43 in NSC-34 cells, and restoring cell viability. Moreover, TRO induces a structural reorganization of neuronal TDP-43 inclusions, without altering their gel-like state. Finally, we demonstrated that TRO can decrease the inclusion formation in a *C. elegans* model overexpressing TDP-43, improving worm motility and viability. This study provides evidence that TRO can prevent the pathological effects induced by TDP-43, putting forwards its potential as a new therapeutic candidate in TDP-43-associated proteinopathies. This study was supported by MUR (PRIN2022PNRR, project P20225ZPYH to R.C.).

P-30-048
The impact of Myrtus communis essential oil on cognition, anxiety and oxidative stress in a zebrafish (*Danio rerio*) model of Alzheimer's disease

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Alzheimer's disease (AD) is the main cause of dementia, accounting for 60-80% of all cases, and is quickly becoming the most expensive, lethal and burdening disease of the century. The AD core symptoms consist of memory impairment and cognitive deficit, but neuropsychiatric symptoms such as anxiety and depression were commonly observed during the clinical course of the illness. The main pathological hallmarks of AD consist of the deposition of amyloid-β (Aβ) in the brain parenchyma and the cerebral vasculature, together with the presence of intraneuronal neurofibrillary tangles and the gradual loss of synapses. *Myrtus communis*, also known as Myrtle, is a flowering plant native to the Mediterranean region that was traditionally used to treat diarrhea, headache, pulmonary and skin diseases. Myrtle was also shown to possess antioxidant and anti-inflammatory properties and can lower Aβ levels leading to cognitive improvement. This study aims to characterize the Myrtle essential oil (MEO) and evaluate its pro-cognitive, anxiolytic and antioxidant potential in a zebrafish model of AD induced by scopolamine (SCOP). For this, the MEO was analyzed by GC-MS and chronically administered to zebrafish previously treated with scopolamine. The cognitive performances and anxiety-like behavior were assessed in specific *in vivo* tasks. The oxidative stress parameters and acetylcholinesterase (AChE) specific activity were measured from the brain tissues and correlated with the behavioral scores. Following the GC-MS analysis, a total number of 14 compounds were detected in MEO with α-pinene, 1,8-cineole, limonene, α-terpineol and linalool found in the greatest quantity. The behavioral assessment showed that MEO significantly ameliorated the SCOP-induced cognitive deficits and anxiety and mitigated the brain oxidative stress and reduced the specific activity of AChE. Taken together, our data suggests that MEO could be used effectively in the amelioration of dementia-related conditions. *The authors marked with an asterisk equally contributed to the work.

P-30-049
Effects of short-term intranasal oxytocin on behavioral parameters and brain neurotrophic factors in adolescent and adult female mice

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Oxytocin is also known for its active role in regulation of anxiety and social behaviours involving bonding or close social interaction between animals. Oxytocin functions through its receptors in the brain. Intranasally administered oxytocin shows similar behavioral effects as centrally administered. The aim of this study was to investigate the effects of short-term intranasal oxytocin on behavioral parametres, brain derived neurotrophic factor (BDNF) and oxytocin receptors (OXTR) in adolescent and adult mice. BALB/c adolescent (PN21-PN59) and 3 months old adult female mice were used in this study. Animals were divided into four groups (n = 6/group): adolescent control (AC), adolescent + oxytocin (AO), adult control (ADC), adult + oxytocin (ADO). Animals received intranasal oxytocin at a dosage of 20 μg/kg 30 minutes before behavioral experiments and sacrification. Behavioral experiments were performed to assess anxiety and sociability. The expression of BDNF and OXTR in the hippocampus were analyzed. As a result of the study, it was found that in the open field test, the time spent in the center by the AC group was significantly higher than the ADC group. The ADC group spent significantly more time in the thigmo zone than the AC group. In the three-chamber sociability test, the time spent in social area was significantly higher in all groups. Sociability was preserved in all groups. There was no significant difference between plasma oxytocin levels. There was no significant difference between the expression of OXTR and BDNF in the hippocampus. In conclusion, short term oxytocin administration had no significant effect on sociability, anxiety and the expression of BDNF and OXTR in the hippocampus. *The authors marked with an asterisk equally contributed to the work.

P-30-050
A novel *ex-vivo* model for studying SARS-CoV-2-induced neuroinflammation and microglial spine pruning

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Recent evidence suggests that COVID-19 infection targets the CNS causing interleukins-mediated brain inflammation [1], activation of microglia, and neuronal damage, reminiscent of what is observed in human neurodegenerative disorders [2]. Despite the social and clinical Neurocovid burden, the study of pathology on animal models lacks significant preclinical data. In particular, *in vitro* and/or *ex-vivo* Neurocovid-like experimental paradigms allowing pharmacological manipulation and electrophysiological assays would represent a good implementation to preclinical research in the field. Recently, intracerebroventricular injection of the human spike protein was regarded to promote inflammation, microglial TLR4 activation, and memory dysfunction in Swiss mice [3]. Nonetheless, the extensive characterization of the morpho-functional pattern of microglia activation induced by the human Spike in the mouse brain awaits further investigations. In line with this, the main aims of this study are: 1) to set up a novel *ex-vivo* Neurocovid model mimicking SARS-CoV-2-driven brain inflammation using mouse brain slices culture, 2) investigate microglial activation/phenotype and neuronal spine clearance by a cutting-edge super-resolution imaging technique, Expansion Microscopy (ExM) [4-5], 3) assess LTP pattern upon Spike incubation in brain slices [6]. In conclusion, a deeper understanding of the role played by microglia in COVID-19 neuroinflammation will potentially enable the evaluation of a novel approach to COVID-19 therapy, including the use of anti-spike monoclonal antibodies like Pronectins [7]. References: [1] Roy D, et al. 2021 doi: 10.1017/cjn.2020.173. [2] Theoharides TC, et al. 2023 doi: 10.3390/cells12050688. [3] Fontes-Dantas FL, et al. 2023 doi: 10.1016/j.celrep.2023.112189. [4] Wassie AT, et al. 2019 doi: 10.1038/s41592-018-0219-4. [5] Bissen D, et al. 2022 doi: 10.1016/j.xpro.2022.101507. [6] Mango D, et al. 2019 doi: 10.3389/fphar.2019.00778. [7] Polerà N, et al. 2023 doi: 10.3390/cancers15061647.

P-30-051
The effects of zinc supplementation on behavior and synaptic proteins in prenatally valproic acid exposed autism model of rat pups

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Autism spectrum disorder (ASD) is defined as a group of neurodevelopmental disorders that begin in childhood, with limited and repetitive behavior patterns as well as deficits in social communication and social interaction. Mineral imbalance in the prenatal and postnatal periods might be one of the environmental factors that cause autism. It has been suggested that maternal zinc deficiency may be a risk factor for autism and can be investigated as a biomarker in autism. Therefore, we aim to investigate the effects of zinc supplementation on synaptic proteins in rat pups of the autism model by biochemical and histological methods, besides the behavioral analysis. Pregnant Spraque Dawley rats were randomly divided into 4 groups (n = 4/group): Control (C), Valproic acid (VPA), Zinc (Zn), and Valproic acid + Zinc (VPA + Zn). On day 12.5 of pregnancy, saline was given to the C group and the Zn group, and valproic acid was given intraperitoneally at a dose of 500 mg/kg to the VPA group and the VPA + Zn group. One hour after the injection, saline was given to the C group and the VPA group, and zinc was given subcutaneously to the Zn group and the VPA + Zn group at a dose of 2 mg/kg. Behavioral changes were analyzed with the three-chambered social approach test on postnatal day 36. To examine the level of synaptic proteins in the hippocampus, Synaptophysin, Neuroligin-3, and SHANK3 expression levels were determined. In the male offspring, the social approach behavior in the VPA group was impaired, while the VPA + Zn group showed normal social exploration levels. We found

decreased SHANK3 expression levels in the VPA group, but no significant difference in Synaptophysin and Neuroligin-3 expression levels. In conclusion, we found that decreased SHANK3 levels in the hippocampus might be related to ASD and zinc may have a protective effect on social behavior but not on synaptic proteins.

P-30-052
Differential effects of chronic magnesium supplementation (citrate, glycinate, and malate) on brain function, behavior, and muscle strength in rats

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Magnesium (Mg) balance is important for the vitality and functionality of cells. The aim of the study is to investigate the effects of chronic Mg use on Mg levels in the brain, skeletal muscle, besides muscle strength, emotional and cognitive functions. Sprague Dawley female rats were divided into 4 groups (Mg citrate (n = 10), Mg glycinate (n = 10), Mg malate (n = 10), control group (n = 8)). Mg was given by oral gavage for 8 weeks (35.4 mg/kg/daily elemental Mg). Learning-memory (Morris water maze test-MWMT)), anxiety (open field test – OFT), motor coordination (rotarod test) and muscle strength (forelimb grip strength test) were measured. BDNF and Mg levels of the whole brain, as well as the amygdala, striatum, prefrontal cortex, hippocampus regions; and Mg levels of the gastrocnemius and soleus muscles were examined. In the MWMT, latency to find the platform in the citrate group was less than the control group, and the time spent in the target quadrant was longer. Mg levels of the amygdala and BDNF levels of the hippocampus were higher in the citrate group compared to the control group. Mg citrate had a positive effect on learning and memory by improving cognitive functions. In the OFT, the glycinate group spent more time in the center of the area than the control group, and all Mg groups showed less thigmotaxis. Corticosterone levels were lower in the citrate and malate groups compared to the control. In this study, the anxiolytic activity of Mg compounds was supported. In the evaluation of skeletal muscle strength, Mg malate increased grip strength, while Mg citrate and glycinate decreased compared to control. Mg levels in muscle tissues were higher in the malate group than the control, and lower in the glycinate group. Locomotor activity was increased in the OFT in the malate group. In conclusion, it is thought that Mg malate may be beneficial, as an increase in muscle strength and performance was observed due to the high muscle Mg levels in the Mg malate group.

P-30-053
Effects of voluntary and regular exercise on irisin levels in skeletal muscle and brain tissue of adolescent male rats exposed to chronic social isolation

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Irisin was initially recognized as a myokine secreted as a product of the FNDC5 protein that regulates exercise-induced energy metabolism in skeletal muscle. Although irisin is a hormone usually produced in muscle cells, some studies show that it is also produced in the hippocampus. This suggests a possible effect of irisin on brain functions. In this study, we investigated the effects of voluntary and regular exercise on the amount of irisin in the brain and muscles in socially isolated adolescent male rats. 42 adolescent male Sprague-Dawley rats were divided into 6 groups: control (C), socially isolated (SI), voluntary exercise (VE), regular exercise (RE), socially isolated + voluntary exercise (SI-VE), and socially isolated + regular exercise (SI-RE). The socially isolated groups were kept in separate cages for 4 weeks. The socially isolated groups were housed in separate cages for 4 weeks without receiving sensory cues from each other, while the others remained in the same cage for 4 weeks. During the social isolation period, the designated groups were exercised. Four weeks later, all rats were sacrificed, the prefrontal cortex, hippocampus regions of the brain and gastrocnemius muscle tissues were dissected. To conclude; the irisin/protein level in the hippocampus of the SI-RE group was higher than that of the VE and SI-VE groups. The irisin/protein level in the gastrocnemius of the SI-VE group was found to be higher than the VE and RE groups and SI-RE group was higher than in the VE, RE and SI groups. Rats exposed to social isolation may show a stronger irisin response than expected when they exercise, as a compensatory mechanism against this stress situation.

P-30-054
Elucidating the functional interaction between the intracellular domain of the glycine receptor α3 and the exocytosis complex protein SEC8

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The glycine receptor (GlyR) is a pentameric ligand-gated ion channel (pLGIC) which play a critical role regulating the neuronal excitability. Its dysfunction is associated with pathological conditions such as hyperekplexia, chronic pain and epilepsy. To date, four alpha subunits and one beta subunit have been identified. The pentameric complex, can exist in two configurations: homopentameric with only alpha subunits or heteropentameric with alpha and beta subunits in a 4:1 stoichiometry. Each subunit has an extracellular (ECD), transmembrane (TMD) and intracellular (ICD) domains. Historically, sites for allosteric modulators have been focused on the ECD and TMD,

meanwhile the ICD has been related only to the synaptic clustering. However, recent research suggests that the ICD is a target for intracellular modulation, exerted by proteins such as Gbeta-gamma, which potentiated the GlyR alpha1 function. In this context, a novel interaction between GlyR alpha3 ICD and the SEC8 protein has been reported. This interaction enhances the transport and regulates axonal trafficking of GlyR alpha 3. Nevertheless, the impact on GlyR alpha3 function is still unknown. This study aims to understand the functional effect of GlyR-SEC8 interaction using biochemical, immunohistochemical and electrophysiological approaches. Our experimental results shown that the interaction of GlyR alpha3 with SEC8 produced a left shift in the dose-response curve, apparently due to an increase in the desensitization rate, without in changes in the membrane expression levels or maximal currents. These results opening new lines of research into the regulation of GlyR by intracellular components.

P-30-055
Myricetin encapsulated in extracellular vesicles as a nanotherapeutic alternative in neuroinflammation

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Microglia are the resident immune cells of the CNS, and they represent the first active defence mechanism against pathogens and non-infectious injuries such as hypoxia and endogenous misfolded proteins. On the other hand, their activation toward the M1 phenotype in response to both environmental and endogenous stimuli results in the release of inflammatory and neurotoxic factors, leading to neuroinflammation and brain damage. In our previous studies, we reported that myricetin, a natural antioxidant and anti-inflammatory flavone, suppresses inflammatory pathways and keeps microglia in resting state, protecting neurons from death in an *in vitro* model of neurotoxicity [Boriero, D et al. (2021) FEBS J 288, 2347-2359]. Nevertheless, its high hydrophobicity and low bioavailability limit its use *in vivo* applications. To overcome myricetin's drawbacks, we propose the use of small extracellular vesicles (EVs) as drug delivery system for myricetin in the context of microglia activation. Recently, EVs have emerged as potential drug delivery systems due to their non-immunogenicity, nanometric size and ability to cross the blood–brain barrier. In this work, EVs have been isolated from a cell-conditioned medium (CCM) of the murine microglia BV2 cell line through differential ultracentrifugation and filtration steps. They have been characterised for size and quantity through nanoparticle tracking analysis (NTA) and western blot for the expression of vesicle markers. Once isolated, the EVs have been encapsulated with myricetin through passive diffusion and purified by ultrafiltration. High-performance liquid chromatography has been used to evaluate the encapsulation efficiency of EVs.

P-30-056
The 75-99 URG7 peptide and its analogs modulate the α-synuclein structures

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Parkinson's disease (PD) is a neurodegenerative disorder characterized by motor and non-motor symptoms, as the results of the death of dopaminergic neurons in the Substantia Nigra pars compacta of the brain. The main hallmark is the presence of intracellular Lewy bodies with fibrillary neurotoxic accumulations of alpha-synuclein. Synuclein, released by dying cells, can be found in extracellular biological fluids, including human cerebrospinal fluid (CSF) and blood. Various therapeutic strategies aim to target alpha-synuclein, including approaches to prevent its aggregation, promote its clearance, and mitigate its toxic effects. Heat shock proteins and proteins from endoplasmic reticulum (ER) play an important role in maintaining cellular homeostasis and can protect neurons by degrading damaged or misfolded proteins through different mechanisms [Guo H. et al. (2023) Neurochemistry 162]. URG7 is a 99 amino acid protein localized in the ER membrane with the N-terminal region in the lumen and the C-terminal region in the cytoplasm [Ostuni A. et al. (2013) FEBS Lett 587(18)]. The C-terminal peptide of URG7 (residues 75-99) and its analogs interact with the β-sheet filaments of aggregated recombinant α-synuclein and consequently disaggregate it [Dandurand J. et al. (2024) Int. J. Mol. Sci 25(2)]. Preliminary studies have shown that anti-URG7 antibodies are correlated with anti-alpha-synuclein antibodies in both controls and PD patients. Assessing whether these can interfere in disaggregation processes is a priority.

P-30-057
Effects of antipsychotic drugs on chromatin condensation level of human neurons

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Clozapine has been identified as the most effective antipsychotic medication, uniquely approved for treatment-resistant schizophrenia. Its use, however, is restricted due to severe side effects. Despite extensive studies the mechanism of action underlying clozapine's unique efficacy still remains elusive. Recent studies have shown clozapine also affects gene expression through epigenetic modifications, like changes in DNA methylation patterns. The aim of this study was to identify genes associated with chromatin regions, which condensation levels are altered by antipsychotic drugs. The effects of treatment with clozapine were compared to other commonly used antipsychotic drug – risperidone to pinpoint genetic regions uniquely regulated by clozapine. Human neurons derived from LUHMES cell line were treated with

antipsychotic drugs and subsequently ATAC-seq assay was performed to identify transposase-accessible DNA regions. Chromatin accessibility analysis revealed enrichment in open chromatin peaks in promoter and 5′UTR regions of genes involved in neuron homeostasis.

P-30-058
miRNAs regulating Tau expression are increased in neuron-derived extracellular vesicles in frontotemporal dementia, but not in Alzheimer's disease

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Great efforts have been made to identify biomarkers to improve the diagnosis of dementias such as frontotemporal dementia (FTD) or Alzheimer's disease (AD), but only few candidates have been described in recent years. Tau protein plays an important role in the pathogenesis of both FTD and AD. We described three microRNAs (miR-92a-3p, miR-320a and miR-320b) regulating Tau protein synthesis which are differentially expressed in plasma of FTD patients with respect to healthy controls and AD patients [Previously published in: Piscopo P et al. (2023) Front Mol Neurosci 16:1127163]. Here we suggest that the changes observed in plasma are due to a deregulation of the three miRNAs in neurons and consequently in neuronal extracellular vesicles (NEVs). The three miRNAs were increased in FTD human induced pluripotent stem cells (hiPSC)-derived neurons and in their conditioned medium, compared to wild-type hiPSC-derived neurons. In the cerebrospinal fluid (CSF) of FTD patients, both miR-92a-3p and miR-320a showed higher levels with respect to controls or AD patients. In NEVs isolated from patients' plasma, both miR-92a-3p and miR-320a levels were triplicated in the FTD group compared with controls or AD patients. Our observations point to roles for Tau, miR-92a-3p, miR-320a and miR-320b which differ in the molecular neuropathology of FTD and of AD. Moreover, we suggest that miR-92a and miR-320a derived from NDEVs might be useful biomarkers for the differential diagnosis of dementias. *The authors marked with an asterisk equally contributed to the work.

P-30-059
Effect of N-acetyl-aspartate on microglia metabolism and inflammation

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Neuroinflammation is an important feature involved in neurodegenerative diseases that initially protects the brain from insults but becomes detrimental when sustained. Microglia are resident immune cells of the central nervous system (CNS) that play a central role in chronic inflammatory processes due to an imbalance between the neurotoxic (M1) and neuroprotective (M2) phenotypes. CNS homeostasis is strictly regulated by intricate communication between neurons and glial cells. N-acetyl aspartate (NAA) is among the most synthesized metabolites in the CNS; it is primarily produced by neurons and cleaved by recipient cells yielding acetate and aspartate. While existing data predominantly focuses on NAA's role in oligodendrocytes, where NAA-derived acetyl-CoA is used for myelin synthesis, we present findings on NAA's impact on microglial metabolism and activation. Our study demonstrates that NAA stimulates the mitochondrial oxidative metabolism sustained by an increase in lipid turnover. In particular, the acetate deriving from the catabolism of NAA can replenish the cell with cytosolic acetyl-CoA which then triggers lipid synthesis used by the cell for ATP production through oxidative phosphorylation. Furthermore, we investigated whether NAA could influence LPS/IFNγ-induced M1 polarization. Our findings reveal that NAA mitigates the expression of pro-inflammatory markers, as evidenced by the reduction in iNOS, TNF-α, and IL-6. These results are of particular interest considering that NAA is often considered as a marker of neuronal health. Reduced NAA levels are linked to neuronal loss and dysfunction, making it a potential prognostic marker in neurodegenerative diseases. This work was supported in part by PNRR "MNESYS" (MUR PE00000006).

Immunobiochemistry

P-31-001
The effect of oxalic acid treatment against varroa mites on honey bees (*Apis mellifera*)

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The honey bee (*Apis mellifera*) populations are declining, mainly due to the effects of various factors on bees. *Varroa destructor* mites are responsible for the most serious problems with honey bee health worldwide. The mites transmit viruses that are mostly lethal to honey bee colonies, typically the deformed wing virus (DWV). There are several treatments against varroosis; however, natural acaricides such as oxalic acid (OA) have become widespread among beekeepers around the world. These miticides are effective against *Varroa mites*; on the other hand, they also

influence honey bees. The aim of this research was to determine the effect of oxalic acid on honey bees (*Apis mellifera*), mainly on the immunity of adult bees and honey bee brood. Molecular biology and proteomics methods were used to study protein components involved in the immune responses of honey bee brood. In adult bees, antimicrobial peptides (AmPs) were quantified by ELISA and LC-MS methods, and the antimicrobial activity of hemolymph was determined by diffusion tests. The results of this study showed that long-term exposure to this type of treatment resulted in induced humoral immune responses in adult bees. The antimicrobial activity of hemolymph was higher after OA treatment, which correlates with higher levels of antimicrobial peptides in hemolymph. In honey bee brood, relative expression of antimicrobial peptides defensin and hymenoptaecin were increased after OA treatment; meanwhile, relative expression of abaecin was decreased. Similar responses of bee immune components were reported after exposure to pesticides. AmPs abaecin, apidaecin, defensin, and hymenoptaecin are peptides produced in responses to bacterial or fungal pathogens. Our results open a question, why is the immune system activated by OA? Further studies are necessary to reveal whether immune activation is a positive or negative effect of OA treatment.

P-31-002
Therapy induced autophagy associated secretome affects antitumor activity of natural killer cells

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The aim of this study was to characterize the chemotherapeutic stress induced autophagic secretome and to investigate whether autophagic secretome affects the NK-mediated immune response to MCF-7 breast cancer cells. Etoposide was used to induce chemotherapeutic stress mediated autophagy in MCF-7 cells. Analysis of autophagy induction was tested by using autophagy markers including LC3I/II and p62 degradation by western blot and immunofluoresence analysis. LC/MS-MS analysis and cytokine array analyses were used to characterize the chemotherapy-induced autophagic secretome content. Whether the chemotherapy-induced autophagic secretome affects the capacity of DNAM1-NK-92 cells to target MCF-7 cells was determined by degranulation assay. LC3I/I conversion and p62 accumulation confirmed that 150 μM Etoposide treatment for 24h induce autophagy in MCF-7 cells. It was also determined that did not cause apoptosis. LC/MS-MS analysis showed that metabolic enzymes, tumor antigens, chaperones and metastasis-related proteins were secreted during chemotherapy-induced autophagy. In addition, 41 different cytokines/chemokines and growth factors were detected in the secretome by cytokine array analysis. The findings revealed that the secretome formed by the combination of a chemotherapy agent and an autophagy inhibitor increased the tumor targeting capacity of NK92 cells more than the other groups. Under *in vitro* conditions, chemotherapy-induced autophagy induction stimulates NK cell effector functions and respond to drug-induced stressors that contribute to anticancer activity.

P-31-003
Affibodies as valuable tool to counteract β2-microglobulin aggregation

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Beta-2 microglobulin (β2m) is the invariant subunit of the Major Histocompatibility Complex I which plays a critical role in the regulation of the adaptive immune system. Although monomeric β2m is stable in physiological conditions, high local concentrations or the presence of mutations may prompt the misfolding of the protein, causing the formation of amyloid fibrils. The prolonged accumulation of such fibrils can trigger the development or exacerbation of different chronic and autoimmune diseases. Therefore, stabilizing the native state of β2m could be the first step towards preventing pathology progression and favoring the recovery. In this framework, a strategy to address the lack of protein stability is to foster protein-protein interactions. Particularly, a new class of antibody-like molecules called affibodies, has been developed to selectively target β2m. Unlike antibodies, affibodies are smaller, easily customizable molecules with both higher solubility and thermal stability. The four best hits in the affibody library were expressed, purified and then incubated with β2m to assess their interaction. The binding was preliminary assessed by size-exclusion chromatography and subsequently confirmed by isothermal titration calorimetry and microscale thermophoresis techniques. The results revealed a dissociation constant in the nanomolar scale for two of the complexes. In parallel, ThT assays were performed to assess whether the interaction between β2m and affibodies can reduce the aggregation propensity of β2m. A significant reduction was observed after incubation with one of the affibodies. Taken together, the collected results suggest that affibodies can bind β2m *in vitro* with high affinity and significantly reduce its aggregation rate.

P-31-004
The new FKBP51s splice isoform in alternative macrophage polarization: multidimensional profiling of tumor-associated macrophages

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Tumor-associated macrophages (TAMs) are pivotal in cancer progression and immunotherapy resistance. Our previous research identified the immunophilin FKBP51s as a potential TAM marker and a potential target for reprogramming strategies. [Previously published in: Troiani et al. (2020) Br J Cancer 122, 1782–1790.] Specifically, alternative splicing of *FKBP5* occurs during M2 macrophage polarization, and FKBP51s silencing impacts intracellular signaling, membrane proteome composition, and cytokine production. As a result, TAM features such as migration, invasiveness, and immunosuppressive capability are impaired. Furthermore, macrophage metabolic activity shifts from the OXPHOS typical of M2-like macrophages to the glycolytic activity characteristic of M1, when FKBP51s is depleted. This study explores how FKBP51s promotes the transition to an oxidative-type metabolism with heightened mitochondrial function. Our investigation into OXPHOS complex expression levels

revealed a reduction of nuclear-encoded subunits in M2 cells upon FKBP51s depletion, while mitochondrial-encoded subunit levels remained unaffected. Immunoblot analysis of fractioned lysates indicated that FKBP51s predominantly localizes to the cytoplasm of M2 macrophages, tightly associated with the cytoplasmic side of the endoplasmic reticulum (ER). Polysome profiling further revealed the association of FKBP51s with polysomes, thus suggesting a potential role in ensuring the proper folding of proteins at the ribosomal tunnel exit. Moreover, a comprehensive differential proteomic analysis of FKBP51s-depleted M2 macrophages unveiled a restoration of an anti-tumor macrophage phenotype. In conclusion, our findings suggest a co-translational role of FKBP51s to help the macrophage switch towards the pro-tumor phenotype. The results of this study will add important pieces of information for the comprehension of TAMs physio-pathology with translational applications in the field of cancer treatment and diagnosis.

P-31-005
Modulation of the endocannabinoid system and anti-inflammatory effects by cannabinol via TRPV1 in human keratinocytes

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Cannabinol (CBN) is one of the non-psychoactive secondary metabolites of cannabis, whose beneficial activity on human skin inflammatory diseases has attracted increasing attention, due to recent studies on its anti-inflammatory properties [previously published in: Walsh et al. (2021) Front. Pharmacol. 12, 777804]. Phytocannabinoids (pCBs) derived from cannabis are known to interact with the complex network of the lipid endocannabinoid system (ECS), fully expressed in human keratinocytes. Based on the ability of other minor pCBs to affect skin ECS elements and inflammation [previously published in: Di Meo et al. (2022) IJMS 23, 5430; Tortolani et al. (2023) IJMS 24, 2721], here, we sought to investigate if also CBN could modulate the major ECS elements, in both normal and lipopolysaccharide-inflamed human keratinocytes (HaCaT cells). As results, CBN increased the gene and protein expression, as well as the activation, of cannabinoid receptor 1 (CB_1) and vanilloid receptor 1 (TRPV1). In addition, CBN modulated the metabolism of the endocannabinoids anandamide (AEA) and 2-arachidonoylglicerol (2-AG), by increasing the activities of NAPE-PLD and FAAH - the biosynthetic and degradative enzyme of AEA - and that of MAGL, the hydrolytic enzyme of 2-AG. CBN also affected keratinocyte inflammation by reducing the release of pro-inflammatory interleukin (IL)-8, IL-12 and IL-31 and increasing that of anti-inflammatory IL-10. Of note, the release of IL-31 was mediated by TRPV1. Finally, a modulation of glycogen synthase kinase 3β (GSK3β) upon treatment with CBN and distinct ECS-directed compounds, through the analysis of the mitogen activated protein kinases (MAPK) signaling pathway, was ascertained. In conclusion, our results demonstrate that CBN modulates distinct ECS elements and exerts anti-inflammatory effects – remarkably *via* TRPV1 – in human keratinocytes, thus holding potential for both therapeutic and cosmetic purposes. This work was supported by InMed Pharmaceuticals. *The authors marked with an asterisk equally contributed to the work.

P-31-006
Simulated microgravity affects pro-resolving properties of primary human monocytes

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Microgravity is a space-related stressor responsible for cellular and molecular alterations of immune and inflammatory homeostasis linked to the disorders that astronauts experience during and after missions [previously published in: Hughes-Hulfort M (2011) FASEB 25, 285-2864]. Among the targets of microgravity, monocytes and lymphocytes are important immune effectors, which produce soluble mediators that control tissue homeostasis, including specialized pro-resolving mediators (SPMs), a novel class of immunomodulatory lipids produced from omega-3 and -6 essential polyunsaturated fatty acids through the action of 5-, 12- and 15-lipoxygenase (5-, 12- and 15-LOX). These molecules shut down the inflammatory surge in the aftermath of the immune event, and their disfunction is linked with many inflammatory diseases. However, to date, microgravity has never been investigated in the field of pro-resolving lipids. Our results show that the 24 h exposure to rotary cell culture system (RCCS)-simulated microgravity rearranges SPM receptors and enzymes both at the gene and protein level, in human monocytes. In particular we show a significant gene and protein upregulation of the SPM receptors GPR32 and GPR18; and a significant decrease in 5-LOX expression simultaneously with a slight increase in its activity. The reduction in 5-LOX expression results in a significantly-reduced biosynthesis of RvD1, a prominent SPM. Also, we show monocytes and lymphocytes display reduced levels of pro- and anti-inflammatory cytokines, following RCCS incubation. Finally, the treatment with RvD1 significantly reduces monocytes' production of TNFα at Earth gravity, but not at simulated microgravity. These results suggest that microgravity can impair activation and function of monocytes in the inflammatory process; so their study is important in the research of pathological processes involved in space-related diseases to develop therapeutic countermeasures. *The authors marked with an asterisk equally contributed to the work.

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P-31-007
Pro-resolving and immunomodulatory properties of *N*-acylethanolamines in innate and adaptive immune cells

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N-Acylethanolamines are a group of endogenous bioactive lipids which have been investigated in several diseases due to their immunomodulatory and pro-resolving-like properties. Key members of this class include endocannabinoids (eCB) – e.g., arachidonoylethanolamide (AEA) – and numerous other eCB-like compounds referred to as autacoid local injury antagonist amides (ALIAmides) [Previously published in: Chiurchiù V et al. (2018) FASEB J 32,5716-5723 and Leuti A et al. (2020) Adv Drug Deliv Rev 159: 133-169]. Of note, AEA act on both innate and adaptive immune cells by blunting their pro-inflammatory features, however, its involvement in the cellular aspects of inflammation resolution, a process typically governed by ω-3-derived lipids, remains largely uninvestigated. So far, the effect of ALIAmide adrenoylethanolamide (DEA) on immune cells is largely unknown. In this study, we characterized the pro-resolving/immunoregulatory properties of AEA on primary human monocyte-derived macrophages (MoDMs) and of DEA on T cells. We found that AEA treatment in the nanomolar range enhances the production of SPMs Resolvin (Rv)D1 and RvE1 in a type 2 cannabinoid receptor (CB_2)-dependent manner and enhances efferocytosis (i.e., the phagocytosis of apoptotic cells) through CB_2 and GPR18, without affecting MoDMs polarization and immunophenotype. Notably, GPR18 is a prominent pro-resolving lipid binding receptor, which can be engaged by AEA, suggesting that this eCB might directly contribute to the resolution process. In a different experimental setup, we also found that administration of AEA or DEA leads to a reduction of TNF-α, INF-γ and IL-17 production in CD4+ and CD8+ T cells in a dose-dependent manner. Interestingly these effects were blocked by CB_1 reverse agonist SR141716A. Our data show for the first time that DEA can directly affect different T-cell subsets and provide proof of direct effect of AEA on the regulation of pro-resolving features in human macrophages. *The authors marked with an asterisk equally contributed to the work.

P-31-008
New perspectives on the immunomodulatory potential of GM1 in cystic fibrosis

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Cystic fibrosis (CF) is a hereditary disease resulting from mutations in the gene encoding the cystic fibrosis transmembrane conductance regulator (CFTR). CFTR acts mainly as a chloride channel located on the apical surface of epithelial cells. In CF, the transport of chloride ions in secretory epithelia is compromised, leading to the production of thick and viscous mucus that primarily affects the respiratory system. Previous research identified GM1 ganglioside as a crucial CFTR interactor, essential for channel stability at the cell plasma membrane (PM). In CF cells, the content of GM1 was observed to be reduced in the absence of CFTR. Interestingly, restoring GM1 levels improved CFTR stability, especially when combined with CFTR modulators, the newest therapeutics. Moreover, GM1, known for modulating immune responses, also plays a role in regulating *Pseudomonas aeruginosa* clearance in CF bronchial cells. Based on this evidence, our study explored the role of GM1 in host-pathogen interactions in CF. Initially, xenophagic clearance activity was measured in CF and non-CF bronchial cells exposed to GM1, revealing reduced clearance of invading pathogen in CF cells. However, a 48-h GM1 pre-treatment rescued bacterial clearance ability. Bacterial invasion assays ruled out reduced invasion capacity as the cause. Subsequently, the safety of GM1 aerosol administration was tested in WT mice, showing no statistically significant toxicity at tested doses (1, 5, and 20 mg/kg/day), as observed through weight, temperature, and well-being assessments. These findings suggest that GM1 has potential therapeutic benefits in enhancing bacterial clearance in CF without inducing significant toxicity when administered via aerosol. Regarding the importance of the role of GM1 in stabilizing CFTR at the PM and its immunomodulatory function, this study may contribute to developing novel therapeutic approaches to enhance the treatment of CF patients, and their immunomodulatory capacities.

P-31-009
Engineering a nanobody structure to increase its avidity and affinity for Aβ42 oligomers: the dimeric-DesAb-O

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Small, soluble amyloid-β_{42} ($A\beta_{42}$) oligomers, rather than mature amyloid fibrils, are regarded as major neurotoxic agents in Alzheimer's disease (AD). In a previous work, we rationally designed a single-domain antibody (sdAb), called DesAb-O, with high specificity for $A\beta_{42}$ oligomeric conformers [Aprile FA et al. (2020) Proc Natl Acad Sci U S A. 117, 13509-13518]. Recently, we showed that the sdAb can selectively detect synthetic $A\beta_{42}$ oligomers in cultured cells, neutralizing their associated neuronal dysfunction. DesAb-O can also identify $A\beta_{42}$ oligomers in the cerebrospinal fluid (CSF) of AD patients, compared to healthy individuals, preventing cell dysfunction induced by CSFs administration [as previously published in Bigi A, Napolitano L et al, (2024) Alzheimers Res Ther. 16,13]. Given the outstanding potentialities of this sdAb, we design a dimeric-structure of DesAb-O, with the aim to further increase its avidity and affinity for toxic $A\beta_{42}$ oligomers. We engineered the dimeric-DesAb-O structure by linking two DesAb-O monomeric domains with a flexible linker region consisting of three repetitions of the sequence GGGGS. We expressed and purified this novel construct and characterized its molecular weight and secondary structure by mass spectrometry and circular dichroism. Then, we performed aggregation assays to monitor the ability of dimeric-DesAb-O to interfere with the $A\beta_{42}$ aggregation process and a real-time based ELISA assay to study its binding for $A\beta_{42}$ oligomers. Results showed that the dimeric-DesAb-O is able to interfere with the $A\beta_{42}$ aggregation process to a greater extent than DesAb-O. Furthermore, the dimeric structure of DesAb-O showed a higher specificity and affinity for $A\beta_{42}$ oligomers compared to DesAb-O. In conclusion, the dimeric-DesAb-O appears to be a promising tool for the future development of sdAb-based immunodiagnostic tests for the early diagnosis of AD.

P-31-010
High fat diet-wheat gliadin interaction and its implication for obesity and celiac disease onset in DQ8 mice

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Obesity is a chronic disease characterized by abnormal or excessive fat accumulation, which may lead to the onset of many other metabolic disorders. Obesity is induced not only by genetic factors but also by environmental factors, especially a high fat and high carbohydrate diet is considered the main triggering agent. Celiac disease (CD) is an autoimmune disease triggered by gluten. Interestingly, several studies in adults and children with CD indicate that obesity/overweight at disease onset is not unusual. However, the mechanisms of interaction with gluten and high fat diet (HFD) are unclear. In this study, AB^0 HLA-DQ8 transgenic (DQ8) mice, a model of gluten sensitivity, were fed with a gluten-free HFD (HFD) or a gluten containing HFD (HFD + G) for 12-weeks to analyze the morphology and molecular markers in the small intestine and liver. We found that HFD + G significantly induced in the small intestine higher mRNA levels of TNF-a, IL-1b, and IL-10 than in HFD DQ8 mice, but not in the liver. Interestingly, HFD + G also induced increased transcription of intestinal tight junction genes (zonula occludens-1 and -2, occludin, claudin-1 and -3). However, we did not identify changes in the intestinal architecture, while an increased number of lipid droplets in liver was detected in HFD + G DQ8 mice. In conclusion, HFD-gluten interaction caused impaired intestinal immunity and permeability, associated with increased liver dysfunction in genetically gluten-sensitive mice. These findings may help to elucidate new aspects of the pathogenetic mechanism in CD, driven by metabolic changes related to diet components other than gluten.

P-31-011
Interactions between WASP and DGKα in Wiskott-Aldrich syndrome

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The Wiskott-Aldrich syndrome (WAS) is an X-linked immunodeficiency characterized by eczema, impaired humoral and cellular immunity, and thrombocytopenia. There are multiple hematopoietic cell defects in WAS, such as impaired T-cell functions, markedly reduced platelet number and size, and decreased migration and response to agonists in several cell lineages. By studying diacylglycerol signaling in X-linked lymphoproliferative disease, we discovered that diacylglycerol kinase alpha (DGKα) acts as a negative regulator of T-cell activation and its activity is limited by the direct binding to the Wiskott-Aldrich syndrome protein (WASp). We are currently investigating the role of WASp and DGKα at the immune-synapse by flow cytometry and live cell imaging. We aim to improve our understanding of the role played by this signaling pathway in the T-cell signaling balancing activation induced cell death and cytokine expression . In addition, we observed that DGKα has an important role in megakaryocyte differentiation and platelet activity acting as a negative regulator. We find out that megakaryocytes silenced for WAS had a defect in size and differentiation and this defect could be rescued by DGKα inhibition. Our data suggest that part of the phenotypes observed in WAS may be due to the hyperactivity of DGKα, that consumes diacylglycerol perturbing glycerophospholipid signaling. In this scenario, the inhibition of DGKα activity may constitute a novel therapeutic approach for WAS patients not undergoing bone marrow transplant.

P-31-012
The pro-healing and anti-inflammatory potential of palmitoleic acid on diabetic wounds

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Chronic inflammation is a hallmark of chronic diabetes wounds, and due to increased demand, cost-effective wound-healing technologies and fundamental science mechanisms have been a focus of research. Palmitoleic acid (ω-7 monounsaturated fatty acid) is found in young people's skin and decreases with age. This fatty acid accelerates wound healing and muscular healing [previously published in Weimann E et al. (2018) PLoS One 13, 1-10; Malvestiti R et al. (2017) Eur J Lipid Sci Technol 119, n. 8]. Herein, we investigated the effects of palmitoleic acid on human and rat neutrophils. Human patients were divided into two groups: non-diabetic subjects and type 2 diabetes non-insulin-dependent subjects. The human neutrophils were collected through blood samples, plated ($2{,}5 \times 10^6$ cells), treated with 0-200 μM palmitoleic acid, in the presence or absence of LPS (5μg/ml), and incubated at 37°C for 4 and 18 h. The toxicity was evaluated by MTT [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide assay], cell membrane integrity, and DNA fragmentation. Moreover, the cytokine release from cultured diabetic neutrophils was evaluated by the ELISA technique. We also investigated the palmitoleic acid effect in 6-8 weeks old streptozotocin (STZ)-induced diabetic male rats. A sterile inflammatory air pouch in the animal dorsal was treated with palmitoleic acid (100 μM) with the presence or absence of LPS (5 μg/ml), where we assessed neutrophil migration and exudate formation. Our findings reveal that palmitoleic acid is not toxic to neutrophils from humans. Palmitoleic acid demonstrated inhibition of LPS-stimulated rat diabetic neutrophil migration and an exudative inflammatory response. We concluded that palmitoleic acid is a proponent in diabetic wounds due to its anti-inflammatory effects, decreasing the neutrophil inflammatory response in people with diabetes. Financial Support: FAPESP (Process 2023/03741-3), CAPES and CNPq (Process 309644/2021-6). *The authors marked with an asterisk equally contributed to the work.

P-31-013
Analysis of the binding mode of S100A6 to RAGE using integrative structural approaches reveals the importance of cysteines for ligand: receptor complex formation

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S100 proteins are small calcium-binding proteins that are released extracellularly upon danger sensing and thereafter act as alarmins, triggering pro-inflammatory responses through receptor-dependent signaling, mostly via the RAGE receptor. Their overexpression and massive release have been implicated in the generation of pathological inflammation with deleterious outcome, notably during carcinogenesis, making the RAGE:S100 interactions valuable therapeutical targets. In particular, increased expression of S100A6 and enhanced signaling through RAGE are correlated with disease progression and poor patient prognosis in various cancers. The exact binding mode of S100A6 to RAGE remains however of debate, which precludes the identification of epitopes to be targeted for blockage of their interaction. Here, we used a combination of X-ray crystallography, *in vitro* binding assays, mass spectrometry and mutational analysis to further unravel the molecular architecture of the RAGE:S100A6 complex and identify the interfacial regions on both proteins. We show that in solution, S100A6 forms a 2:2 complex with the RAGE ectodomain, in a calcium-dependent manner. Furthermore, SEC binding assays reveal that S100A6 can bind to both the V and C1 domains of RAGE, while our previous structural data on the full-length complex suggested a major binding site for S100A6 on the RAGE C1 domain. Finally, we show that Cys3, present at the beginning of helix H1 of S100A6, is crucial for complex formation since its replacement by a serine completely abrogates the interaction with RAGE. Interestingly, complex formation is restored if a cysteine is reintroduced at position 84, in the second half of helix H4. These findings suggest that the novel S100A6 homodimeric conformation observed in the structure of the RAGE:S100A6 complex may be stabilized by two distinct mechanisms, one of which involves the formation of a disulfide-crosslinked S100 dimer.

P-31-014
Kinetic analysis of molecular interactions directly on living immune cells using real-time interaction cytometry

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Understanding the kinetics of molecular interactions is pivotal in immunobiochemistry as it illuminates the dynamic nature of immune responses, including antibody-antigen binding and receptor-ligand interactions. These interactions are not only essential for immune system function, but also for developing targeted immunotherapies like therapeutic antibodies. Notably, the majority of antibody targets, such as PD-(L)1, CD3, and HER2, are transmembrane proteins whose binding kinetics are influenced by factors like membrane density and mobility, transmembrane domain folding, and the presence of coreceptors. To obtain physiologically relevant kinetic data with high *in vivo* predictability, it is pivotal to characterize the molecular interactions of therapeutic antibodies within their native cellular environment. Here, we introduce Real-Time Interaction Cytometry (RT-IC), a technology facilitating the direct measurement of kinetics on living cells. RT-IC utilizes polymer-cage-functionalized chips, enabling label-free immobilization of individual cells within a microfluidic channel. Leveraging RT-IC, we investigated the real-time binding kinetics of anti-CD3 and anti-PD-1 antibodies with their targets directly on T cells. Our findings show reproducible measurements of association and dissociation rates, shedding light on the differential binding behavior of anti-CD3 antibodies to living *versus* fixed Jurkat cells. The interaction data reveals distinct dissociation rates, exhibiting a biphasic dissociation behavior for fixed cells and a monophasic behavior for living cells. We attribute this phenomenon to the preserved membrane fluidity in living cells, which likely facilitates bivalent interactions

through the clustering of receptor molecules. These results underscore the criticality of directly measuring binding kinetics on cell surfaces to anticipate the therapeutic efficacy of antibodies, thereby advancing antibody-based therapeutics.

P-31-015
Production of semi-synthetic glycoproteins for the development of novel and effective glycoconjugate vaccines for tuberculosis (Immuno-HUB project)

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Tuberculosis (TB) is still the leading cause of death by an infectious disease worldwide: it kills more than 1 million people every year. TB prevention and treatment are hampered by the low efficacy of the sole vaccine available at present, the old bacille Calmette-Guérin, and by the increasing presence of antibiotic resistant *Mycobacterium tuberculosis* strains. Thus, new, effective vaccines against TB are urgently needed. The use of glycoconjugate vaccines is a successful strategy employed to fight various pathologies. In order to combine the antigenic properties of the proteins with the antigenic and/or immunogenic characteristics of the linked oligosaccharides (double hit approach), our work is aimed at the design and production of a series of subunit vaccines produced from selected recombinant antigenic proteins of *M. tuberculosis* (TB10.4 and Ag85B) and from their fusion (chimeric) proteins, chemically glycosylated and engineered to remove unwanted glycosylation sites. Starting from previous results [published in: Rinaldi F. et al. (2018) RSC Adv 8, 23171-23180], novel chimeric TB10.4-Ag85B proteins have been designed by inserting appropriate flexible linkers. Under optimized expression conditions, up to 10 mg of pure soluble chimeric proteins per L of *E. coli* culture medium have been now obtained. Glycosylation of the new chimeric proteins by employing different oligosaccharides (mainly arabinomannans) is now carried out using a novel approach. This project represents an innovative strategy for developing TB vaccines and shows the potential for application to other pathologies (e.g., COVID-19). This work is part of the Immuno-HUB Project (Immunoterapia: cura e prevenzione di malattie infettive e tumorali, project number T4-CN-02) supported by the Italian Ministry of Health.

P-31-016
Sex- and age-associated proteasome dynamics in BTBR mice

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The proteasome is a multicatalytic complex composed of a 20S core whose activity can be regulated by capping with regulatory particles (11S and 19S). The immunoproteasome is a proteasome variant abundant in immune cells where it shapes the repertoires of antigenic peptides, regulates CD8+ T cell expansion, and promotes cytokine production and differentiation of Th1 cells. BTBR mice exhibit an autism-like phenotype, elevated oxidative stress, and an abnormal immune response with an activated T-cell system. Autism syndrome has been linked to immune dysregulation that is a hallmark of advancing age. Previous studies on aged BTBR mice have focused on changes in the composition of immune cell populations in the brain and peripheral blood, however little is known at the molecular level and in the lymphoid organs which are heavily affected by aging. We studied the assembly and activity of proteasome complexes in the lymph nodes of young and old BTBR mice. Differences between young and old animals, and old males and females were found. In general, old males showed decreased chymotrypsin-like activity while trypsin-like was upregulated and caspase-like downregulated both in males and females compared to young animals. By contrast old females showed increased immunoproteasome activity. Activity was equally distributed between 11S and 19S capped proteasomes in young mice, while it was mostly associated with 19S-capped proteolytic particles in old males due to the loss of 11S capping. Conversely, old females displayed more 11S-capped 20S and immunoproteasome activity associated with this complex than young animals. Interestingly, aging was accompanied by a tendency to preferentially assemble 19S capped proteasomes. Overall, these results highlight intriguing differences in proteasome assembly and activity between young and old males and females. Further experiments are planned to test whether and which of these changes are age-related or are peculiar traits of the autism-like phenotype.

P-31-017
Exploring discrepancies between autoantibody levels and neutralizing capacity in pulmonary alveolar proteinosis (PAP) of autoimmune origin

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Pulmonary alveolar proteinosis (PAP) is an uncommon condition characterized by the abnormal accumulation of alveolar surfactant. It arises from a range of disorders disrupting surfactant balance and is categorized into primary PAP (either autoimmune or hereditary), secondary PAP, and congenital PAP. Autoimmune PAP (aPAP), accounting for 90% of cases, stems from autoantibodies that block granulocyte macrophage colony-stimulating factor (GM-CSF), impairing alveolar macrophage function and leading to surfactant buildup. (1) The precise mechanism behind PAP development necessitates specific diagnostic assessments. When PAP is suspected, the anti-GM-CSF autoantibody enzyme-linked immunosorbent assay (ELISA) test serves as a valuable diagnostic tool for aPAP, as it detects the sole elevated biomarker in serum associated with this condition. However, serum autoantibody levels do not reliably indicate disease severity. (2) Thus, our current study explores the capacity of sera from aPAP patients to impede alveolar macrophage maturation via functional assays. These assays revealed that sera from aPAP patients significantly hindered TF-1 cell line proliferation

compared to sera from healthy individuals. (3) Nevertheless, the results did not definitively establish a direct correlation between neutralizing capacity and autoantibody levels. In this work we shed light to the reasons behind the differences between the two parameters by mapping the cytokine regions implicated in the autoantibodies inhibitory capacity (unpublished). Previously published in: (1) McCarthy C et al. (2022) Am J Respir Crit Care Med 205, 1016–1035; (2) Inoue Y et al. (2008) Am J Respir Crit Care Med 177, 752–762; (3) Uchida K et al. (2004) Blood 103, 1089–1098

P-31-018
Immune aquaporins and inflammation: implications in sepsis and targeted therapy

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The ability of immune cells to communicate and to shift shape for migration, phagocytosis or antigen uptake is supported by crucial membrane proteins, such as aquaporins (AQPs), that maintain water homeostasis and regulate volume changes. AQP3 and AQP9 are water channels that also facilitate glycerol, and hydrogen peroxide diffusion through membranes. Although highly expressed in macrophages and neutrophils, AQPs role in the inflammatory process is still unclear. Here, we activated the NLRP3 inflammasome in macrophages to investigate AQPs role in inflammation. AQP3 contribution to cell volume changes was assessed by measuring cell membrane water and glycerol permeability. Moreover, we took advantage of the selective AQP3 inhibitor Auphen to study the role of AQP3, the most expressed AQP in THP-1 monocytic cells, in the production of IL-6 and activation of NLRP3 inflammasome. AQP3 was silenced as a complementary approach. To study the influence of AQP3 in membrane changes during priming cells were stimulated with LPS, while for inflammasome activation cells were stimulated with nigericin, ATP or glycerol hyperosmotic challenge. AQP3 inhibition/silencing partially blocked LPS-priming and decreased production of IL-6, suggesting an involvement of AQP3 in macrophage priming via NF-κB. Moreover, AQP3-dependent cell reswelling increased IL-1β release through caspase-1 activation. Inflammasome activation was also blocked when AQP3 was inhibited/silenced. AQP3 involvement in priming and inflammasome activation points towards AQPs as potential players in the setting of the inflammatory response. These findings prompted us to develop an antibody against the most representative AQP in human primary monocytes and neutrophils, anti-AQP9, to reduce inflammation and with potential applications in clinics. Such therapy may represent a promising approach to target diseases where AQP9 has a pathophysiological role, such as malaria, and ovary cancer.

P-31-019
Evaluation of the immunomodulatory effects of the BanLec-Bet v 1 chimera and its mutants on antigen-presenting cells

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Improving allergen vaccines involves enhancing their safety and efficacy by the modulation of helpful immune responses, especially, antigen presentation to specific T cells by antigen-presenting cells (APC). Exploring novel adjuvants, like banana lectin (BL) known for its immunomodulatory potential, is essential to direct immune cells toward allergy management. BL potential mitogenicity was reduced by induction of a histidine replacing threonine at amino acid position 84 -BL_{H84T}. This study examined the modulation of APC using chimeras combining the major birch pollen allergen Bet v 1a and its hypoallergenic isoform Bet v 1l with BL and BL_{H84T} (Bet v 1a-BL, Bet v 1l-BL_{H84T}, and BL_{H84T}-Bet v 1l). After the production of chimeras in *E. coli* and purification, they were used to stimulate macrophages differentiated from THP-1-monocytes isolated from peripheral blood of an acute monocytic leukemia patient, and peripheral blood mononuclear cells derived from birch pollen-allergic patients. Their effects were compared to BL, BL_{H84T}, Bet v 1a, and Bet v 1l stimulated cells. Obtained results indicated that Bet v 1a-BL prompted the secretion of the anti-inflammatory cytokine IL-10 and augmented the IFN-γ/IL-4 ratio. Furthermore, antigen uptake was assessed using co-cultures of transgenic Jurkat T cell reporter cells that express a T cell receptor recognizing the immunodominant epitope Bet v $1_{142\text{-}153}$ in the context of HLA-DR7 ($Jurkat_{tg}$) and HLA-DR7$^+$ Epstein-Barr virus-transformed B cells (EBV). EBV incubated with proteins were co-cultured with $Jurkat_{tg}$ revealing strong activation of $Jurkat_{tg}$ by all chimeras. In summary, our data show that chimeras efficiently stimulate allergen-specific $Jurkat_{tg}$, and modify the cytokine milieu produced by bona fide APC making them suitable tools to steer T cell responses towards a tolerogenic/Th1 phenotype. Acknowledgment: Ministry of Science, Technological Development and Innovation of Republic of Serbia and EFIS-IL Short-term Fellowship 2023.

P-31-020
Elucidation of the structure of NKp30 receptor oligomers

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NKp30 is one of the main human natural killer (NK) cell activating receptors used in directed immunotherapy. The oligomerization of the NKp30 ligand binding domain depends on the length of the C-terminal stalk region, but our structural knowledge of NKp30 oligomerization and its role in signal transduction remains limited. Moreover, ligand binding of NKp30 is affected by the presence and type of N-glycosylation. In our study [1], we assessed that NKp30 oligomerization depends on its N-glycosylation. Our results show that NKp30 forms oligomers when expressed in HEK293S GnTI$^-$ cell lines with simple N-glycans. However, NKp30 was detected only as monomers after enzymatic deglycosylation. Furthermore, we characterized the interaction between NKp30 and its best-studied cognate ligand, B7-H6, with respect to glycosylation and oligomerization, and we solved the crystal structure of this complex with glycosylated NKp30, revealing a new glycosylation-induced mode of NKp30 dimerization. Another challenge is to reveal the real structure of oligomers on the NK cell membrane. We approach this by comparing the structure of the oligomeric ectodomain of NKp30 in solution, which we solve by transmission cryo-electron microscopy, with the results obtained by super-resolution microscopy techniques. Overall, this study provides new insights into the structural basis of NKp30 oligomerization and furthers our understanding of the molecular mechanisms involved in NK cell activation. This work was supported by the Czech Science Foundation (23-08490L, and 24-12553O). We acknowledge CMS-Biocev ("Biophysical techniques, Crystallization, Diffraction, Structural mass spectrometry") of CIISB, Instruct-CZ Centre, supported by MEYS CR (LM2018127) and CZ.02.1.01/0.0/0.0/18_046/0015974. This work used the Integrated Structural Biology platform of the Strasbourg Instruct-ERIC centre IGBMC-CBI supported by FRISBI (ANR-10-INBS-0005-001). Reference: [1] Skořepa et al. (2020), Cancers, 12(7):1998.

P-31-021
Human monocyte-derived dendritic cells can be used as carriers for the broad range of oncolytic viruses

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Oncolytic viruses currently emerge as a promising therapeutic approach for cancer treatment that provides selective elimination of cancer cells and stimulation of anti-tumor immune response. However, upon intravenous administration, innate and adaptive immunity limits the spread of oncolytic virus, decreasing the number of infectious viral particles that can reach the tumor site. One of the possible ways to solve this problem is to use cell carriers that can internalize virus, protecting it from neutralizing antibodies. Some types of cell carriers also support viral replication, thereby increasing the amount of virus delivered to the tumor. Different cell types were investigated as potential virus carriers: neural and mesenchymal stem cells, transformed cell lines and various subsets of immune cells, including monocyte-derived dendritic cells (mo-DC). Despite promising proof-of-concept studies featuring mo-DC as carriers, these works were focused on several specific virus types. Therefore, it is hard to evaluate the versatility of this approach for various oncolytic viruses. In this work, we performed a large-scale investigation of mo-DC in the role of potential carriers for 10 oncolytic viruses from different families (mammalian orthoreovirus type 1,2,3, Sendai virus, Newcastle disease virus, echovirus type 1,7,12, enterovirus B75, poliovirus type 1). Infection with different doses and further replication kinetics analysis revealed that mo-DC supported replication of all tested viruses and produced remarkable virus yields. Notably, replication efficiency varied for different viruses and serotypes. In addition, we assessed the effects of viral infection on mo-DC viability and induction of maturation markers using flow cytometry. Overall, these findings highlight mo-DC as potential carriers for different types of oncolytic viruses. This work was supported by the Ministry of Science and Higher Education of the Russian Federation (agreement #075-15-2019-1660).

P-31-022
STAT1 levels determine response to chemotherapy and immune-killing in lung cancer via paracrine mechanisms

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Up to 60% of non-small cell lung cancer (NSCLC) patients that initially respond to chemotherapy, or immunotherapy develop resistance. The JAK/STAT pathway modulates the response to chemotherapy and immune-killing1,2. Among STAT members, STAT1, although highly expressed in NSCLC, is poorly investigated. The aim of this study is to investigate the molecular mechanisms that link STAT1 with sensitivity/resistance to chemotherapy and immune-killing in NSCLC. After screening on 6 human NSCLC cell lines, we focused on the top STAT1 and

PD-L1-expressing cells (Calu-3 and NCI-H2228) for chemosensitivity and immune-killing assays. Protein arrays to detetect cytokines and proteomic analysis of extracellular vesicles (EVs) of wild-type (WT) and STAT1-silenced (sh) cells were performed. STAT1 silencing increased the sensitivity to cisplatin and pemetrexed, the proliferation of T-lymphocytes co-cultured and the immune-killing of tumor cells by T-cells. Although JAK/STAT axis controls the expression of PD-L1 in NSCLC, we did not detect significant variation of its expression between WT and shSTAT1 cells. Among the differentially released cytokines controlled by JAK/STAT axis, CXCL1, CXCL8, CCL2, Angiogenin and TIMP-2 were downregulated in shSTAT1 cells. Since cytokines are often carried by EVs, we performed a proteomic analysis of EVs from WT and shSTAT1 NCI-H2228 cells. CXCL1 and STAT1 were identified in EVs isolated from WT, but not from silenced cells. We are investigating if there is a horizontal transfer of STAT1/CXCL1 and how these factors mediate resistance to chemotherapy and immune-killing. STAT1 mediates resistance to chemotherapy and immune-killing in NSCLC by changing the cytokines released. CXCL1 and STAT1 itself could be horizontally transferred by EVs. Targeting the paracrine CXCL1/STAT1 circuitry may represent a new chemo-immuno-sensitizing strategy. References: 1. Tuli H et al, doi: 10.1007/s11033-022-07399-w 2. Mouritzen MT, doi:10.3390/cancers15184480.

P-31-023
Different H1sD2 glycoforms influence the cytokine gene expression in THP-1 differentiated macrophages

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[Correction added on 28 August 2024, after first online publication: The affiliation 'South Denmark University' was changed to 'University of Southern Denmark'.]

Post-translational protein modifications play a crucial role in various aspects of human biology, including the immune system. In particular, N-glycans interact with pattern recognition receptors to exert a substantial influence on the innate immune response. Allergen-specific immunotherapy (AIT) is an effective therapeutic approach, but it has certain drawbacks that necessitate the development of creative ways to increase its effectiveness. Using adjuvants to activate the innate immune response more successfully is one such strategy. The main influenza virus antigen, hemagglutinin, is a surface glycoprotein that has gained interest as a possible adjuvant candidate for AIT. By using hemagglutinin, we evaluate how N-glycans impact THP-1 macrophage activation. This study focused on the production and characterization of *in silico* designed H1sD2 glycoforms, which combine the receptor-binding domain of hemagglutinin (H1s) with a major allergen derived from house dust mite denoted as Der p 2 (D2). To produce these glycoforms, the *Pichia pastoris* expression system was utilized. The H1sD2 glycoforms were purified and characterized by mass spectrometry. Immunomodulatory potential was investigated by stimulation of THP-1 derived macrophages and measurement of the level of gene expression for different cytokines (IL-4, IL-10 and IFN-γ). Interactions of H1sD2 with the corresponding receptors expressed on M0 macrophages (THP-1 monocytes primed with PMA) and M2 macrophages (THP-1 monocytes primed with PMA and differentiated with IL-4) were analyzed by flow cytometry. The results of this investigation show that the glycan decoration of H1sD2 glycoproteins influences both the binding to corresponding mannose-binding receptors and the expression profile of cytokine genes. Supported by the Ministry of Science, Technological Development and Innovation of Republic of Serbia; Contract number: 451-03-65/2024-03/200168 and FEBS Collaborative Developmental Scholarship 2022.

P-31-024
The endocannabinoid anandamide activates pro-resolving pathways in human primary macrophages by engaging both CB2 and GPR18 receptors

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Resolution of inflammation is the cellular and molecular process prevents widespread and uncontrolled inflammation, while restoring tissue function in the aftermath of acute immune events. This process is orchestrated by specialized pro-resolving mediators (SPM), a class of bioactive lipids that reduce immune activation and promote removal of tissue debris and apoptotic cells by macrophages. Although SPMs are the lipids that have been best studied for their role in facilitating the resolution of self-limited inflammation, a number of other lipid signals, including endocannabinoids, also exert protective immunomodulatory effects on immune cells, including macrophages. These observations suggest that endocannabinoids may also display pro-resolving actions. Interestingly, the endocannabinoid anandamide (AEA) is not only known to bind canonical type 1 and type 2 cannabinoid receptors (CB1 and CB2), but also to engage SPM-binding receptors such as GPR18. This suggests that AEA may also contribute to the governing of resolution processes. In order to interrogate this hypothesis, we investigated the ability of AEA to induce pro-resolving responses by classically-activated primary human monocyte-derived macrophages (MoDM). We found that AEA, at nanomolar concentration, enhances efferocytosis in MoDMs in a CB2- and GPR18-dependent manner. Using lipid mediator profiling we also observed that AEA also modulates SPM profiles in these cells, including levels of Resolvin (Rv) D1, RvD6, Maresin (MaR)2, and RvE1 in a CB2-dependent manner. AEA also modulated expression of SPM enzymes involved in the formation and further metabolism of SPM such as 5-lipoxygenase and 15-Prostaglandin dehydrogenase. Our findings show, for the first time, a direct effect of AEA on pro-resolving pathways in human macrophages and provide new insights into the interaction between different lipid systems controlling pro-resolving responses that reestablish homeostasis in the aftermath of acute inflammation. *The authors marked with an asterisk equally contributed to the work.

P-31-025
Investigation of prolactin-induced protein interaction with galecin-3 in seminal plasma

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Human seminal plasma provides a unique milieu for maturation, protection, and function of spermatozoa, which contains an array of biologically and immunologically active compounds, including endogenous lectins and their glycoprotein ligands. Seminal plasma prolactin-inducible protein (PIP), can be complexed with several proteins, including actin, serum albumin, zinc-α2-glycoprotein and immunoglobulin G, can also bind to CD4 receptors present on the surface of macrophages, T lymphocytes and spermatozoa, thus might perform an immunomodulatory function by initiating the adaptive immune response. PIP has been implicated as a ligand for galectin-3 (Gal-3) in prostasomes, although little is known about this interaction in seminal plasma. The aim of the study was to examine the integration between PIP and galectin-3 in the seminal plasma of men with reduced semen parameters, through determination the occurrence of LacNAc (*N*-acetyllactosamine, Galβ1-4GlcNAc) and LacdiNAc (*N*, *N*-diacetyllactosamine, GalNAcβ1-4GlcNAc) residues present in the oligosaccharide chain of the PIP, as ligands recognized by galectin-3. Lectin ELISA assay with using RCAI, DSL, WFL lectins, which recognise Gal/GalNAc residues were used to analyse galactosylation profile of PIP, additionally affinity chromatography was applied to verify whether PIP could be a galectin-3 binding protein in seminal plasma. Finally, statistical analysis showed decreased expression of Gal/GalNAc residues in the subfertility groups compared to the control group. Moreover, the correlation between the occurrence of the determined LacNAc and LacdiNAc glycoepitopes and the reactivity of the PIP protein with galectin-3 was determined. Our study provides new data on the galactosylation profile of the prolactin-inducible protein and highlights the importance of PIP interaction with galectin-3 in the context of male infertility. This study was supported by Wroclaw Medical University, grant no. SUBK.A411.23.066.

P-31-026
Mannosylation of antigenic proteins as a strategy to increase their immunogenic properties

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Many microorganisms capable of activating the acquired immune system during pathological conditions and triggering an immune response against specific antigens display mannose-containing glycans on their surfaces. In the last years, mannose-based glycoconjugates have been explored to enhance antigen uptake mediated by mannose receptor (MR), a transmembrane glycoprotein highly expressed on antigen-presenting cells. Thus, protein mannosylation emerges as a viable strategy to improve their antigenic properties and facilitate their active targeting to the immune system. Previous research has identified the disaccharide manα1,2 man as a crucial epitope for sugar–MR interaction and established a correlation between α(1,6) mannans chain length and affinity binding towards the receptor. Consequently, we developed mannose-based oligosaccharides incorporating the aforementioned sugar frameworks and carrying thiocyanomethyl and 3-aminopropyl groups at the anomeric position. Thiocyanomethyl sugars, upon conversion into iminomethoxyethyl derivatives, have been shown to be reactive towards the ε-amino groups of lysine residues. Conversely, sugars endowed with an anomeric amino group, following their intermediate transformation into active esters through the use of a disuccinimidyl homobifunctional linker, were employed to form amide bonds with the lysine residues of a given protein. Ribonuclease A (RNase A) and human serum albumin (HSA) served as model proteins for developing the conjugation protocol. Then, with the final goal of developing a vaccine against tuberculosis, we applied these strategies to glycosylate two recombinant *Mycobacterium tuberculosis* antigens, native Ag85B and its variant Ag85B-dm. The resulting mannosylated proteins are expected to exhibit enhanced immunogenic properties compared to their non-glycosylated counterparts. Following initial evaluation via surface plasmon resonance (SPR) analysis, the most promising candidates will proceed to *in vivo* assessments.

P-31-027
Human RNase 7 and neurons: moving towards an unexpected new functional link

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Human ribonuclease 7 (RNase 7) is a member of the RNase A superfamily (1) with remarkable antimicrobial properties on a wide spectrum of pathogens (2). RNase 7 expression, mainly at the skin level, can be induced by proinflammatory cytokines and bacterial challenge, clearly confirming its contribution to the innate antimicrobial defence in the areas where it is released. However, all these properties, linked to current studies on RNase 7 mostly dedicated to the innate immunity of the lining epithelia, do not yet allow us to better clarify the real properties of this molecule in the human body. This is the inspiration that animated the present study which is focused on identifying a functional connection between immunomodulatory properties of RNase 7 and human tissues not yet explored. In this regard we have chosen as the object of study the nervous system, a very delicate network in which the finding of natural molecules potentially able in combating infections that may affect this system, could be extremely useful. Starting from this premise, here we show the first functional link among RNase 7 and brain by using neuroblastoma cells (SHSY5Y) thanks to which we were able to detect both the expression and localization of endogenous protein but also that they are positively responsive to recombinant form of RNase 7 at different growth conditions. Our most interesting data indicate that recombinant RNase 7 exerts powerful immunomodulatory effects on SHSY5Y cells stimulated with LPS isolated from different bacterial strains. These pioneering data open an appealing scenario in which a new investigative front emerges for RNase 7 and its potential therapeutic role in the defence of the nervous system against microbial infections. References: 1. Sun D, et al. (2022) iScience25,105284. 2. Rademacher F, et al. (2019) Front Immunol., 10:2553.

P-31-028
Verification of the transport function of Langerhans cells during TBEV infection

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Langerhans cells (LC) are a type of immune cell that potentially play a role in the transmission of *Orthoflavivirus encephalitidi*s, formerly known as tick-borne encephalitis virus (TBEV), throughout the body. TBEV is a tick-borne virus that, after entering the human body via a tick bite, spreads through the body to the brain, where meningitis can develop. Once the TBEV virus enters the human body, it targets immune cells present in the skin, mainly dendritic cells, and macrophages. Langerhans cells (LCs) are a subset of dendritic cells located in the skin, known for their role in antigen presentation and for transmitting certain viruses to local lymph nodes. We chose to investigate the transport function of the LC in the dissemination of TBEV in the body since it has not yet been thoroughly described. Firstly, the LCs were derived from mouse (C57Bl/6N) bone marrow using specific growth factors (GM-CSF, IL-4, and TGF-β1). After derivation for 7 days, cells were sorted to obtain a population of Langerin+ and MHC-II+ cells. LCs were then infected with TBEV. After one day of incubation, LCs were subcutaneously injected into the mice. Mice were dissected at time intervals of 1-7 days post-infection and serum, local lymph nodes, spleens, and brains were obtained and analysed by qRT-PCR and plaque assay for virus presence. We conclude that LCs transport the TBEV into the lymph nodes and thus participate in the spread of infection in the body. *The authors marked with an asterisk equally contributed to the work.

P-31-029
Immunological effects of venoms from endemic species of Cyprus

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The complement system plays an important role during an immune response, including the activation of inflammatory processes as a consequence of the generation of proteolytic fragments of its components. Numerous studies have highlighted the impact of venoms on processes such as hemolysis, inflammation, and also the activation of the complement system. Understanding these mechanisms could significantly contribute to developing strategies to address the effects of envenomation. Futhermore, venoms of endemic species offer a valuable but underutilized source of bioactive compounds with potential applications across various fields. We sought to study the effects of venoms from Cypriot endemic species, the Cypriot blunt-nosed viper, *Macrovibera l. lebetina* and the scorpion *Mesobuthus cyprius* on the classical (CP), lectin (LP) and alternative (AP) pathways. Analysis revealed that varying concentrations of crude venoms promoted significant degradation of components of the complement pathways, generating anaphylatoxins. Our results therefore indicate the activation of the complement system by direct proteolytic action of the venoms on several components. Ongoing studies aim to characterize the specific venom peptides and proteins that are responsible for these pronounced effects on the complement system.

P-31-030
Inactivation of complement cascade by *Streptococcus anginosus* proteases

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Streptococcus anginosus is a Gram-positive bacterium, classified within the Streptococcus anginosus group. Initially considered commensal bacteria in the colon, oral cavity, and vagina. *S. anginosus* is now recognized as an opportunistic pathogen that can lead to brain or liver abscesses. Despite an increasing number of clinical reports, the molecular mechanisms underlying *S. anginosus* pathogenesis remain poorly understood. One of the virulence strategies employed by *S. anginosus* involves the inactivation of components of the innate immune system. Our research has revealed the bacterium's efficient attenuation of the antibacterial activity of humoral factors. We identified its capability to deactivate the complement system while remaining insensitive to its antibacterial effects. The molecular mechanism responsible for this phenomenon relies on the proteolytic activity of the examined bacteria. Our studies have established that a key player in this process is an enzyme exhibiting metalloprotease activity. Genomic analysis of clinical strains of S. *anginosus*, isolated from acute infections, confirmed the presence of sequences encoding a zinc metalloprotease with a secretion domain. Importantly, these genes were absent in strains that did not exhibit complement inactivation. By introducing mutations into the complement-inactivating strain, we demonstrated the primary role of this protease. Detailed analysis revealed that C1q, C2, C4,and C5 are the molecular targets of this protease. This study represents the first report demonstrating that *S. anginosus* deactivates the complement system through the expression of proteases. The discovery of the complement inactivation mechanism by *S. anginosus* can have dual effects. Firstly, it serves to protect the microorganism itself. Secondly, it may suggest that *S. anginosus* supports the survival of complement-sensitive pathogens, shedding light on its involvement in mixed infections. Supported by NCN, Poland 2018/29/B/NZ6/00624.

P-31-031
Evaluation of immunoassay interferences of thyroid function tests

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Immunoassay (IA) platforms are the preferred methods in clinical laboratories for the measurement of thyroid function tests (TFT). However, they are vulnerable to different types of interference. Potential interferences in the TFT should always be suspected when clinical and biochemical consistencies arise. In this study, the results of interference studies performed in patients with suspected interference in TFT were evaluated. It was aimed to raise awareness about how the interference study should be perform and in which cases there may be interference. Patients who underwent interference research for TFT (Thyroid stimulation hormone, free T4, free T3) in the Medical Biochemistry Laboratory of Gazi University Faculty of Medicine between June 2017 and January 2021 were evaluated retrospectively. The interference studies were evaluated in 77 patients, who were consulted with suspicion of interference from different departments, mainly from the Department of Endocrinology and Metabolic Diseases of Gazi University, and whose results were suspected by the biochemistry doctors' validations. For interference research were used: analysis with IA method using different antibodies (ECLIA) or not using biotin (CLIA), analysis after precipitation with PEG, analysis by serial dilution, analysis after removal of excess biotin using streptavidin beads, analysis after treatment with heterophile antibody removal methods, analysis after scavenger ALP addition, analysis after incubation with the serum of patients with true TSH elevation. When the data were evaluated, interference was observed in 10 of 77 patients. Free T4 interference was detected in 7 of 10 patients, free T3 and free T4 in 1, and free T3 interference in 2 patients. Biotin interference was observed in 5 patients. It was emphasized how important the communication between clinicians and clinical biochemists. There is a need to carry out studies using broader and advanced analysis methods, for increased awareness.

P-31-032
Role of the domain II of human serum albumin in bacteria toxins recognition: from biochemical studies to biotechnological implications

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Serum proteins are emerging as key players in the host-pathogen interactions, thus influencing the onset and the severity of infections. Human serum albumin (HSA) consists of three homologous domains and represents the major plasma transporter displaying extraordinary ligand-binding properties. HSA is also excreted at mucosal surfaces and enters tissues when inflammation increases vascular permeability. Our research group contributed to define a novel role of HSA as a key player in the recognition and inactivation of different pathogen-associated molecular patterns. We identified the domain II (DII) of HSA as a potential toxin-binding site able to recognize and neutralize *Clostridioides difficile*[1], *Streptococcus pyogenes*[2], and *Staphylococcus aureus* toxins. Here, we aim to demonstrate the role of the only DII in bacteria toxins neutralization. We set up an efficient protocol for DII expression and purification in BL21 *E.coli* cells, producing the DII fused to an MBP-tag that allowed its purification by affinity chromatography. The capability of the recombinant DII to bind bacteria toxins was evaluated by ELISA and *in vitro* experiments using human cells. Notably, the calculated binding affinity between DII and each toxin closely mirrors that observed for the full-length HSA. These results demonstrated that the DII is implicated in bacteria toxin recognition and clearly highlights a role of HSA in the innate immunity. Furthermore, we report the development of an HSA-based biosensor using ultra-high doped Germanium-based plasmonic antennas arrays on standard silicon/silicon-on-insulator substrates for the label-free detection of little amounts of biomolecules by THz-Time domain spectroscopy[3]. Ultimately, our goal is the development of a low-cost lab-on-chip biosensors for the early detection of toxins in liquid biopsies. References: [1] di Masi et al., 2018; J Infect Dis (9):1424–35. [2] Vita et al., 2020; Front Immunol.;11: 507092. [3] Hardt et al., 2021; Opt. Express 29(5) 7680-7689.

P-31-033
Engineering a dual-action TB vaccine: MTB fusion protein after glycosylation with LAM mimetic oligosaccharides

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Tuberculosis (TB) is a transmissible disease caused by *Mycobacterium tuberculosis* and it is the second leading cause of death from a single infectious agent. It is estimated that approximately 10 million people develop TB each year; in 2022 about 1.4 million people died from TB worldwide. TB is a serious public health concern and new effective vaccines are needed. In our previous work, oligosaccharides derived from arabinomannan (AM) have been created and conjugated with recombinant Human Serum Albumin (rHSA) [Previously published in: Z. Li et al. (2020) Eur J Med Chem 204:112578]. rHSA was glycosylated with AraMan-IME and Ara3Man-IME. Bio-panning experiments revealed that patients with active tuberculosis exhibited a higher antibody response to Ara3Man, a sugar found in lipoarabinomannan (LAM), which is a major component of the mycobacterial cell wall. In this way, through ELISA assays and bio-panning experiments, we have been able to demonstrate the importance of the Ara3Man as an immunodominant epitope in LAM and support its role in eliciting protective immunity against tuberculosis [Previously published in: Bernardini et al. (2024) Biol

Direct 19:11]. These works aim to develop, produce, and evaluate a series of subunit vaccines from chimeric fusion proteins from *Mycobacterium tuberculosis* (rTB10.4 and rAg85B). This work is part of the Immuno-HUB Project (Immunoterapia: cura e prevenzione di malattie infettive e tumorali, project number T4-CN-02) supported by the Italian Ministry of Health.

Molecular Basis of Diseases – Part A

P-32-001
Lack of trimeric intracellular cation channel B (TRIC-B) in osteoblasts impairs cell junctions and β-catenin cellular localization

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Osteogenesis imperfecta (OI) is a family of rare inherited connective tissue diseases sharing bone deformities and fragility. OI type XIV is caused by mutations in the *TMEM38B* gene, encoding the trimeric intracellular cation channel B (TRIC-B), a K^+ channel in the rough endoplasmic reticulum membrane necessary to regulate intracellular Ca^{2+} flux. This form of OI is characterized by defects in osteoblast (OB) differentiation and activity, but little is known about the molecular basis of the phenotype. Cell-cell and cell-matrix junctions play essential roles in regulating OB activity and bone homeostasis. So, primary OBs from a *Tmem38b* osteoblast specific knock-out mouse (*Runx2Cre; Tmem38b*$^{fl/fl}$, cKO) were used to dissect the effect of lack of TRIC-B on cell junctions. Interference reflection microscopy revealed an increased OB adhesion sites area and length and an increased number of adhesion sites' clusters. An accumulation of actin at cell junction sites was found by total internal reflection fluorescence (TIRF) revealing an abnormal cytoskeleton. Also, the actin binding protein vinculin was found reduced and differentially organized in cKO OBs compared to controls. Analyses of cell-cell cadherins showed a reduced expression of both N- and E-cadherin at mRNA and protein levels. TIRF analysis revealed no differences in the expression level of phospho-focal adhesion kinase (pFAK), pivotal in cell-matrix contacts, but an increased number of shorter pFAK clusters in cKO OBs. Immunofluorescence analysis of β-catenin, that is physiologically bound to cadherins, highlighted an abnormal accumulation at cell junctions and a reduced nuclear translocation, the latter confirmed in TRIC-B KO human fetal OBs. Taken together, our data deepen the relationship between Ca^{2+} homeostasis, cell junctions and osteoblastogensis paving the way for a better understanding of OI type XIV pathophysiology.

P-32-002
The role of SMTNL1 in the physiological and pathological functions of the female reproductive system

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The incidence of infertility is higher in women displaying diseases linked to impaired glucose homeostasis. The defect of glucose metabolism interferes with the process of fertilization, however, the molecular mechanism of the underlying causes is yet to be uncovered. Smoothelin-like protein-1 (SMTNL1) was isolated from muscle and steroid hormone-responsive tissues. SMTNL1 regulates contractile functions of various cells by the inhibition of myosin phosphatase (MP) holoenzyme. In addition, upon phosphorylation at Ser301 by protein kinase A, SMTNL1 translocates to the nucleus, where it functions as a transcriptional co-activator of the progesterone receptor-B. SMTNL1 null mice exhibited reduced reproductive fitness, and were more prone to manifest type 2 diabetes mellitus. *In vitro* human endometrial model was established to investigate the effect of SMTNL1 in pregnant (medoxyprogesterone treatment) and in gestational diabetes (GDB) models, applying hyperglycaemic-hyperinsulinaemic conditions. In decidualised human endometrial stromal cells (HESC) SMTNL1 overexpression aided differentiation, analyzing several differentiation markers' mRNA expression (IGFBP-1, HAND2 and PRL) by RT-PCR. SMTNL1 had not modified the expression of the elements of insulin signaling pathway, such as IRS-1, Akt and ERK1/2 but their phosphorylation and activity were altered through the expressional regulation of the novel type protein kinase C ε (nPKCε). Moreover, SMTNL1 mimicking peptide was introduced to HESC culture. Secreted prolactin levels were examined by ELISA, and significant elevation of prolactin secretion upon peptide application was registered. Inflammatory cytokine (IL-6) mRNA levels were reduced upon SMTNL1 application, examined by RT-PCR. Our data collectively suggests the insulin-sensitizing effect of SMTNL1, as well as a potential target to improve fertility disorders through the regulation of decidualization and prolactin secretion.

P-32-003
FOXM1 sustains DNA repair processes of epidermal stem cells

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Epithelial stem cells (ESCs) are endowed with impressive regenerative capacities that sustain epithelia regeneration during homeostasis and wound healing *in vivo*. They progressively give rise to transient amplifying cells (TAC) that proceed the onset of terminal differentiation. Bulk and single-cell transcriptional profiling allowed to highlight that ESC display stem cell features and to identify FOXM1 as a key transcription factor required for stem cell maintenance in human keratinocytes [Previously published in: Enzo et al. (2021), Nat Commun 12, 2505]. ESCs are enriched in genes of the DNA repair pathway, as it is crucial to prevent the propagation of genotoxic mutations in derived progenitor cells. Together with that, it is known that FOXM1 has an important role in DNA repair processes in pluripotent stem cells and many cancer types [Previously published in: Chen et al. (2021),

Genes, 12, 1548]. Therefore, we studied if ESC and TAC display different DNA repair dynamics after irradiation and if FOXM1 is involved in this process. At basal levels, proteins involved in the DNA damage response, specifically in the homologous recombination, are more expressed in ESC as compared to TAC. In addition, we observed that ESC-derived cells expressed apoptotic markers where DNA lesions could not be repaired, suggesting the activation of a specific death program. Of note, FOXM1 enforced expression could restore the ability to undergo a regulated cell death upon irradiation. This study uncovers a key biochemical mechanism regulating stem cells in healthy keratinocytes, probably needed to guarantee genomic fidelity during ESC proliferation and differentiation. A better understanding of the network acting to repair the DNA is instrumental to improve error-free based gene editing techniques. Moreover, since the skin is constantly subjected to genotoxic insults, this knowledge will add insights on skin cancer progression, to develop early diagnostic tools or personalized therapies. *The authors marked with an asterisk equally contributed to the work.

P-32-004
A molecular rationale for the variable penetrance of CASQ2-related cardiac arrhythmias

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Catecholaminergic polymorphic ventricular tachycardia type 2 (CPVT2) is a hereditary heart disease linked to the sudden death of very young individuals. This syndrome predisposes to fatal cardiac arrhythmias and is associated with mutations in the cardiac Calsequestrin protein (CASQ2), essential for the coordinated contraction of the heart. Unfortunately, available treatments are not specific to the pathological mechanism, and one in three individuals remains susceptible to sudden arrhythmias. The uncertainty about the risk to which these individuals and their families are exposed makes it urgent to identify and correct the specific CASQ2 defect. CASQ2 is a 45 kDa, low-affinity Ca^{2+}-handling protein able to reach an enormous Ca^{2+}-binding capacity (up to 60 ions per monomer) upon Ca^{2+}-dependent polymerization. Our characterization of the kinetics of polymerization of the wild-type and mutant CASQ2 proteins for the first time defines two groups of mutants, which in turn underlie the recessive and the dominant forms of the CPVT2 syndrome. Unexpectedly, CASQ2 Ca^{2+}-specific dimer is in a conformational equilibrium with a Ca^{2+}-independent dimer, where the latter competes with Ca^{2+}-specific polymerization. In the context of pathological dominant CASQ2 mutants, the equilibrium between these two dimeric forms is disrupted, and abnormal polymers form. The dominant negative effect is exerted through sequestering of the wild-type protein into complexes with non-physiological Ca^{2+}-binding properties. Our results provide for the first time a rationale for stratification of CPVT2 patients. Leveraging on our validated gene therapy for a recessive form of CPVT2, we will now be able to propose the most efficacious therapies for the most appropriate group of CPVT2 patients. In addition, our molecular insights will trigger the development of a complementary gene therapy strategy for the dominant form of the disease.

P-32-005
New pathogenetic pathways of Dupuytren's contracture and alternative treatment options

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Dupuytren's contracture (DC) is a nonreversible thickening of the palmar aponeurosis of the hand worsening the life quality. The precise etiology of the pathology remains unclear, and the main (single) way to solve the problem is surgery. Researchers tried to understand the etiology and pathogenesis of the disease and to find a more appropriate remedy for the disease. Present study aimed to find the biochemical bases for pathology development. To this end, the enzymatic activities of dipeptidyl peptidases 2 (DPP2) and 4 (DPP4), as well as of adenosine deaminase (ADA) were compared in the tendon systems of healthy individuals (n = 14), those with Carpal tunnel syndrome (CTS) (compression of the median nerve by the carpal ligament, n = 11) and in DC disease people (n = 33). The significant differences in activities of the named enzymes in the studied groups were registered. Notably, DC disease samples exhibited significant activities of DPP4 and DPP2 enzymes which are absent in healthy and CTS samples. Then, the mean of ADA activity in DC disease samples markedly surpassed that in healthy cohorts by 11.22 times (0.5486 ± 0.057 μmol/min per μg protein and 0.0489 ± 0.00508 μmol/min per μg protein in the DC and healthy groups, respectively ($p < 0.0001$)). The mean of ADA activity in the DC group was higher by 1.17 times than in the CTS patients (0.4695 ± 0.0328 μmol/min per μg protein). Besides, an interesting phenomenon has been registered connected with the ADA activity: a wave-like dependence of ADA activity from the patient age was observed. These findings contribute to the understanding of possible important role aforementioned enzymes in DC etiology. The studies are being continued for elucidation the cause of observed phenomena. The newfound insights might pave the way for the development of innovative non-surgical treatment modalities.

P-32-006
Structural dynamics drives catalytic efficiency of AADC: basis for genotype-phenotype correlation in AADC deficiency

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Aromatic amino acid decarboxylase (AADC) is the enzyme that synthesizes the neurotransmitters dopamine and serotonin. Mutations in its gene lead to AADC deficiency, whose genotype-phenotype relationship is unclear. We used a combined approach of crystallography, MD, SAXS, limited proteolysis, and pH/kinetic studies to understand the structural determinants that govern kcat and Km in AADC. AADC is an obligate dimer with the two active sites located at the interface. Crystallographic data and MD show that the active sites are connected to each other

by a network of interactions and a previously unknown water tunnel. When the ligand is bound at one active site a highly flexible catalytic loop (CL) closes upon it, stabilized by several intra-CL interactions and with surrounding regions. Interestingly, the integrity of these distinct protein regions is crucial for acquiring the catalytically active conformation. Moreover, AADC flexible regions are generally evolutionary variable, while the dimer interface constitutes a rigid and conserved core. The biochemical characterization of more than 50 AADC pathogenic variants provided a powerful tool for mapping the alteration of the kinetic parameters on the protein structure. We found that the variants affecting protein flexibility led to a drop in kcat due to impairment in CL mobility while the variants mapping at the interface led to an increase in Km. Finally, since AADC activity presents a strong pH dependence (regarding both kcat and Km), we obtained isomorphic crystals at different pH values whose solved structures showed that CL flexibility and dimer interface are coupled and pH sensitive. Altogether this approach represents a step forward in predicting the effect of missense pathogenic mutations, may provide support to the best therapeutic choice, and could constitute an additional step in defining the genotype-phenotype correlates in AADC deficiency.

P-32-007

Abstract withdrawn.

P-32-008

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P-32-009
microRNA-mediated control of metastatic behaviour in colorectal cancer

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Colorectal cancer (CRC) is one of the most prevalent cancer types globally. Despite significant advancements in diagnosing and treating the disease, CRC remains a substantial health concern. There's a pressing need for a deeper comprehension of the molecular processes underlying CRC development, progression, stress responses, and resistance to drugs. During the quest for new CRC markers, we identified an upregulated microRNA in CRC tumors compared to non-tumoral tissues in a significant cohort of cancer patients. Investigating this microRNA's role, we examined its impact on CRC tumor development, progression, cellular stress, and responses to cell death using K-RAS mutant CRC cellular models. We scrutinized its involvement in cancer-related cellular functions like growth, migration, invasion of the extracellular matrix, autophagy, and responses to chemotherapy-induced cell death. Furthermore, we analyzed the genes targeted by this microRNA that contributed to the observed characteristics to enlighten the underlying molecular mechanisms. Among these, a metalloprotease emerged as a transcriptionally deregulated target of the microRNA. Given the metalloprotease's enzymatic activities on Wnt ligands, it also holds the potential to play crucial roles in CRC metastasis. Lastly, the transcriptional regulation of the microRNA itself focuses on the same Wnt axis completing the metastasis-associated signaling loop.

P-32-010
Platelet C3G has a dual role in the development of hepatocarcinoma through the regulation of the immune system

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The hepatocarcinoma (HCC) is usually developed in a chronic liver disease context characterized by inflammation, being frequently associated with fibrosis. Platelets regulate both fibrosis and HCC development and progression having opposite effects that might depend on the context. Their role in the control of the liver immune response is remarkable. C3G, a guanine nucleotide exchange factor for Rap1, plays distinct roles depending on the stage and/or type of tumour. In HCC, it promotes tumour growth, while decreases migration and invasion. Previous results from our group have also demonstrated that C3G plays a key role in platelet activation and secretion, regulating both angiogenesis and tumour metastasis. In this work, to determine the function of platelet C3G in the development of HCC, we have used a model of HCC associated with fibrosis induced by DEN + CCl4 treatment in megakaryocyte-platelet C3G knockout and wt mice. We found that platelet C3G promotes hepatocarcinogenesis, while protects from fibrosis and modulates the expression of chemokines like CXCL7 or CCL2. In addition, C3G regulates the secretion of factors, such as CD40L, which is involved in the recruitment of immune cells to the liver in this context. In agreement with this, platelet C3G regulates monocyte and neutrophil infiltration in HCC. Both proteomic analysis of platelet-rich plasma and transcriptomic analysis of livers showed differences in immune system response, as well as in processes related to angiogenesis, blood coagulation or metabolic and stemness pathways. Therefore, our results indicate that platelet C3G has a complex and relevant role in HCC, promoting HCC development but protecting from liver fibrosis. This role might be mediated by the regulation of the immune liver response likely through platelet activation and secretion of components from their granules.

P-32-011
The role of ELK3 transcription factor in triple negative breast cancer cell migration in 3D confined spaces

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Metastasis remains the primary cause of death in breast cancer (BC), lacking targeted therapies. Unraveling the molecular mechanisms driving this process is crucial for developing treatments aimed at metastatic tumor cells. To this end, the migration capacity of seven phenotypically diverse BC cell lines was evaluated in 3D microfluidic devices and their migration capacity was associated with their molecular signature. Among the 715 genes whose expression was correlated with the migratory phenotype (rho > ±0.7; $p < 0.05$), the transcription factor ELK3 emerged as a potential key regulator of BC cell migration in confined environments. Therefore, this research aims to explore ELK3 role in triple negative breast cancer (TNBC) metastasis by investigating its impact on three interconnected processes: cell migration in confined spaces, the epithelial-to-mesenchymal transition (EMT) and the acquisition of the cancer stem-like cell (CSC) phenotype. MDA-MB-231 TNBC cell lines in which *ELK3* is either overexpressed or knocked-down were generated by a lentivirus-assisted cell transduction procedure. The genetic transformation was validated by PCR, RT-qPCR and western blot at DNA, RNA and protein level, respectively. Modulation of *ELK3* significantly affected cell migration in 3D microfluidic devices: its overexpression boosted migration speed (+31.2%) and persistence (+38%), while its downregulation inhibited cell migration (-22.1% for speed and -23.4% for persistence). *ELK3* expression also impacted the stemness potential of the cells, as its upregulation increased both spheroid size (+17.8%) and number (+49.2%) in mammosphere formation assays. No effects on the EMT process were observed, based on E-cadherin, N-cadherin and pan-cytokeratin expression by flow-cytometry. In conclusion, ELK3 exerts a substantial influence on the confined migration of TNBC cells, though further exploration is necessary to fully comprehend the molecular connections underlying this impact.

P-32-012
Role of hyaluronic acid in endometrial receptivity

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Despite long-term research, the percentage of successfully implanted embryos during the *in vitro* fertilization (IVF) process remains low (approximately 30%), resulting in a significant number of patients with a condition known as non-receptive endometrium. Glycosaminoglycans on the surface of both the embryo and endometrial epithelial cells play a key role in their interaction. Among these, hyaluronic acid (HA) is a major component. Estrogen and progesterone, the primary hormonal regulators of changes in the endometrium during the menstrual cycle, also influence HA metabolism and the expression of HA-related genes. Our main hypothesis is that the modulation of HA metabolism and the expression of HA receptors are key components of successful embryo implantation, and that pathological alterations in these processes are the cause of endometrial non-receptivity. To test the importance of HA-related genes and the hormonal regulation of their expression, we established a functional receptivity assay using Jar cells (a choriocarcinoma cell line commonly used as trophoblast surrogates). We employed two endometrial epithelial cell lines as models of receptive and non-receptive endometrium: RL95-2 (CD44+, representing receptive endometrium) and AN3CA (CD44-, representing non-receptive endometrium). We used molecular and cytometric approaches to study the differences between receptive and non-receptive endometrium. Functional testing of different receptivity levels in these two cell lines, and after estrogen or estrogen/progesterone treatment, was performed using Calcein-stained Jar cells, and the rate of adhesiveness was determined. The expression of CD44, RHAMM, HAS3, Hyal2, and TMEM2 in epithelial cells was analyzed using RT-PCR (for gene expression), western blot, and flow cytometry (for protein expression). The presence of HA on the cell surface was analyzed by ICC and flow cytometry.

P-32-013
Peptidyl arginine deiminase-2 effects on the myelin basic proteins charge isomers

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The deamination of arginine and its conversion to citrulline is a modification observed in positively charged proteins such as histones or myelin basic protein (MBP). This reaction is catalyzed by peptidyl arginine deiminase (PAD), whose abnormal activation is associated with autoimmune diseases like rheumatoid arthritis and multiple sclerosis. However, the mechanisms that trigger PAD activation and the pathophysiological processes involved in hypercitrullination remain unknown. In this study, we investigated the interaction between PAD and various

charged isomers of MBP, each differing in the degree of post-translational modification. Immunoprecipitation experiments were conducted to examine the binding between PAD and the different charge isomers of MBP. Our findings revealed that the phosphorylated forms of MBP (C3 and C4) exhibited a higher affinity for PAD compared to the unmodified (C1) and fully citrullinated forms (C8). Additionally, we observed that only in the presence of the unmodified C1 isomer did PAD undergo autocitrullination, which was inhibited by the endogenous guanidine-containing component, creatine. In the presence of other isomers, PAD did not undergo autocitrullination. Furthermore, we found that the unmodified isoform of MBP-C1 contains methylated arginines, which were not affected by the pre-treatment with PAD. Based on our findings, we propose that the increased phosphorylation of central threonines in the original MBP may trigger PAD activation, leading to increased citrullination of the protein and subsequent disorganization of the myelin sheath. These insights contribute to a better understanding of the underlying mechanisms in autoimmune diseases associated with hypercitrullination, potentially opening new avenues for therapeutic interventions.

P-32-014
Exploring the role of peroxisome proliferator-activated receptor γ in the regulation of endometrial receptivity

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Endometrial receptivity, essential for embryo implantation, is potentially influenced by the peroxisome proliferator-activated receptor γ (PPARγ). PPARγ activation negatively impacts estrogen, progesterone, and angiogenic signaling in the endometrium while reducing inflammation and oxidative stress. Its connection to receptivity biomarkers, however, is not fully understood. Our study, using RNA-seq data from the Gene Expression Omnibus (GEO) database, explored PPARγ's role in endometrial receptivity. We analyzed datasets GSE214872 and GSE171032, representing receptive and non-receptive endometrial cells. To minimize bias, we focused on control samples. A key observation was the absence of PPARγ expression in non-receptive cells, while it was present in receptive cells, highlighting its potential significance in receptivity. The lack of the β3 subunit of integrin αVβ3 in non-receptive cells, a known receptivity biomarker, further underscores this point. Interestingly, homeobox A10 (HOXA10) was more expressed in non-receptive cells, suggesting a complex role or different regulatory mechanisms in endometrial cells. Insulin growth factor binding protein 1 (IGFBP1) was absent in both cell types, raising questions about its role in endometrial function. Bone morphogenetic protein 2 (BMP2), found only in receptive cells, is linked to tissue remodeling and may play a part in establishing receptivity. However, similar levels of mucin 1 (MUC1) in both cell types suggest its limited role or varied regulatory patterns in receptivity. In summary, our findings highlight PPARγ's role in endometrial receptivity and provide the basis for more research to clarify the relationship between PPARγ and receptivity biomarkers. This work is supported by projects APVV-22-0357 and VEGA 1/0435/23. *The authors marked with an asterisk equally contributed to the work.

P-32-015
Gremlin-1 controls inflammation and fibrosis in an *in vitro* model of dysfunctional adipocytes

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In obesity, during the abnormal expansion of the adipose tissue (AT), adipocytes loose "adipo" markers and transdifferentiate into myofibroblasts. This event is paralleled by increased production of extracellular matrix and stiffening of the tissue. Such dysfunctional/fibrotic state of the AT is exacerbated by increased inflammation, and it is associated with insulin resistance and difficulty in weight loss. Among factors that may control the function of AT during obesity, the soluble factor gremlin-1 could play a pivotal role. Specifically in the AT, gremlin-1 regulates white/brown adipogenesis and insulin sensitivity and its local and circulating levels are increased in obesity and in its complications (type 2 diabetes, non alcoholic fatty liver disease, etc). Of note, gremlin-1 is a central driver of inflammation and tissue fibrosis in multiple tissues. However, its possible involvement in the dysfunction, inflammation and fibrosis of the AT and its effects on the metabolic health of patients remain only poorly understood. Therefore, here we evaluated the effects of gremlin-1 expression/administration exploiting *in vitro*/*vivo* models. Our results show that gremlin-1 is overexpressed in 3T3-L1-derived adipocytes *in vitro* under stress conditions (i.e. hypoxia, pro-inflammatory stimuli). Furthermore, gremlin-1 transfection in 3T3-L1-derived adipocytes induces the expression of inflammation/fibrosis markers like S100A4, TNF-alpha, alpha-SMA, Vimentin, and Snai1. Consistently, high levels of ectopic gremlin-1 promote cell transdifferentiation processes to a mesenchymal/fibrogenic phenotype in the subcutaneous tissue of nude mice and induce interstitial fibrosis *in vivo*. Our data point to a pro-inflammatory/fibrotic role of gremlin-1 in AT, that need further confirmation. This would eventually suggest gremlin-1 as an attractive target to reduce AT dysfunction, improve the metabolic function of patients with obesity and reduce the risk of the associated complications.

P-32-016
The open dimeric conformation of cardiotropic light chains: a key feature underlying soluble toxicity in cardiac AL amyloidosis

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Immunoglobulin light chain (AL) amyloidosis is a protein misfolding disease caused by the conversion of immunoglobulin light chains (LC) from their soluble dimeric functional states into highly organized amyloid fibrils. Fibrillar deposition of LCs severely affects organ function and results in poor prognosis for patients, especially when heart involvement is severe. In addition to fibrillar deposition, a further pathogenic factor is represented

by the direct toxicity of soluble pre-amyloid LCs. Even though the molecular mechanisms underlying LC toxicity remain largely unclear, several biochemical and biophysical traits were found to be typical of amyloidogenic LCs. In particular, we have recently depicted an unreported partially monomeric LC dimer representing a conformation likely specific for toxic LCs. In this peculiar conformation, LC variable domains are far from each other and expose highly hydrophobic patches that can aberrantly interacts with the surroundings (1). Here, we explore the role of this open dimeric conformation in the context of the pathology by studying five additional patient-derived cardiotoxic LCs from a biophysical and biochemical point of view. We found that these LCs are characterized by remarkable fold instability, increased dynamics, flexibility, and susceptibility to protease cleavage. Interestingly, fluorescence-based experiments revealed the high surface hydrophobicity of the LC homo-dimeric folds, corroborating the existence of the open conformation previously observed. Indeed, the existence of closed-open equilibrium typical of these cardiotropic LCs is at the basis of their cardiotoxicity, as pointed out by specific assays on human cardiac fibroblasts. Overall, we ascribed to the closed-open equilibrium a pivotal role in driving LC cardiotoxicity, paving the way for the design of future therapeutics directed towards the stabilization of this conformation. (1) Previously published in: Broggini L et al. (2023) JMB 435, 24

P-32-017
Association between GPER polymorphism rs3808350 and risk of prostate cancer in Slovak population

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Prostate cancer (PCa) is the most frequently diagnosed malignancy among men in more than half of the countries in the world with Slovak population having one of the highest all-age mortality in Europe. Therefore, understanding the genetic factors contributing to its development is crucial for effective detection and personalized treatment strategies. GPER (G protein-coupled estrogen receptor 1) is estrogen receptor associated with several hormone dependent malignancies, including breast cancer, ovarian cancer and endometrial cancer. GPER has been associated with pro-tumorigenic role in prostate cancer. GPER polymorphism rs3808350 is located in the 5'-regulatory region of *GPER* gene. Our research aims to investigate protentional association between GPER polymorphism rs3808350 and prostate cancer using tetra-primer allele-specific polymerase chain reaction. Based on our results, GPER is associated with risk of prostate cancer. Moreover, rs3808350 is associated with clinical parameters, such as lower serum PSA (<10 ng/ml), higher Gleason score (≥7) and pT3/pT4 staging. Obtained results will help to understand the genetic factor affecting prostate cancer.

P-32-018
Mitochondrial complex I deficiency perturbs peroxisomal biogenesis and function

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Mitochondrial damage is a hallmark of ageing and age-related disorders. Amidst several inter-organelle communication pathways, the crosstalk between mitochondria and peroxisomes is poorly understood despite these organelles are interdependent, as dysfunction in one lead to dysfunction in the other. Mitochondrial abnormalities are found in peroxisomal (PEX)-mutants, strengthening the hypothesis that this axis is crucial for correct functioning of both organelles. However, few data directly link peroxisomal dysfunction to a pre-established mitochondrial altered environment. We characterized peroxisomal biogenesis using mouse embryonic fibroblasts (MEFs) from mitochondrial respiratory complex I deficiency (NDUFS4 KO) versus WT. Transcript levels of several important peroxins involved in PEX biogenesis and maturation, as well as PEX-related genes for antioxidant and β-oxidation pathways were found increased in NDUFS4 KO. We also observed increased number of peroxisomes despite lower abundance of peroxisomal matrix proteins in these peroxisomal particles. In particular, protein levels of Pex19 and ACOX1, involved in PMP70 transportation and β-oxidation, are also decreased, suggesting diminished PEX-β-oxidation in NDUFS4 KO. Despite total protein catalase levels are increased in KO cells, CAT/PEX colocalization do not differ and, total cellular hydrogen peroxide (H_2O_2) levels are increased while no changes were observed in mitochondrial-H2O2, suggesting PEX-involvement in the increased H_2O_2-generation in KO cells. Supplementation of the cellular medium with very long chain fatty acids, which rely on peroxisomal beta-oxidation, increases basal oxygen consumption rates and impairs cell response to mitochondrial uncoupling. Our study reveals novel data in the complex mitochondria-peroxisome crosstalk, and the impact that mitochondrial dysfunction as in peroxisomal homeostasis.

P-32-019
Role of autophagy in carcinoma-associated fibroblast activation and signaling

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Tumors do not only consist of cancerous cells, but they also harbor several normal-like cell types and non-cellular components. Carcinoma-associated fibroblasts (CAFs) are one of these cellular components that are found predominantly in the tumor stroma. Autophagy is an intracellular degradation and quality control mechanism, and recent studies provided evidence that autophagy played a critical role in CAF formation, metabolic

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reprogramming and tumor-stroma crosstalk. However, communication with surrounding cells and the mechanism of autophagy stimulation in the tumor microenvironment are not fully documented. For this purpose, we first focused on CAFs and their role in tumor stroma. We identified a factor called CTF1 (cardiotrophin 1) secreted from cancer cells. Our previous data showed that CTF1 is an activator of autophagy in fibroblasts and breast cancer-derived CAFs. We also discovered that CTF1 acted as an important tumor-derived factor regulating breast cancer cell migration and invasion (1). In line with these in vitro observations, we discovered that elevated levels of CTF1 in patient-derived tumors showed a positive correlation with lymph node metastasis (1). Our current analyses suggested that CTF1-associated cellular events may also be dependent on the autophagic capacity of fibroblasts. We revealed that loss of autophagic activity in fibroblast causes diminished activation and transdifferentiation of CAFs, and alteration in fibroblast activation signaling. Therefore, our results suggest that CTF1 is a crucial player in stromal autophagy, fibroblast activation and signaling. Previously published in: 1-Akkoc et al. (2023) Autophagy 19(1):306323.

P-32-020
From deleterious to mild – the diverse effects of missense mutations on the human SCD1

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Many of the syndromes associated with excessive dietary intake and sedentary lifestyle are due to systemic disturbances in lipid metabolism. A key enzyme in the defense against lipotoxicity is stearoyl-CoA desaturase-1 (SCD1), which catalyzes the synthesis of unsaturated fatty acids. The expression of SCD1 is regulated at several levels, but the modulating effect of genetic variations must also be considered. In the present work, we investigated whether natural missense mutations in SCD1 could affect the expression or function of the enzyme. The effects of missense variants in SCD1 were predicted by SIFT, PolyPhen-2, VEP and I-Tasser programs and analyzed in a cell culture system. Mutation-dependent SCD1 expression and possible changes in ER stress markers were monitored at both protein and mRNA levels by immunoblotting and qPCR. Fatty acid profile was investigated by MS. Based on our results, the severity of SCD1 missense mutations was ranked. The c.257Tins proved to be the most deleterious, causing structural, activity, quantitative and ER stress changes. Consistent with unfavorable structural predictions, p.H125P showed significantly reduced protein abundance and desaturation activity with mild ER stress. p.A333T was found to be moderately deleterious, showing reduced activity and expression but no evidence of ER stress. In contrast, p.M224L did not cause any changes in the parameters tested and slightly increased protein production. From our results, we concluded that missense variations in the SCD1 may affect the amount and function of the enzyme to different extents. This work was supported by FK_138115 and ÚNKP-23-3-I grants.

P-32-021
CRISPR/Cas9-mediated knockout of DDC in SH-SY5Y cells: a promising *in vitro* model for aromatic amino acid decarboxylase (AADC) deficiency

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DDC gene encodes aromatic amino acid decarboxylase (AADC), the pyridoxal 5-phosphate-dependent enzyme that catalyzes the last step of dopamine and serotonin synthesis. Mutations in this gene cause a severe form of infantile Parkinsonism known as AADC deficiency. AADC-deficient patients show a reduced synthesis of serotonin and catecholamines (dopamine, norepinephrine, epinephrine), leading to a complex movement disorder and global neurodevelopmental delay, with a high risk of premature mortality. Currently, there is no effective treatment for AADC deficiency and no suitable models to investigate the molecular mechanisms of the disease. In this study, we established single-cell-derived knockout clones of *DDC* in SH-SY5Y cells, taking advantage of the CRISPR/Cas9 technology. The presence of an inserted cassette or indel mutations was verified at each cut site by Sanger sequencing. *DDC* KO clones display a reduced expression of *DDC* at mRNA level, while the absence of the protein was confirmed by western blot. Likewise, the activity of AADC is null in *DDC*-KO clones. The metabolites of the dopamine pathway were measured by mass spectrometry after treatment with L-DOPA. *DDC*-KO clones show high levels of 3-O-methyldopa (3-OMD) and a lack of dopamine and downstream metabolites, while the transient expression of AADC wild type rescues this phenotype. Notably, SH-SY5Y cells can differentiate into cells with morphological and biochemical characteristics of mature neurons by sequential exposure to brain-derived neurotrophic factor in a serum-free medium. Therefore, *DDC*-KO SH-SY5Y cells may represent a novel *in vitro* model to investigate the intracellular effects of pathogenic AADC variants and could provide further insights into the molecular basis for the metabolic phenotype of homozygous and compound heterozygous patients, a prerequisite to develop pharmacological interventions for AADC deficiency.

P-32-022
The role of the NAD-related pathways in the pathophysiology of alfa-sarcoglycanopathy

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Nicotinamide adenine dinucleotide (NAD) is an essential cofactor whose repletion ameliorates muscular dystrophy by improving the mitochondrial and muscle stem cell function. Indeed, NAD deficit represents a deleterious feature of the dystrophic muscle resulting in muscle degeneration. Indeed, the NAD-ase CD38, the major consumer of extracellular NAD, was demonstrated to exert a key role in the pathophysiology of Duchenne muscular dystrophy. Sarcoglycanopathies (SGCs) are recessive inherited limb-girdle muscular dystrophies (LGMD) due to mutations in sarcoglycan genes, where the role of NAD/CD38 axis in the pathophysiology is unknown. Our data demonstrate that in skeletal muscles of Sgca-/- mice, a mouse model resembling (LGMDR3), NAD(P)(H) and ATP content are significantly lower than in WT animals. Accordingly, muscle biopsies from LGMDR3 patients display the same pattern of NAD(P)(H) reduction in comparison to control subjects. We demonstrated that NAD reduction in Sgca-/- mice is associated to a decreased expression of Nampt, the enzyme mediating NAD salvage pathway from nicotinamide, and increased NAD-ase activity and CD38 expression in muscle, due to infiltrating cells RNA sequencing was performed in 17 patients affected by LGMDR3, classified as severe or mild on the basis of clinical parameters, and 10 unaffected individuals. Unsupervised hierarchical clustering analysis of 5000 genes with the highest variance demonstrates that the gene expression profile for severe LGMDR3 is strongly different compared to controls, whereas mild LGMDR3 shows no consistent differences to controls. The analysis of genes related to NAD metabolism reveals significant downregulation of NAD salvage pathway enzymes, whereas several NAD consuming enzymes are overexpressed in severe LGMDR3patients compared to controls. In conclusion, this study provides a first picture of alterations in NAD-related metabolic pathways, suggesting novel promising therapeutic targets for LGMDR3 patients.

P-32-023
Role of dietary trans fatty acids in the regulation of stearoyl-CoA desaturase-1 enzyme

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Since unsaturated fatty acids (FAs) play important role in crucial biological processes including energy storage, membrane construction or gene expression, the significance of stearoyl-CoA desaturase-1 enzyme (SCD1), introducing the first double bond into FAs, is undeniable. While the double bonds generated by SCD1 and further human endogenous desaturases are in *cis* position, an average diet not only contains saturated and *cis* unsaturated FAs, but *trans* unsaturated FAs as well. However, health effects of *trans* FAs (TFAs) and whether there is a difference between industrially produced and ruminant-derived TFAs are controversial. We previously demonstrated that other types of FAs can modify the intracellular level of SCD1 and that the only amino acid changing polymorphism, rs2234970 (p.M224L) responds differently to these FAs. In this study, we examined the impacts of the two most common TFAs, the natural vaccenic acid (18:1 *trans* Δ11) and the artificial elaidic acid (18:1 *trans* Δ9) on SCD1 expression in an *in vitro* cellular system. We used immunoblotting, qPCR and luciferase reporter system to monitor the protein and RNA level and the promoter activity of endogenous and transiently transfected SCD1 in two cell lines treated with each TFA. We observed significant difference and opposing effect of the two TFAs: while vaccenic acid decreases, elaidic acid increases the amount of SCD1 on all three levels. We further investigated how TFAs affect the amount of available SCD1 in the case of the p.M224L polymorphism and observed a time dependent response. This raises the possibility of long-term physiological effects of dietary intake depending on genetic variations, as well as the need for personalized treatment/diet. This work was supported by the Hungarian National Research, Development and Innovation Office (NKFIH grant number: FK138115).

P-32-024
Functional characterization of novel PAX9 mutations causing isolated oligodontia

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Hypodontia, missing one or more teeth, is a relatively common human disease; however, oligodontia, missing six or more teeth excluding third molars, is a rare congenital disorder. Many genes have been shown to cause oligodontia in non-syndromic or syndromic conditions. In this study, we identified two novel *PAX9* mutations in two non-syndromic oligodontia families. Mutational

analysis identified a silent mutation (NM_006194.4: c.771G>A, p.(Gln257=)) in family 1 and a frameshift mutation caused by a single nucleotide duplication (c.637dup, p.(Asp213Glyfs*104)) in family 2. A minigene splicing assay revealed that the silent mutation resulted in aberrant pre-mRNA splicing instead of normal splicing. The altered splicing products are ones with an exon 4 deletion or using a cryptic 5' splicing site in exon 4. Mutational effects were further investigated using protein expression, luciferase activity assay and immunolocalization. We believe this study will not only expand the mutational spectrum of *PAX9* mutations in oligodontia but also strengthen the diagnostic power related to the identified silent mutation.

P-32-025
In-vitro SMA model; SMN1 gene knockdown in SH-SY5Y cells

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Spinal muscular atrophy is an autosomal-recessive disorder characterized by degeneration of motor neurons in the spinal cord and caused by mutations in the survival motor neuron 1 gene, SMN1. The phenotype is extremely variable, and patients have been classified in type I- II and III. SMA based on age at onset and clinical course. All three types of SMA are caused by mutations in the survival motor neuron gene (SMN1). Therefore, in this study we aimed to knockdown of SMN1 gene in human neuroblastoma cells to generate SMA model for *in-vitro* studies. For this aim, target sequences of SMN1 for two regions were analyzed using bioinformatic tools and these target sequences were cloned into shRNA-based vector system (pLKO.1). Fist, the vector was restricted with EcoRI and AgeI restriction enzymes. Then, the primers were annealed and ligated into EcoRI and Age I restricted pLKO.1 vector. Transformation of ligation reaction was performed into DH5α competant cells. The colonies were picked and plasmid isolation was performed using plasmid isolation kit (Mn). Recombinant colonies were screened using restriction procedure with EcoRI and NcoI enzymes. The recombinant colonies were verified with sequence analyses. In a result, we obtained two plasmid vectors namely pLKO.1shRNA1 (target region 1) and pLKO.1 shRNA2 (target region 2). The vectors were transfected using Turbofect transfection reagent as manufacturer's instructions. Puromycin selection was carried out after 48 h of transfection. Then the pellet of cells was used for RNA isolation and cDNA was obtained from RNA templates. The mRNA level of SMN1 was determined with real-time PCR analyses. We obtained that, the mRNA level of SMN1 was decreased approximately 25-fold in SMN1 knockdown ShSy-5y cells when compared to control cells (SMN1 positive). This work was supported mainly by the Scientific and Technological Research Council of Turkey (TUBITAK) with project number 2210300.

P-32-026
The hepatic fibrogenic effect of aberrant IgG-Fc glycosylation

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Liver fibrosis is the major cause of end-stage liver diseases, including cirrhosis and hepatocellular carcinoma. Hepatic stellate cell (HSC) is the main cell type that contributes to liver fibrogenesis. HSCs can be activated by injury elements for the liver or inflammatory stimuli during chronic hepatitis, thereby producing excessive matrix proteins and accumulating them in the liver tissue. We have previously demonstrated the relationship between aberrant *N*-glycosylation on the crystallizable fragment (Fc) of serum IgG with the severity of chronic hepatitis. Herein, we addressed the clinical relevance of aberrant IgG-Fc *N*-glycosylation to liver fibrosis. IgG-Fc *N*-glycosylation pattern in patients with chronic hepatitis was analyzed using liquid chromatography-tandem mass spectrometry. We found that Ishak liver fibrosis scores of the patients were correlated positively with the proportion of IgG-Fc with fucosyl-agalactosyl (G0F) and agalactosyl (G0) glycoforms, and inversely with sialyl-fucosyl-fully galactosyl (G2FS), fucosyl-*N*-acetylglucosamine bisected-fully galactosyl (G2FN), and G2FNS glycoforms. Moreover, we detected the expressions of messenger RNA and protein of human Fc gamma receptors in human HSCs. Treatment of agalactosyl IgG proteins induced the activation, transforming growth factor-β1 secretion and collagen production in human HSCs. In conclusion, aberrant IgG-Fc *N*-glycosylation, particularly agalactosylation, enhances the hepatic fibrogenic properties of human HSCs.

P-32-027
Study of the interactions among UGGT and Tdark glycoprotein missense mutants

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Some patients carry a missense mutation in a gene for a secreted glycoprotein which retains some residual activity. The disease is caused by the endoplasmic reticulum (ER) quality control checkpoint enzyme: UGGT (UDP-glucose-glycoprotein glucosyltransferase). UGGT has two isoforms, both aiding a glycoprotein to dwell in the ER until folded. UGGT recognises a misfolded glycoprotein in the ER and reglucosylates, then dispatches it to ER lectins to cause its retention. UGGT also prevents the secretion of misfolded proteins with missense mutations that, if secreted, would have some activity. Patients with responsive mutation may benefit from rescue-of-secretion therapy by inhibition of UGGT, but at the moment the scope for this approach is not known as it is impossible to estimate if a missense mutant is responsive. This is true for rare disease associated mutations in 1/10 glycoprotein genes about which little is known: few publications describe the gene, the function of the protein is not known and

reagents to study it are scarce (antibodies). Each of these 10 glycoproteins is classified as T(target) Dark by the Pharos Consortium. Telethon foundation is funding a project whose aims are: 1) assess *in vitro* or *in cellula* if the 10 Tdark mutants are reglucosylated by either UGGT isoform and, if so, characterise the interaction between that UGGT isoform and the Tdark mutant; 2) investigate *in cellula* whether the 10 Tdark mutants are flagged for ER retention by either isoform of UGGT and if deletion of either isoform can rescue Tdark mutant secretion. The 10 Tdark genes were cloned into vectors for secreted expression in mammalian cells, in frame with various tags (*e.g* TGP, HALO, 6xHIS). Rare disease associated missense mutants in the same genes were selected on the basis of the likelihood they are responsive, and the mutants cloned in the same DNA vectors. Reglucosylation assays are being carried out to check if any of the mutants is a UGGT client while the WT is not.

P-32-028
Alterations of endocannabinoid signalling in an organ of Corti cell-line upon cisplatin-induced ototoxicity

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Cisplatin is a widely used and potent chemotherapeutic to treat several human tumors; however, it has a plethora of adverse effects including ototoxicity in adults and children. Currently, there are no approved therapeutics to counteract these adverse effects. Endocannabinoids (eCB) are a class of bioactive lipids playing a vital role in several organs including the cochlea. To date, only a very few studies have interrogated the role of eCBs in cochlear patho-physiology. Here, we sought to investigate for the first time the *in vitro* expression of the primary eCB system components (i.e., receptor targets and metabolic enzymes), at the protein level in the UB/OC1 mouse organ of Corti cells, along with their alterations in cisplatin-mediated ototoxicity. Firstly, we established an ototoxic model by treating UB/OC1 cells with the IC_{50} (30 μM) of cisplatin. By western blotting, we found the expression of the following receptors- cannabinoid receptors 1 and 2 (CB_1, CB_2), transient receptor potential vanilloid 1 (TRPV1), peroxisome proliferator-activated receptor δ (PPAR δ); and enzymes diacylglycerol lipase α/β (DAGL α/β) and fatty acid amide hydrolase (FAAH) in the cell line. Interestingly, we found that the CB_2 receptor and the DAGL β enzyme were significantly reduced in cisplatin-treated compared to vehicle-treated (CB_2 by ~22%, $p = 0.0058$; DAGL β by ~40%, $p = 0.0041$), while no differences were observed in the others. Overall, our findings demonstrate for the first time the presence of key eCB system components in UB/OC1 cells and document the selective involvement of CB_2 and DAGL β in cisplatin-mediated ototoxicity. This investigation was supported by Dompé Farmaceutici S.p.A. under a collaborative research agreement with University of L'Aquila (P.I. Professor M. Maccarrone). The funder had no role in the design of the study, in the collection, analyses or interpretation of data, in the writing of the manuscript or in the decision to publish the results.

P-32-029
Mechanisms involved in proteostasis failure and amyloid deposition in osteoarthritis

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Proteostasis is fundamental to maintain cellular homeostasis and its impairment can lead to a defective folding of proteins followed by deposition of amyloid aggregates. Our study aims to investigate the mechanisms that regulate protein misfolding in osteoarthritis (OA), the most common degenerative and progressive joint disease thus representing the leading cause of disability in the elderly. The mechanisms involved in OA onset are only partially known and currently there is no targeted OA therapy. Primary cells (chondrocytes and synoviocytes), organ cultures (articular cartilage), and *ex vivo* tissues were isolated from knee joints of OA patients undergoing arthroplasty. Cells were treated with chloroquine (CQ) and/or lipopolysaccharide (LPS) for *in vitro* assessment of amyloid deposition after blockage of autophagy and/or induction of ER stress. Protein aggregation was investigated through western blotting (WB), using an amyloid fibril-conformation dependent antibody and cytofluorimetry using Thioflavin-T (ThT), an amyloid-specific dye. Markers of autophagy (p62, LC3), ER stress (calnexin and HSPs) and UPS (ubiquitin) activation were also investigated by WB given the role these pathways play in protein quality control. Also, cells were pre-treated with nutraceuticals with anti-inflammatory and anti-amyloidogenic properties to test their preventive effect on amyloid formation. The presence and localization of amyloid deposits in *ex vivo* samples and organ cultures were confirmed by immunohistochemical and histochemical assays (Congo Red and ThT). Overall, our data show that amyloid deposition in OA tissues follows the impairment of protein folding and degradation mechanisms (autophagy, UPR and UPS) and homeostasis disruption (senescence and inflammation). These effects are partially mitigated by nutraceuticals. In conclusion, amyloidosis could play a role in OA progression and failure of proteostasis, a hallmark of aging, represents a promising therapeutic target.

P-32-030
In vitro effect of hCG on cryptorchid patients' gubernacular cells: a predictive model for adjuvant personalized therapy

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Cryptorchidism is the absence of one or both testicles in the scrotum at birth, being a risk factor for testis cancer and infertility. The most effective method to treat cryptorchidism is orchiopexy followed by human chorionic gonadotropin (hCG) therapy; however, a portion of patients does not show a significant improvement of testis volume and vascularization after adjuvant therapy. In this study, we generated an *in vitro* model to predict the patient response to hCG by cultivating and treating primary cells derived from four cryptorchid patients' biopsies of gubernaculum testis, the ligament that connects the testicle to the scrotum. Gubernaculum shares many properties with testicular cells, including the expression of the hCG-receptor (LHCGR), due to their common embryonic origin and analogously respond to hormonal therapy *in vitro*. Here, we show that hCG stimulates gubernacular cells to proliferate and to form vessel-like structures with a different extent among the four patients derived cells, together with a decrease of both oxygen consumption and reactive oxygen species generation. Furthermore, we show that gubernacular cells that present the lower response extent to hCG also express at high levels the LHCGR short variant, which has been reported to be non-functional. Hereby, we evince that the diverse patient response to hCG may be ascribable to their age, since young patients better respond in vitro to the hormone, together with the expression level of the short variant of the hCG receptor.

P-32-031
Dissecting Annexin A11 in its domains: structural and functional characterization

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Annexins are a family of Ca^{2+}-dependent phospholipid-binding proteins with several functions. Among these, there is Annexin A11, whose mutations have been found in patients with ALS, a neurodegenerative disease. Annexin A11 has the longest N-terminus domain within Annexins, formed by 197 amino acids of which most are glycine, proline, and tyrosine, and a very conserved C-terminus that hosts four calcium-binding motifs that has been suggested to be involved in the binding to phospholipids[1]. One of the functions recently attributed to this protein has been the ability to passively transport RNA granules, via its C-terminus that tethers to lysosomes, from the neuronal soma to the intrasynaptic space to enable protein translation *in situ*[2]. The aim of the present work has been the dissection of the Annexin A11 in its structural and functional domains, with a particular focus on measuring the affinity of the C-terminus for the calcium and the effect of the latter on the binding between the C-terminus and membrane phospholipids. A purification protocol has been set up to obtain the two domains soluble, on which circular dichroism has been performed showing that the C-terminus has mostly alpha-helices whereas the N-terminus is unstructured. This domain undergoes phase separation, which is why different concentrations of protein, salt and different temperature conditions have been tested to construct a phase diagram and control this phenomenon. The binding affinity of calcium to the C-terminus domain has been measured by fluorescence spectroscopy leading to a Kd of 0,6 μM. Lastly, pull down assays have been conducted in vitro with lysosomes-like liposomes, confirming that the purified C-terminus domain is able to interact with membrane phospholipids in a calcium-dependent manner, as suggested *in vivo*[2]. Previously published in: 1) Gerke V. and Moss S.E (2002) Physiological Reviews 82, 331-371. 2) Liao Y. et al. (2019) Cell 179, 147-164.

P-32-032
Sphingosine-1-phosphate receptor 3 is a non-hormonal target to counteract endometriosis-associated fibrosis

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Endometriosis is an inflammatory and fibrotic disease affecting 6-10% of reproductive age women, defined by the presence of endometrial glands and stroma outside the uterus. The presence of fibrotic tissue leads to adhesions, scarring and loss of normal tissue architecture, representing one of the causes of persistent pain and infertility. The molecular mechanisms underlying the development of fibrosis in endometriosis are still elusive. Recently we demonstrated that the signalling of the bioactive sphingolipid sphingosine 1-phosphate (S1P) is dysregulated in endometriosis [Previously published in: Bernacchioni et al. (2021) Fertil Steril115(2):501-511]. In particular, $S1P_3$, one of the five S1P receptors, was increased at mRNA level in endometriotic lesions compared to healthy endometrium and required to mediate the pro-fibrotic action of TGFβ in uterine adenocarcinoma cells. Here, we analyzed tissue samples of endometriotic lesions from various locations comparing them to healthy endometrium. $S1P_3$ was found by immunohistochemistry to be upregulated in all types of endometriotic lesions considered, and its expression positively correlated with the extent of fibrosis, measured by Masson Trichrome staining. In addition, we provided evidence that S1P induces epithelial to mesenchymal transition and fibrosis in endometriotic epithelial 12Z cells, upregulating the expression of vimentin, N-cadherin, SNAI1 and transgelin. Using RNA interference and pharmacological approaches, we found that this pro-

fibrotic effect is mediated by $S1P_3$ and that it relies on downstream activation of ERK1/2 and ERM (ezrin-radixin-moesin), since when these signaling pathways were pharmacologically blocked, the S1P action was significantly reduced. Taken together these findings add new information to the understanding of the molecular mechanisms involved in endometriosis pathogenesis and identify $S1P_3$ as possible new pharmacological target to counteract the fibrotic trait of the disease.

P-32-033
Studying the activation of blood coagulation induced by staphylococcal biofilms: unraveling the link between infections and thrombosis

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Infective endocarditis (IE) is an infection of the cardiac endothelium. It has an annual incidence of 3–10/100,000 of the population with a mortality of up to 30%. *Staphylococcus aureus* is the most prevalent cause of IE. After endothelial injury, bacterial colonisation is facilitated, thus triggering additional endothelial injury and thrombus formation. Production of a biofilm (multicellular community of microorganisms, enclosed in a self-produced extracellular matrix) assists bacterial persistence and contributes to antibiotic tolerance. Considered that thrombotic complications are often associated with IE, here we explore the possibility that biofilms produced by *Staphylococcus aureus* (coagulase +) and *Staphylococcus epidermidis* (coagulase -), could induce fibrin generation in human plasma and investigate their ability to directly convert fibrinogen into insoluble fibrin *in vitro*. *S. aureus* and *S. epidermidis* were cultured and added to a 96-well microtiter plate, leading to the formation of bacterial biofilms. To test fibrin generation in plasma, diluted (1:2) human plasma was added to each biofilm and to empty wells as a blank experiment. To test fibrin generation from purified fibrinogen, a solution of fibrinogen (0.68 mg/ml) was added to each biofilm and to empty wells as a control. In both assays, fibrin generation was monitored by turbidimetry. The data obtained indicate that: both biofilms of *S. aureus* and *S. epidermidis* efficiently and similarly induce fibrin clotting in human plasma; both biofilms of *S. aureus* and *S. epidermidis* do not convert isolated fibrinogen solutions into fibrin. The results of this study point out that bacterial biofilms can trigger blood coagulation, thus providing the molecular basis for explaining the positive relationship between IE, biofilm formation and an increased thrombotic risk. Further studies are needed to elucidate the biochemical mechanisms underlying biofilm-induced activation of blood coagulation.

P-32-034
Amyloid fibrils induce blood coagulation by activating FXII: pathogenesis of intracardiac thrombosis in TTR and AL amyloidosis

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Transthyretin amyloidosis (ATTR) and immunoglobulin light chain amyloidosis (AL) are amyloid diseases characterized by fibril deposition in several organs, associated with cardiomyopathy and intracardiac thrombosis. However, the link between amyloid diseases and coagulation is still unclear. We demonstrated ability of amyloid fibrils to induce plasma clotting, and we established the molecular mechanisms resulting in activation of blood coagulation. The effect of the net charge of the fibril components was investigated by comparing the effect of fibrils made with similar precursors carrying opposite net charges (λ1 and λ6). All fibrils (both from *ex vivo* specimens and from recombinant origin, characterized by ThT, DLS, TEM) were added to human plasma or whole blood, monitoring clotting by turbidimetry, Thrombin Generation Assay or Thromboelastometry. Enzymatic assays were performed with isolated zymogens and chromogenic substrates. Binding of coagulation factors to fibrils was quantified by ELISA. Our data indicate that both natural and artificial fibrils similarly induce clotting. Addition of either TTR in a non-fibrillar state or of the amorphous precipitate of ß-casein does not trigger clotting, while λ1 amyloid fibrils, carrying a positive net charge at pH 7.4, partially lose the ability to trigger blood clotting. All fibrils induce activation of Factor XII (FXII), both in the presence of Factor XI (FXI) and prekallikrein (PK). Fibrils are also able to increase the efficiency of Prothrombin (FII) activation by activated Factor X (FXa). Fibrils represent a surface on which FXII, FII, FV, and FXa can anchor and become activated. The ability to activate FXII and blood coagulation appears to be correlated with the net charge of the amyloid protein components, with negatively charged fibrils being the most efficient in triggering blood coagulation. These mechanisms can explain the hypercoagulable state that can lead to thrombosis in cardiac amyloidosis.

P-32-035
Induction of E-cadherin expression by deficiency of steroid sulfatase is a key factor in hyperkeratinization for X-linked ichthyosis

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X-linked ichthyosis (XLI) is a genetic disorder caused by a deficiency of steroid sulfatase (STS), leading to an excessive accumulation of cholesterol sulfate and excessive keratinization. The hyperkeratinization seen in STS knockout (KO) mice demonstrates that this model is suitable for studying XLI. Additionally, RNA-seq analysis of skin tissues from STS KO and transgenic

mice revealed an enrichment of genes associated with calcium signaling such as calcium-sensing receptors, and keratinocyte differentiation markers such as keratin 1 and keratin 10. In our studies in STS-deficient cells, we observed an upregulation of E-cadherin, involved in keratinocyte differentiation and stratification through calcium interaction, along with increased levels of the keratinized envelope proteins and keratinization markers such as involucrin and loricrin. Intriguingly, the downregulation of STS and the subsequent accumulation of cholesterol sulfate inhibited the expression of Hakai, a ubiquitin E3 ligase of E-cadherin, thus prohibiting E-cadherin endocytosis and ubiquitin degradation, thereby stabilizing E-cadherin. Furthermore, our finding indicated that the inhibition of E-cadherin led to decreased levels of involucrin and loricrin in HaCaT cells. Specifically, mutations occurring in the N-terminal region of the E-cadherin domain, which is crucial for cell adhesion and calcium interaction, significantly reduced the expression of involucrin and loricrin. Moreover, treatment with miR-6766, known to impede E-cadherin expression, resulted in the suppression of keratinization marker proteins. Consequently, STS deficiency activates the calcium signaling system, promoting keratinocyte differentiation, and fostering excessive keratinization via the E-cadherin upregulation. Therefore, E-cadherin emerges as an important key factor in the hyperkeratinization observed in XLI, and these findings provide valuable insight into potential therapeutic targets for addressing XLI.

P-32-036
Histone deacetylase 3 regulates the life and death cycle of adipocytes in white adipose tissue

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Understanding adipose tissue physiology is crucial in the context of obesity epidemic. Chemical inhibition or genetic inactivation of histone deacetylase 3 (HDAC3) causes metabolic rewiring of white adipocytes toward browning through a futile cycle of lipolysis and lipogenesis [Previously published in: Galmozzi A et al. (2013) Diabetes 62, 732–742; Ferrari A et al. (2017) Nat Commun 8, 93]. Current research focuses on phenotypic alterations in white adipose tissue (WAT) from HDAC3 KO (H3fatKO) mice with emphasis on the impact of enhanced fatty acid metabolism on adipocyte health. To achieve our aim, we conducted immunohistochemistry, RNAseq, ChIPseq (H3K27ac) and FACS analyses on WAT of H3fatKO mice and floxed control mice. Adipocytes in which HDAC3 was silenced were assayed for cell proliferation and gene expression. Surface marker analysis was performed on bone marrow macrophages (BMDM) co-cultured with adipocytes. Dead adipocytes surrounded by macrophages are present in the visceral WAT of H3fatKO mice. FACS analysis shows a 2.48-fold change increase ($p = 0.0311$) in infiltrating macrophages in WAT, which exhibit polarization towards an M2-like population. Moreover, HDAC3 silencing in cultured adipocytes prompts macrophage activation. Integrated analysis of transcriptomic and epigenomic data unveils ferroptosis as one of the enriched pathways in H3fatKO mice, suggesting ferroptosis as a mechanism for eliminating metabolically exhausted adipocytes. Concurrently, evidence of progenitors undergoing differentiation supports a potential renewal process in WAT. Gene expression and FACS analyses validate increased adipocyte proliferation. In conclusion, HDAC3 ablation in adipocytes remodels WAT immunophenotype and may serve as a metabolic rheostat, influencing the rejuvenation of WAT by eliminating aging adipocytes and fostering progenitor differentiation. Insight into the role of HDAC3 may offer the opportunity of improving the pathophysiology of metabolic disorders.

P-32-037
Exploring the associations of multifaceted proteins to diseases

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The characterization of multifunctional proteins is an expanding research area aiming to elucidate the complexities of biological processes. We recently developed MultifacetedProtDB (Bertolini et al, 2024, https://multifacetedprotdb.biocomp.unibo.it), a curated database providing a collection of 1103 multifunctional human proteins, of which 812 are enzymes. We annotate this collection for disease associations, merging information from UniProt, Humsavar, Monarch, and ClinVar, reporting disease nomenclatures as MONDO, ICD10, OMIM and Orphanet catalogues. Some 30% of proteins in our database (321 enzymes and 110 non-enzymes) are associated with 895 MONDO diseases classified into 213 ICD10 categories and in 17 out of the 19 ICD10 main chapters (excluding chapters without a genetic component). The most represented chapter is XVII: "Congenital malformations, deformations and chromosomal abnormalities" accounting for 226 diseases associated with 135 multifaceted proteins, followed by IV: "Endocrine, nutritional and metabolic diseases", VI: "Diseases of the nervous system", II: "Neoplasm", with 134, 105, and 48 associated multifaceted proteins, respectively. Out of the 895 diseases, 323 are included in the Orphanet catalogue of rare diseases. Over the 431 multifaceted proteins with MONDO disease annotation, 212 are associated with multiple diseases, and 56% are associated also with multiple Reactome pathways. Performing different functions in different pathways could explicate why a protein is associated with different diseases, giving insight to the molecular mechanism leading to disease insurgence. MultifacetedProtDB is useful for characterizing the involvement of a multitasking protein in the cell molecular complexity and associated disease, and possibly future research will enlarge the actual collection and improve its annotation. Bertolini et al. MultifacetedProtDB: a database of human proteins with multiple functions. Nucleic Acids Res. 2024 Jan 5;52(D1): D494-D501.

P-32-038
ACE2 signalling drives the activation of hypoxia inducible factor 1 subunit alpha (HIF1α) during lung injury: a focus on a possible ACE2 nuclear fraction activity

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Angiotensin-converting enzyme 2 (ACE2) represents a key receptor for SARS-CoV-2 cell entry [1]. Interestingly, ACE2 undergoes a proteolytic cleavage of the ectodomain; then, a second cleavage of the transmembrane domain promotes the internalization of the intracellular carboxy-terminal fragment (ICD) [2]. Despite the ACE2 enzymatic activity has been widely investigated, no direct evidence of the role of ACE2 intracellular signalling on lung cells has been reported. Herein, the ACE2 intracellular activity and its correlation with HIF-1α activation were deeply investigated on lung epithelial cells (16HBE). Firstly, cells were treated with an ACE2 enzymatic inhibitor that caused a decrease of transmembrane ACE2 protein in concomitant with an increase of the soluble protein fraction release in culture medium. Then, the activation of the hypoxia-inducible factor-1-alfa (HIF-1α) as well as the transcription of HIF-1α target genes were investigated. ACE2 inhibition increased not only the HIF-1α protein level but also the transcription of its target genes, because of the increase of HIF-1α gene expression. Subsequently, the ability of the ICD to localize into the nuclei of 16HBE was investigated through an immunofluorescent analysis using an ACE2-GFP protein, demonstrating its nuclear localization in response to the ACE2 inhibitor. Finally, the gene expression of the pro-fibrotic factor TGFβ1 was increased in correspondence to the ACE2 cleavage and nuclear translocation. Together, these results strengthen the knowledge on a non-conventional activity of the ACE2 enzyme as a possible mechanism contributing to the pathological consequences of SARS viral infection in the lung. This research project is funded by Tuscany Region "Bando ricerca covid-19" OPTIMISED. Previously published in: 1. Jackson, C.B. et al. Nat Rev Mol Cell Biol. (2022) 23, 3-20. 2 Bartolomé A. et al. Sci Rep. (2021) 11.

P-32-039
A novel quinolone-based HDAC6 inhibitor (QBC) promotes apoptosis in chronic lymphocytic leukemia by activating STAT4

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Chronic lymphocytic leukemia (CLL) is a hematological malignancy characterized by uncontrolled proliferation of mature $CD5^+$ B cells, largely due to apoptosis defects. Prolonged leukemic cell survival has been linked to impaired expression of the pro-apoptotic adaptor p66Shc [Capitani N. et al. (2010) Blood 115(18), 3726-36] and of its transcription factor STAT4, which is typically observed in patients with poor prognosis [Cattaneo F. et al. (2016) Oncotarget 7(35), 57086-57098]. Of note, a mechanistic explanation for the STAT4 defect in CLL has not yet been provided. Normalization of STAT4 expression, or activation of the residual STAT4, might represent a valuable therapeutic strategy for CLL treatment. It has been reported that histone deacetylase 6 (HDAC6) directly deacetylates and inhibits STAT4 in T lymphocytes [Zhang Y.S. et al. (2022) Cell Death Differ., 29 (11):2303-2315]. We hypothesized that this mechanism is also operational in CLL cells and contributes to promote CLL cell survival. Here we show that the new potent and selective HDAC6 inhibitor QBC enhances p66Shc expression and rescues the apoptosis defect in CLL cells, results that were reproduced by siRNA-mediated HDAC6 silencing. Flow cytometric analysis revealed that QBC activates residual STAT4 by enhancing its acetylation and subsequent phosphorylation. Moreover, by transiently transfecting vectors encoding STAT4-luciferase or the STAT4-GFP fusion proteins in CLL cells, we found that QBC enhances both nuclear translocation and transcriptional activity of STAT4. Altogether, these data confirm the role of STAT4 as an innovative target for drug design in CLL and suggest that selective HDAC6 inhibition represents a promising therapeutic strategy for CLL treatment.

P-32-040
Modelling the aging-associated loss of proteostasis leading to proteinopathy

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Aging is the major risk factor for the development of neurodegenerative diseases, including Alzheimer's disease (AD) and frontotemporal lobar degeneration (FTLD). These conditions are characterized by a generic failure of the proteostasis network, with specific proteins losing solubility, gaining propensity to misfold and aggregate into neurotoxic species and inducing many others to aggregate. Among them, the transactive response DNA-binding protein 43 (TDP-43) is recognized as the major neuropathological hallmark of FTLD with TDP-43 inclusions (FTLD-TDP) and other TDP-43 proteinopathies, and also reported to accumulate in the brain of AD patients in addition to amyloid-β (Aβ) and tau proteins. In this study, we set up two neuronal models of brain aging characterized by the progressive decline of protein homeostasis. The first one was obtained by treating cultured neuronal cells overexpressing human full-length TDP-43 with sub-threshold concentrations of autophagy and proteasome inhibitors. The second is a model of protein co-morbidity between tau and TDP-43, obtained by co-expressing them in neuronal cells and investigating their pathological synergy. We show that cells overexpressing human full-length TDP-43 undergo a progressive pathogenic accumulation of cytoplasmic self-assemblies visualized by confocal microscopy upon chronic exposure to autophagy and proteasome inhibitors. We also demonstrate that the concomitant overexpression of tau and TDP-43 worsens TDP-43 pathology. In conclusion, these results suggest the employment of our experimental approaches as good model systems to mimic the aging-associated functional decline of the proteostasis machinery. They also suggest the association between TDP-43 and tau pathologies, demonstrating the existence of a pathogenic synergy resulting in increased TDP-43 cytoplasmic accumulation.

P-32-041
Effects of escitalopram on mTORC1 signaling in 3D cortical spheroid model of depression with dexamethasone treatment

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3D *in vitro* models of depression are essential for studying the pathophysiology of depression, mechanisms of antidepressants action, drug efficacy, and new drug development. 3D cortical spheroids exposed to the synthetic glucocorticoid dexamethasone showed impaired neuroplasticity contributing to one of the key mechanisms of depression. The purpose of this study is to determine if this model can be used as an *in vitro* 3D model of depression with escitalopram treatment. To this end, to explore the molecular mechanisms by which escitalopram enhances neuroplasticity, I investigated whether escitalopram modulates the mTORC1 signaling pathway in 3D cortical spheroids exposed to dexamethasone. Primary rat cortical cell-based spheroid cultures were treated with escitalopram (0.1, 1, and 10 μM) under dexamethasone (100 μM) conditions. Western blot analyses were performed to assess changes in BDNF, mTORC1-mediated proteins, and synaptic proteins PSD-95, GluA1, and synapsin 1. Neurite outgrowth was analyzed by immunofluorescence. Dexamethasone significantly reduced BDNF expression and neurite outgrowth, and escitalopram restored these reductions in a dose-dependent manner. Dexamethasone significantly decreased the phosphorylation levels of mTORC1 as well as downstream proteins 4E-BP1 and p70S6K, along with reduced levels of synaptic proteins. Under dexamethasone condition, escitalopram significantly increased the phosphorylation levels of mTORC1, 4E-BP1, and p70S6K as well as synaptic protein levels, with significant increases at 10 μM. Escitalopram enhances neuroplasticity by upregulating the mTORC1 signaling in cortical spheroids exposed to dexamethasone, demonstrating the usefulness of this model as an *in vitro* model of depression underlying impaired neuroplasticity.

P-32-042
Effects of sirtuin 1 on dendritic outgrowth and spine formation through mTORC1 signaling

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There is growing evidence that one of main mechanisms of depression is related to impaired neuroplasticity. Sirtuin 1 plays an important role in neuroplasticity, but its mechanisms are not fully elucidated. Activation of mechanistic target of rapamycin complex 1 (mTORC1) signaling in neurons, is known to enhance neuroplasticity. In this study, we investigated how sirtuin 1 affects mTORC1 signaling and dendrite outgrowth and spine formation in rat primary cortical cells under dexamethasone-induced neurotoxic conditions. Cortical cells were treated with SRT2104 (0.1, 1, and 10 μM), a selective sirtuin 1 activator, along with dexamethasone (500 μM). Changes in levels of sirtuin 1, mTORC1-mediated proteins, and synaptic proteins (PSD-95 and GluA) were measured by western blotting analysis. Changes in dendritic outgrowth and spine density were assessed by immunofluorescence. SRT2104 significantly increased the levels of sitruin 1 expression and phosphorylation of ERK1/2 (a downstream target of sirtuin 1) in a concentration-dependent manner under dexamethasone conditions. SRT2104 also significantly increased the phosphorylation levels of mTORC1 and 4E-BP1 and p70S6K (downstream targets of mTORC1). Moreover, SRT2104 significantly increased dendritic outgrowth and spine density. In contrast, sirtuin 1 knockdown through sirtuin 1 siRNA transfection significantly reduced dendritic outgrowth and spine density as well as phosphorylated levels of ERK1/2 and mTORC1. These results suggest that sirtuin 1 increases dendritic outgrowth and spine density by activation of mTORC1 signaling. Therefore, siturin 1 may represent a novel beneficial therapeutic target for depression. *The authors marked with an asterisk equally contributed to the work.

P-32-043
Tackling the NAD-related signaling pathways to counteract doxorubicin-induced cardiotoxicity

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NAD is a coenzyme involved in mitochondrial metabolism and epigenetic regulations. NAD levels decrease in several pathologies and during aging: restoring its levels with NAD precursor administration, including nicotinamide mononucleotide (NMN) or nicotinamide riboside, is a promising therapeutic and anti-aging strategy. Doxorubicin (DOX) is a widely used chemotherapeutics, with serious toxic side effects, such as cardiomyopathy. DOX causes oxidative stress, mitochondrial dysfunction and DNA damage, ultimately leading to cell death, with a negative impact on patient prognosis/survival. However, there are no effective therapeutic strategies to limit these side effects. NMN administration is promising, as it significantly protects from DOX-induced cardiotoxicity and loss of physical function in mice (Margier et al, Cells 12(1):108, 2022). Similarly, another compound (herein undisclosed compound, UC) efficiently protected against DOX-induced cardiotoxicity. Our aim is to characterize UC effects, and its underlying mechanisms, in DOX-induced cardiac damages. UC or NMN were administered to DOX-treated mice: NMN prevented the NAD decrease induced by DOX in heart, whereas UC increased ATP and cADPR (a Ca^{2+}-mobilizing messenger derived from NAD) levels without impacting NAD content. We aim to decipher UC mechanism of action, possibly by identifying potential receptors/enzymes it interacts with, in a proper *in vitro* model. Our results suggest that both NMN and UC can protect *ex vivo* murine neonatal cardiomyocytes and human cardiac fibroblasts from DOX-induced apoptosis and senescence, respectively. Regarding the mechanisms of action, UC is neither modulator, nor substrate of the NADases CD38 and SARM1, and of NMNAT, involved in NAD synthesis, as tested on the recombinant enzymes. Instead, NMN is a substrate for CD38 and NMNAT, and a modulator for SARM1. The characterization of UC mechanism(s) of action could provide fundamental hints in the prevention DOX-induced toxicity in heart.

P-32-044
Disrupted protein interaction dynamics in a genetic neurodevelopmental disorder revealed by structural bioinformatics and genetic code expansion

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Deciphering the structural effects of gene variants is essential for understanding the pathophysiological mechanisms of genetic diseases. Using a neurodevelopmental disorder called Bosch-Boonstra-Schaaf Optic Atrophy Syndrome (BBSOAS) as a genetic disease model, we applied a combination of structural bioinformatics, cellular biochemistry and synthetic biology approaches to assess the pathogenic impact of human NR2F1 (Nuclear Receptor Subfamily 2 Group F Member 1, an evolutionary conserved orphan nuclear receptor acting as a transcriptional regulator during neurodevelopment) variants and the binding with known and novel partners. The computational structural analyses of NR2F1 variants, consisting of homology modeling, rigid-body docking and molecular dynamics simulations, delineated the molecular consequences on the isolated and complex structures. The Genetic Code Expansion (GCE) approach allowed, by taking advantage of the amber codon suppression, to introduce the photosensitive cross-linker Azido-Phe in specific positions within the protein structure, thus enabling covalent and site-specific capture of transient supramolecular interactions in living cells. This revealed the variable quaternary conformations of NR2F1 variants and highlighted the disrupted interplay with dimeric partners and the newly identified co-factor, CRABP2. The integration of such approaches also highlighted that the heterogeneous disease spectrum of BBSOAS arises from different impacts of pathogenic mutations on NR2F1 conformation, supramolecular interplay, cell cycle, viability, and subcellular localization, thus constituting a framework for unveiling the complexity of neurodevelopmental diseases, such as BBSOAS (Marino V et al. (2024), Protein Sci 33(4):e4953 doi: 10.1002/pro.4953). *The authors marked with an asterisk equally contributed to the work.

P-32-045
Development of an *in vitro* system for mycobacterial ribosome reconstitution

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Stablishing an *in vitro* system for cell-free synthesis and assembly of translationally competent ribosomes is a powerful technique for understanding molecular translation and ribosome mutability. *Mycobacterium tuberculosis* is the causative agent of human tuberculosis (TB), which remains a threat to the health of people worldwide. More than a quarter of global deaths due to antimicrobial resistance are due to drug-resistant *M. tuberculosis*. Most antibiotics commonly used in the treatment of TB target the ribosome. Traditionally, both the function and structure of the ribosome have been considered highly conserved. Nevertheless,

species specific features and some degree of structural heterogeneity of mycobacterial ribosomes, which could confer antibiotic resistance and adaptability to hostile environments, have been recently acknowledged. In our work, we are setting up an *in vitro* system for the reconstitution of mycobacterial ribosomes that can later be used to *in vitro* evolve the ribosome under different conditions. Using the non-pathogenic *Mycobacterium smegmatis*, we have successfully optimized protocols for the isolation of all the components necessary for the molecular assembly of active ribosomes, these include the isolation of total ribosomal proteins and cytosolic components needed for ribosomal assembly, as well as the *in vitro* T7 transcription of the rDNA operon. Moreover, we have been able to detect reporter sfGFP production carried in an mRNA template, although at lower levels than that produced by mycobacterial ribosomes directly isolated. Future work is centered in the optimisation of the reaction buffer to maximise near physiological conditions, as well as depicting a system for ribosome display that will allow the capture of the active ribosomes. Once this is achieved, we will use this system to screen a library of *M. tuberculosis* rRNA mutants under antibiotic pressure to better understand its mechanisms of ribosomal resistance and translation. *The authors marked with an asterisk equally contributed to the work.

P-32-046
Piecing together the puzzle: source tracing for pediatric tuberculosis patient with a multi-faceted approach

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Tuberculosis (TB) remains a global public health concern, with limited reductions in disease incidence rates. Investigating TB transmission chains is crucial for improving control measures. We retrospectively studied a cluster of eight epidemiologically linked TB patients (S1-S8) using whole genome sequencing (WGS) data of *Mycobacterium. tuberculosis* (Mtb) isolates integrated with clinical and epidemiological information. We focused on identifying the possible source of infection for a pediatric TB patient diagnosed at four months of age (S7). All Mtb isolates represented one active TB episode, except for one case of a single prolonged TB episode (S4.1 and S4.2). One patient had three episodes (S1.1-S1.3). Active TB cases linked to the S7 included relatives and neighbors. *In silico* Mtb isolate spoligotyping revealed three genotypes: SIT42 (LAM9) for three isolates (S2, S4.1, and S4.2), SIT254 (LAM-RUS) for three isolates (S5, S6, and S8), and SIT1 (Beijing) for four isolates (S1.2, S1.3, S3, and S7). It also indicated the presence of mixed strain infection in the S1.1 case (SIT1 and SIT254), confirmed by differing SNV analysis, showing small genetic distances of 2-6 SNVs with all three SIT254 and two SIT1 isolates (S1.2 and S1.3). Phenotypic and WGS-based drug susceptibility testing revealed differing resistance patterns among SIT1 isolates: S1.1, S1.2, and S1.3 isolates were isoniazid-resistant, S3 was multidrug-resistant, and only S7 isolate was drug-susceptible. WGS data showed distances of 113-165 SNVs between S7 and other SIT1 isolates, confirming that the source of TB infection for S7 was not among the identified contacts. This study highlights the possibility of wide Mtb strain variability within epidemiologically linked TB patient clusters and underscores the importance of a comprehensive approach in TB source case investigation. Funding: Academic career doctoral grant of the European Union Recovery and Resilience Mechanism Plan 5.2 reform and investment direction.

P-32-047
Understanding the modulators of tau amyloid aggregation pathways

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Aggregation of the intrinsically disordered protein tau is a hallmark of several diseases, called tauopathies, including Alzheimer's disease. Amyloid aggregates are solid-like protein assemblies that are highly ordered and stable. Strikingly, different aggregate structures are involved in distinct tauopathies, revealing a structure-pathology relationship. Yet, the basic mechanisms and factors that drive tau aggregation and structural differentiation remain unknown. Here we explore the modulators of tau aggregation. We intend to provide a unified view on how factors such as disease-associated mutations, truncations and interaction with molecular cofactors modulate tau aggregation. Using a range of biophysical and biochemical methods, we show that aggregation propensity of tau is encoded in its structure at a monomer level. Furthermore, we explore how aggregation inducers, such as nucleic acid, lipids or sugars, modulate aggregation pathways to navigate between different forms of tau amyloids. These results help to understand how different diseases-specific tau strains can emerge and how they can be reconstituted *in vitro*.

P-32-048
Unraveling the transport mechanisms from the blood stream performed by seminal vesicle epithelial cells against a concentration gradient to enrich seminal plasma of low molecular weight compounds

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Human seminal plasma (SP) has a peculiar composition of low molecular weight metabolites. Compared to serum, ascorbic acid is in concentrations up to 8 times higher, uridine is at levels 1000 times higher and other purines (guanine and guanosine) show similar striking differences. Recently, we demonstrated that infertile males have decreased SP concentrations of the aforementioned compounds, compared to the SP values of control fertile males. This suggests that the biochemical composition of SP in crucial for male fertility. Production of SP is ensured by the contribution of the seminal vesicles, the prostate, and the bulbourethral glands, with the seminal vesicles accounting for about the 70-80% of SP. To date, there are no information on the mechanisms through which the seminal vesicles epithelial cells (SVEC)

accumulate these compounds in SP. Since some of these compounds (ascorbic acid) cannot be synthesized by the cells, SVEC capture them from the circulating blood, enriching SP against a concentration gradient. In these experiments, SVEC were layered onto a septum, permeable to low molecular weight compounds. SVEC were then covered with a cell medium supplemented with either ascorbic acid (5 to 100 mmol/l) or uridine (1 to 20 mmol/l). Cell medium, free of both ascorbic acid and uridine, was placed below the septum. After incubation at 37°C (0.5, 1, 2, 4 and 6 hours), concentrations of the compounds under evaluation were determined by HPLC in upper cell medium, in lower cell medium and in deproteinized SVEC extracts. Results indicate that SVEC efficiently perform ascorbic acid and uridine transport, as indicated by the time- and dose-pendent increase of both compounds in lower cell medium. In future experiments, we are planning to characterize the transporters of these molecules and the modulation exerted by different stimuli. This work has been supported by PRIN 2022 grant "Counteracting Human Infertility Pathophysiology (CHIP)", number 2022KREEEF. *The authors marked with an asterisk equally contributed to the work.

P-32-049
Cereblon regulates osteogenic differentiation in mouse primary cultured bone cells

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Cereblon (CRBN) is a multifunctional protein located in the cytoplasm, nucleus, and peripheral membranes of various tissues. CRBN is closely related to the proliferation and metabolism of normal cells as well as tumor cells and is a direct target of immunomodulatory drugs. It can lead to several diseases including cardiovascular disease, obesity, diabetes, fatty liver, and neurodegenerative diseases by abnormal cellular metabolism of CRBN. However, the role and the molecular mechanism of CRBN in differentiation of bone cells remain unknown. In this study, we investigated the role of CRBN in osteogenic differentiation using CRBN knock-out (KO) mice. The deletion of CRBN gene was markedly increased the bone density of the femur (~1.30-fold), especially in male CRBN KO mice (~1.51-fold). We found that CRBN KO cells enhanced potency of osteoblastic differentiation compared to wild-type (WT) mice using methods of alkaline phosphatase and alizarin red staining. We also identified that activations of osteoblastic differentiation by cyclosporin A in CRBN KO cells were enhanced more than WT cells in time-dependent manner. These results suggest that CRBN plays a critical role for osteoblastogenesis in bone remodeling. This research was supported by Basic Science Research Program through the National Research Foundation of Korea (NRF) funded by Ministry of Education (RS-2023-00237812), and by the Korea government (MSIT) (2020R1A2C1004942).

P-32-050
Administration of the chaperone HSP47 as a new collagen specific therapeutic approach for osteogenesis imperfecta

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Osteogenesis imperfecta (OI) or brittle-bone disease is a group of heritable rare collagenopathies affecting collagen type I, the most abundant protein of bone, and mainly characterized by reduced bone mass and increased skeletal fragility. Most of the dominant and several recessive OI mutations delay collagen I chains folding, increasing their exposure to post translational modifications and affecting collagen secretion and fibrils assembly in the extracellular matrix. Several chaperones and multiprotein complexes are involved in collagen biosynthesis, and among them heat shock protein 47 (HSP47) is the only one specific for fibrillar collagen. Using primary fibroblasts from individuals with defect in the prolyl-3-hydroxylation complex (OI type VII and VIII), we tested the therapeutic potential of recombinant HSP47 (rHSP47) *in vitro*. We demonstrated that rHSP47 can be taken up by the cell, localizing at the endoplasmic reticulum (ER) exit sites and ER Golgi intermediate compartment. rHSP47 increased collagen secretion, reduced collagen post translational modifications and intracellular collagen retention and ameliorated the general ER proteostasis, leading to a substantial improvement in cellular homeostasis and vitality. These positive changes were also supported by an increased collagen I content in the OI matrix. *In vivo*, the administration of rHSP47 improved the skeletal development in the zebrafish p3h1$^{-/-}$ OI model, supporting HSP47 beneficial effect on osteoblast anabolic activity.

P-32-051
A single amino acid substitution in fibronectin binding protein A (FnBPA) of *Staphylococcus aureus* strongly reduces cross-linking with fibrin by bacterially-activated FXIII

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Multi-drug resistant (MDR) *Staphylococcus aureus* has swiftly emerged as a significant danger to public health, prompting a critical need for research into novel drug targets and virulence factors. Von Willebrand factor binding protein (vWbp), initially identified as a bacterial receptor for the human von Willebrand factor (vWF), plays a crucial role in the pathogenicity of *S. aureus*. This mechanism involves the binding and non-proteolytical activation of prothrombin, leading in turn to Factor XIII (FXIII) activation and subsequent fibrin cross-linking. In our prior work, we investigated the ability of bacterially-activated FXIII to cross-link an *S. aureus* adhesin, Fibronectin binding

protein A (FnBPA), to the fibrin network, resulting in the formation of complex heteropolymers with elevated molecular weights. In particular, we discovered that only the N1 subdomain inside region A is directly involved in this covalent binding. [Previously published in: Motta C et al, (2023). Sci Rep. 13, 11683.] To further investigate this mechanism, we produced recombinant single amino acid mutant proteins of this region. Glutamine 103 was previously identified as a major cross-linking site and directly participates in the reaction: we proved that substituting only that residue with an alanine the production of high molecular weight heteropolymers through the action of vWbp-activated FXIII is strongly reduced. These discoveries highlight a distinctive pathogenic mechanism, wherein the transglutaminase activity of bacterially activated FXIII could play a relevant role in the persistence of *S. aureus* inside the host. *The authors marked with an asterisk equally contributed to the work.

P-32-052
A vicious cycle: deconstructing the timeline of DNA damage response and mitochondrial alterations

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DNA damage and impaired mitochondrial activity have been extensively correlated in many neurodegenerative diseases, including amyotrophic lateral sclerosis (ALS). The exact mechanisms underlying this association has not been completely elucidated, although production of reactive oxygen species (ROS) by defective mitochondria are considered a cause of DNA damage. We are investigating the connection between mitochondrial function and nuclear genome integrity, and in particular whether nuclear DNA damage is responsible for impaired mitochondrial activity or vice versa. To this end, we use two different cellular models that enable us to monitor the cellular damage over time: (1) a stable isogenic motor neuron-like NSC-34 cell model with tetracycline (Tet)-inducible expression of the cytotoxic hexanucleotide GGGGCC repeat expansion (i.e., a mutation typically found in the ALS-linked C9orf72 gene) [1]; and (2) an inducible model of U2OS cells, called DiVA (for DSB Inducible via AsiSI), which relies on the inducible expression of the AsiSI restriction enzyme upon 4-hydroxy tamoxifen (4-OHT) induction, generating DNA double-strand breaks (DSBs) at known genomic positions [2]. Our results suggest that nuclear DNA damage precedes mitochondrial dysfunction in both our cellular models. Moreover, the prolonged expression of GGGGCC repeat expansion in NSC-34 cells, as well as of the AsiSI restriction enzyme in the DiVA model determined a marked reduction of cellular vitality. We are currently assessing the effect of DNA damage inhibitors and mitochondrial ROS scavenger drugs on this pathological crosstalk. This project highlight the timeline of DNA damage, mitochondrial alteration, and cellular mortality, thus elucidating the importance of organelle crosstalk in neurodegenerative diseases. Previously published in: [1] Stopford MJ et al. (2017) Hum Mol Genet 26, 1133-1145; [2] Aymard F et al. (2014) Nat Struct Mol Biol 21, 366-374.

P-32-053
Helical superstructures between amyloid and collagen VI in heart-derived fibrils from a patient with light chain amyloidosis

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Systemic light chain (LC) amyloidosis (AL) is a disease where organs are damaged by an overload of a misfolded patient-specific antibody-derived LC, secreted by an abnormal B cell clone. The high LC concentration in the blood leads to amyloid deposition at organ sites. Indeed, cryogenic electron microscopy (cryo-EM) has revealed unique amyloid folds for heart-derived fibrils taken from different patients. Here, we present the cryo-EM structure of heart-derived AL amyloid (AL59) from another patient with severe cardiac involvement. Its structure displays a stable core and two flexible segments adopting alternative conformations. Two confirmations are sterically incompatible and typically distributed on separate fibrils. Noteworthy, the fibril core harbours an extended constant domain fragment, thus ruling out the variable domain as sole amyloid building block. Surprisingly, the fibrils were abundantly concatenated with a proteinaceous polymer, here identified as collagen VI (COLVI) by immuno-electron microscopy (IEM) and mass-spectrometry. Cryogenic electron tomography (cryo-ET) showed how COLVI wraps around the amyloid forming a helical superstructure, likely stabilizing and protecting the fibrils from clearance. Thus, here we report the first structural evidence of interactions between amyloid and collagen, potentially signifying a novel pathophysiological mechanism of amyloid deposits.

P-32-054
GABA-transaminase deficiency: an insight on the molecular effects of pathological variants of the GABA-T protein

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The human γ-aminobutyrate aminotransferase (GABA-T) is a mitochondrial pyridoxal-5'-phosphate (PLP) dependent enzyme belonging to the ω-transaminases, which catalyses the degradation of γ-aminobutyric acid (GABA) to semialdehyde succinate (SSA). Pathogenic mutations on the *ABAT* gene may result in the outcome of GABA transaminase deficiency. According to the Human Gene Mutation Database (HGMD), of ten missense mutations on the GABA-T protein, eight have been identified as responsible for the disease. Among the list, mutations Leu211Phe, Arg220Lys and Gln296His have been chosen as

models of interest: bioinformatic predictions suggested that while the L211F alteration could mainly affect structural properties, R220K and Q296H mutations are expected to hit the catalytic activity. This study investigated the effects of the three mutations on the human GABA-T, expressed as a recombinant protein in HEK293 transfected cells, by analysing expression levels and enzymatic activity in the cell lysate. Protein expression was also evaluated in the presence of pyridoxine (PN), a precursor of the PLP often used as a pharmacological treatment for disorders associated with PLP-enzyme deficits. Finally, another goal was to assess whether the three mutations affect the localization and/or maturation of the protein. The data collected so far showed that (I) the L211F mutation strongly reduces the GABA-T expression level, while the R220K and the Q296 mutations have a minimal effect on the expression but a strong impact on the catalytic activity; (II) None of the evaluated variants showed an altered subcellular localization. Moreover, data collected in the presence of PN allowed us to verify the effects of an increased intracellular concentration of the coenzyme on the expression level and specific activity of the investigated variants and the wild-type GABA-T. *The authors marked with an asterisk equally contributed to the work.

P-32-055
Amyloid-β peptide alters VDAC1 electrophysiology and reduces mitochondrial respiration in an *in vitro* model of Alzheimer's disease

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Voltage-dependent anion-selective channel 1 (VDAC1) is the most abundant pore-forming protein of the outer mitochondrial membranes (OMM). VDAC1 allows the passive diffusion of ions such as Cl^-, K^+, Na^+, and small metabolites, including ATP/ADP and NAD/$NADH^+$, thus participating in the proper maintenance of the organelles' bioenergetic functions. Being a channel, VDAC1 has peculiar electrophysiological properties: when reconstituted in artificial planar lipid bilayer (PLB), VDAC1 forms pores with a conductance of about 3.5 nS in 1 M KCl, which decreases with the application of both positive and negative voltages. From several studies, VDAC1 stands out as a preferential mitochondrial binding site for misfolded proteins in neurodegenerative contexts. In Alzheimer's disease (AD), intracellular oligomers deriving from amyloid-β (Aβ) peptide aggregation form toxic aggregates by directly binding VDAC1 on OMM, promoting thus mitochondrial dysfunction and an increase of the oxidative stress. To deepen this aspect, here we analyzed the effect of $A\beta_{1\text{-}42}$ oligomers on VDAC1 channel activity at the PLB. We found that, at the transmembrane potential around ±10 mV, Aβ increases the conductance of VDAC1 of about 30%, as well as the channel propensity to switch towards closed states already at low voltages, contrary to what observed in the presence of VDAC1 alone. To analyze the effect of VDAC1-$A\beta_{1\text{-}42}$ interaction for the overall mitochondrial functionality, we investigated the respiratory profile of permeabilized SH-SY5Y cells exposed to Aβ by high-resolution respirometry. We found that Aβ reduces the basal oxygen consumption and the maximal respiratory capacity, affecting the oxygen flow linked to the ADP phosphorylation. Although further experiments with VDAC1-antagonizing peptides are ongoing, this work could shed a light on the molecular mechanisms behind the VDAC1-Aβ interaction and, at the same time, offer the basis for the development of pharmacological tools in AD treatment.

P-32-056
Multi-omics approach identified a network of lipids, small molecules and proteins associated to lysosomal and mitochondrial metabolism in Parkinson's disease patients carrying TMEM175 mutations

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Parkinson's disease (PD) represents one of the most frequent neurodegenerative disorders for which clinically useful biomarkers remain to be identified and validated for early and precise diagnosis and for stratification of disease subtypes, which may require different treatments. Here, we adopted an integrated multi-omics approach to disclose lipidomic, metabolomic and proteomic alterations in plasma and in dermal fibroblasts of PD patients carrying mutations in TMEM175 gene. We revealed a wide dysregulation of lysosome, autophagy, and mitochondrial pathways in these patients, supporting a role of this channel in regulating these cellular processes. The most significant altered lipid classes (CAR, Cer, FA, HexCer, PC, PC O-, SM, PI), and enzymes (PAG15, PP4P1, GALC, FYV1, PIGO, PGPS1, PLPP1) were involved in phosphosphingolipids and glycerophospholipids biosynthetic pathways. We also disclosed alterations of proteins involved in the insulin pathway (IGF2R), mitochondrial metabolism (ACD10, ACD11, ACADS) and autophagy (RAB7L). Altered amino acids metabolic pathways were observed in PD patients. Interestingly, we highlighted that the levels of CAR 18:2, HexCer 42:1;3O, and HexCer 42:2;3O, negatively correlated with age and age at onset (AAO) in PD patients, while the levels of PI 34:1 showed a significant correlation ($r = -0.5509$; $p = 0.006$) with age and earlier AAO of disease only in TMEM175 PD patients. Increased level of L-glutamate strongly correlated ($p < 0.0001$) with the severity of motor and non-motor symptoms in PD all and PD_TMEM175 patients. All together, these data provide novel insights into the molecular and metabolic alterations underlying TMEM175 mutations and may be relevant for PD prediction, diagnosis and treatment.

P-32-057
6-Hydroxy-L-nicotine alleviates cognitive impairments in 5XFAD transgenic mice

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6-Hydroxy-L-nicotine (6HLN) is a nicotinic derivative from the nicotine metabolism within *Paenarthrobacter nicotinovorans* that possess cognitive-improving abilities and antioxidant properties,

eluding the side-effects of nicotine, the parent molecule. The present study was conducted to examine the effects of 6HLN on cognitive impairments in 5XFAD transgenic mice with five familial Alzheimer's disease (AD) mutations. 6HLN (0.3 mg/kg and 0.6 mg/kg, b.w., i.p.) was administered daily to 5XFAD mice for 7 days and 30 min before behavioral testing. Cognitive function was evaluated using Y-maze and radial arm maze tests, while anxiety-depressive-like behaviors were assessed by elevated plus maze and forced swimming tests. To elucidate the possible mechanism underlying the memory-improving effects of 6HLN in 5XFAD mice, Aβ1-42 and DNA fragmentation levels in mice hippocampus were evaluated. Vehicle-treated 5XFAD mice exhibited hippocampus-dependent memory deficits as compared with non-transgenic mice, which were reversed in 6HLN-treated 5XFAD mice. In addition, reduced hippocampal Aβ1-42 and DNA fragmentation levels in 6HLN-treated 5XFAD mice as compared to non-transgenic mice were noticed, indicating positive effects of 6HLN on cognitive function. Collectively, findings from this study support the positive effects of 6HLN against AD. This work was supported by a grant from the Ministry of Research, Innovation and Digitization, CNCS-UEFISCDI, project number PN-III-P4-PCE-2021-1692, within PNCDI III. *The authors marked with an asterisk equally contributed to the work.

P-32-058
Characterization of the transcriptome and proteome effects of glucose in the L3 and L4 developmental stages of *Anisakis simplex*

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Anisakis simplex is a cosmopolitan parasitic nematode of marine organisms characterised by a complex developmental cycle. Consumption of fish containing larvae can pose a serious health risk, as these parasites can penetrate the mucous membranes of the gastrointestinal tract and cause damage to the stomach and intestinal walls, as well as trigger allergic reactions. According to the European Food Safety Authority (EFSA), *A. simplex* has been classified as a biohazardous organism. Most developmental stages of parasitic nematodes occur under anaerobic conditions, and larvae obtain most of their energy from saccharides. We compared and identified differentially expressed genes (DEGs) and proteins (DEPs) of the transcriptome and proteome of L3 and L4 larval stages of *A. simplex* exposed to glucose (10 mg/ml) *in vitro*. High-throughput sequencing (Illumina), proteomic analysis (LC-MS / MS), and bioinformatics were used to achieve this goal. To understand the molecular functions of the identified DEGs (3,843), enrichment analysis was performed using Gene Ontology (GO) (359) e.g., oxidoreductase activity and extracellular spaces pathways. KEGG analysis revealed several changes in the pathways (42), including the enrichment of lysosomes and ECM receptor interaction pathways. In addition, we identified long non-coding RNAs that are mainly expressed to regulate the expression of other genes. The proteome results yielded a total of 1,229 proteins, which were filtered for further analyses. The final repository consists of 199 proteins that were identified in one group as upregulated DEPs (5), e.g. phosphoglycerate kinase, and downregulated DEPs (12), e.g. ankyrin. The results obtained should lead to a better understanding of the molecular processes underlying the development of A. simplex infection in humans and will complement the existing knowledge on the role of nutrients of this parasite. Work supported by the National Science Centre of Poland, grant no.2018/31/B/NZ9/01683. *The authors marked with an asterisk equally contributed to the work.

P-32-059
Preservation of the fertile potential of cryptorchid boys by generating *in vitro* functional *ex-novo* Leydig-like cells starting from gubernaculum testis biopsies

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Cryptorchidism is when one or both testes fail to descend from the abdomen into the scrotum, representing a pathology of andrological interest in pediatric age mostly associated with impaired fertility. To support the fertile potential of cryptorchid patients, a surgical treatment, called orchiopexy, is recommended within the first 18 months of life, followed by the hormonal stimulation with subcutaneous injections of human-chorionic gonadotropin (hCG), which has been demonstrated to increase testicular volume and vascularization. The first aim of this study was to culture *in vitro* cells derived from biopsies of gubernaculum testis, a para-testicular ligament, of cryptorchid patients to obtain stem-like cells through a de-differentiation protocol optimized in our laboratory. The obtained gubernacular stem cells (GSC) present a spheroidal shape and express stem markers. Hence, the second aim was to drive GSC to further differentiate into *ex-novo* testicular cells, specifically Leydig cells, since gubernaculum shares many properties, including LHCGR expression. Indeed, by treating GSC with specific compounds, we obtained cells that grow in adhesion and present lipid droplets, a typical sign of Leydig cells. To further characterize these cells, we analyze the expression of the steroidogenic enzyme CYP11A1 and the production of testosterone. The potential future clinical application of this project is to obtain fully functional Leydig-like cells directly from patients' biopsy with the aim to (1) generate *in vitro* organoids to support spermatozoa maturation in case of spermatogenesis alterations in adult age; (2) reimplant them in the patients with hypogonadism due to congenital causes or oncological diseases. In conclusion, this project aims to preserve the fertile potential of males with andrological diseases at pediatric age.

P-32-060
Photoreactivity of polycyclic aromatic hydrocarbons (PAHs) and its mechanisms of phototoxicity against human immortalized keratinocytes (HaCaT)

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Polycyclic aromatic hydrocarbons (PAHs) are a group of environmental pollutants ubiquitously present in the environment. They are produced by many anthropogenic sources of different origins and are known for their toxicity, carcinogenicity, and mutagenicity. Sixteen PAHs have been identified as Priority Pollutants by the US EPA and are often associated with particulate matter, facilitating their dispersion through air and water. Exposure of human skin to PAHs might occur simultaneously with solar radiation, potentially leading to phototoxic effects. Phototoxic mechanisms may involve the generation of singlet oxygen and reactive oxygen species, DNA damage under specific light wavelengths, and the formation of charge transfer complexes. Although phototoxic properties for some PAHs were calculated before, there remains a paucity of experimental data. The study examined the photoreactive and phototoxic properties of the 16 PAHs enlisted in the Priority Pollutants list. They were found to efficiently photogenerated singlet oxygen and superoxide anion in simple solutions. Furthermore, singlet oxygen phosphorescence was detected in PAH-loaded HaCaT cells. Phototoxicity against human keratinocytes was evaluated using various assays. At 5 nM concentration, examined PAHs significantly reduced viability and mitochondrial membrane potential of HaCaT cells following the exposure to solar simulated light. Analyzed compounds induced a substantial peroxidation of cellular proteins after light treatment. The results revealed that a vast majority of the examined PAHs photogenerated reactive oxygen species under UVA and violet-blue light, with their phototoxicity corresponding to their photoreactive properties. These findings improve our comprehension of the interactions between PAHs and human skin cells under environmental conditions, particularly when exposed to solar radiation.

P-32-061
Truncation of the constant domain drives amyloid formation by immunoglobulin light chains

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Light chain amyloidosis is a life-threatening disease caused by fibrillar deposition of immunoglobulin light chains (LCs). Amyloid deposits in this disorder contain both the full-length monoclonal LC and fragments encompassing its variable domain (V_L) plus different length segments of the constant one (C_L), thus highlighting the potential role of proteolysis in fibrillogenesis. Here we aimed to develop a biocompatible model of LC amyloidogenesis, accounting for the major species present in natural deposits. Focusing on the amylodogenic λ LC AL55, we studied structure, molecular dynamics, stability and amyloid-forming ability of a prototypic fragment containing both the V_L and part of the C_L (133-AL55), in comparison with the full-length protein (FL-AL55) and to its variable domain (V_L-AL55). The three proteoforms were produced recombinantly. Fibrillogenesis kinetics, cross-seeding, aggregate structure and molecular dynamics were assessed under biocompatible, non-denaturing conditions by biochemical techniques, electron microscopy, isotope-edited Fourier-transform infrared spectroscopy and high-resolution NMR. In contrast to the full-length LC, which forms exclusively amorphous aggregates under shear stress, both fragments are amyloidogenic *in vitro*, but with different kinetics, structure of aggregates and interplay with the unfragmented protein. In particular, 133-AL55, with its dynamic truncated C_L, under appropriate conditions entirely converts into amyloid fibrils microscopically and spectroscopically similar to their *ex vivo* counterpart. Although not incorporated into structured fibrils, amorphous aggregation of FL-AL55 is increased by the presence of 133-AL55 fibrils. These data can help us interpret the kinetics of amyloidogenesis in vivo and highlight how specific LC truncations influence amyloid onset and growth. This work raises the issue of the relation between truncated and full-length LC species, which needs further exploration.

P-32-062
The structure of human Orc6 protein – humanized Drosophila model of the Meyer-Gorlin syndrome

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Orc6 is an important component of the origin recognition complex (ORC) and has functions in both DNA replication and cytokinesis. We solved a solution structure of the full length human Orc6 alone and in a complex with DNA by NMR methods. We have shown that in eukaryotes, Orc6 has a homology with transcription factor TFIIB. The detailed analysis of the structure revealed amino acid clusters important for DNA binding. We further showed that human Orc6 is composed of three independent domains: N-terminal, middle and C-terminal. A mutation in the conserved C-terminal motif of Orc6 impedes the interaction of Orc6 with core ORC and results in Meier-Gorlin syndrome (MGS). MGS is a rare autosomal recessive disorder characterized by microtia, primordial dwarfism, small ears, and skeletal abnormalities. Patients with MGS often carry mutations in genes encoding the subunits of ORC, components of the pre-replicative complex (pre-RC) and replication machinery. Recently, additional MGS mutations in Orc6 were identified. One mutation is localized in the N-terminal domain of the protein. Another is a compound heterozygous mutation that includes a deletion of 20 amino acids from the N-terminus as well as a mutation within a splice site of the gene potentially resulting in a short deletion in the middle of the protein. Based on the structural information we created a hybrid human-Drosophila gene to rescue the orc6 deletion in Drosophila and to study the functions of the protein in a living organism. Using this "humanized" Drosophila model of the Meier-Gorlin syndrome we discovered the molecular mechanisms underlying the observed MGS phenotypes. Our

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studies revealed the importance of evolutionarily conserved and variable domains of Orc6 protein and allowed the studies of human protein functions and the analysis of the critical amino acids in live animal heterologous system.

P-32-063
Iron dyshomeostasis and disrupted inositol metabolism unveiled: insights from the yeast model of human N88S seipinopathy

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Seipin, encoded by the *BSCL2* gene in humans and the *SEI1* gene in yeast, forms an endoplasmic reticulum (ER) bound homo-oligomer targeted to ER-lipid droplet (LD) contact sites for triacylglycerol delivery to nascent LDs. Gain-of-function mutations in BSCL2 (N88S and S90L) lead to motor neuron diseases known as seipinopathies. Our well-established yeast model of N88S seipinopathy [1] exhibits inclusion bodies (IB) composed of WT-N88S and N88S-N88S oligomers, and reduced viability due to increased reactive oxygen species (ROS) generation, oxidative damage, lipid peroxidation, and diminished antioxidant activity. Proteomic and lipidomic profiling to this model revealed changes in phosphatidic acid (PA) levels, which is associated with disrupted inositol metabolism and decreased flux towards phospholipid biosynthesis. Altogether, they also contribute to ER stress beyond the formation of IBs. In addition, we report alterations in iron homeostasis. During growth, we observe that N88S seipin-expressing cells display impaired ability to cope with iron deficiency. This is associated with changes in the expression of Aft1-controlled iron regulon genes, including the mRNA binding protein CTH2 and the member of the high-affinity iron transport system FET3, in a p38/Hog1- and stress responsive Msn2-dependent manner. Although these cells display iron accumulation, this is not associated with changes in oxidative stress. This research will work as a major stepping-stone to define the role of proteins and signaling pathways contributing to these disease-related features, and novel therapeutic targets to be translated in human model studies. Funding Work funded by national funds through Foundation for Science and Technology (FCT), under project 2022.02305.PTDC and CEECIND/00724/2017, CEECIND/00724/2017/CP1386/CT0006. References: [1] Nogueira V et al. Free Radic Biol Med. (2022) 192:165-181.

P-32-064
Role of ADAMTS4 in the recruitment of myeloid-derived immune cells during liver fibrogenesis

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Hepatic fibrosis is characterized by impaired cell-to-cell interactions within the liver tissue due to abnormal deposition of proteins like collagen and elastin. Therapeutic strategies have been developed based on the activity of matrix metalloproteinases (MMPs), which play a role in the degradation of the extracellular matrix. A disintegrin and metalloproteinase with thrombospondin motifs 4 (ADAMTS4) is a secreted protein that is a member of the ADAMTS subfamily. It contains a protease domain with zinc-dependent proteinase activity, similar to MMPs, but unlike MMPs, it possesses an ancillary domain with thrombospondin motifs, enabling it to bind more effectively to proteoglycans. Thus, these structural differences allow ADAMTS to more selectively degrade chondroitin sulfate proteoglycans, including versican and aggrecan, contributing directly to the generation of matrikines. Our study revealed an increased expression of ADAMTS4 in the liver tissue of human metabolic dysfunction-associated steatotic liver disease (MASLD) patients and mouse liver fibrosis models. The most significant increase was observed in hepatic stellate cells (HSCs) among non-parenchymal cells under the context of fibrosis. Diet- or chemical-induced liver fibrosis in *Adamts4* knockout mice resulted in an improvement in fibrosis markers, accompanied by a significant reduction in the recruitment of myeloid-derived immune cells. Furthermore, we demonstrated that TNFα secreted during MASLD progression accelerates the expression and secretion of ADAMTS4 in HSCs and hepatocytes. ADAMTS4 increased the secretion of versikine from HSCs, and the generated versikine contributed to the recruitment of macrophages and their differentiation into the M1 phenotype, thus accelerating hepatitis and involvement in fibrogenesis. These findings propose ADAMTS4 as a novel therapeutic target for the inhibition of hepatic fibrosis.

P-32-065
The effect of hemorphin on antioxidant status in pathophysiology of STZ-induced diabetes

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The major role of oxidative stress in development of diabetes and progression of diabetic complications is well known. Diabetes is accompanied by enhanced generation of reactive oxygen species, low levels and reduced activity of endogenous antioxidant system. Hemorphins, members of the endogenous protective system of the organism, act as homeostatic agents in response to severe disease conditions, including diabetes. Earlier, we have established that Ca^{2+}/calmodulin (CaM)-dependent protein phosphatase calcineurin is involved in molecular mechanisms of antidiabetic effect of LVV-hemorphin-3 (LVV-H3). Calcineurin closely interacts with components of oxidant/antioxidant systems. It was suggested that hemorphin via modulation of Ca^{2+}/CaM/calcineurin-dependent pathway may affect antioxidant status in pathophysiology of diabetes. We investigated the effects of hemorphin treatment on activity of antioxidants, i.e. superoxide dismutase (SOD), catalase (CAT), glutathione (GSH), and thioredoxin (TRX) in plasma of streptozotocine (STZ)-induced diabetic rats. Results showed that intraperitoneal injection of synthetic LVV-H3 (1 mg/kg) significantly increased the activity of SOD (34%), CAT (81.4%) and TRX (33.7%), but not GSH in plasma of diabetic rats compared to control. Injection of calcineurin inhibitor cyclosporine A prior to hemorphin treatment demonstrated involvement of calcineurin signaling pathway in LVV-H3 effects on SOD, CAT and TRX, but not GSH activity in STZ-induced diabetic rats. Hence, hemorphin may improve

antioxidant profile impaired in diabetes and recover the redox homeostatic disturbance. These results provide additional evidence about antidiabetic efficiency of LVV-H3. Summarizing our findings, we suggest that LVV-H3 can be considered for development of antidiabetic drugs, which along with hypoglycemic effect will also possess antioxidative effect to eliminate diabetic complications. *The authors marked with an asterisk equally contributed to the work.

P-32-066
The BAS, a SWI/SNF-type chromatin remodeling complex, affects organ-specific transcription start site choice in Arabidopsis

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Eukaryotic DNA is packaged into the nucleus in the form of chromatin, enabling its storage in limited space. However, chromatin formation restricts DNA accessibility to proteins involved in DNA-dependent processes, such as transcription. To maintain proper gene expression, chromatin must be remodeled. One subfamily of the chromatin remodelers is ATP-dependent SWI/SNF (SWItch/Sucrose Non-Fermentable) chromatin remodeling complexes (CRCs). The SWI/SNF CRCs are proved to impact transcription rates through chromatin structure change, however still little is known on their role in alternative transcription regulation. Alternative transcripts arise from alternative splicing or alternative start/termination site choice and lead to different protein structure and abundance, increasing genome capacity. Therefore, unraveling the impact of SWI/SNF CRCs on alternative transcription provides a valuable information on its contribution beyond gene expression level control. This research aims to determine the role of the BAS (BRM-associated SWI/SNF) CRC in organ-specific alternative transcripts formation through transcription start site (TSS) choice in Arabidopsis. Differences in TSS choice in leaves and flowers were analyzed for wild-type plants and two mutants of the BAS complex subunits – *brm* and *swi3c*. Both mutants showed decreased number of organ-specific TSSs indicating an important role of SWI/SNF CRCs in TSS choice. Chosen genes were verified to confirm direct influence of BAS CRC on chromatin structure and histone modification landscape of their promoter regions, as well as biological effects of particular TSS choice to reveal potential mechanism of SWI/SNF CRCs action and its importance for organ identity. Our results prove the direct functional relationship between chromatin remodeling executed by the BAS CRC and organ-specific TSS choice in Arabidopsis. Project foundation: National Science Centre DEC-2018/29/B/NZ1/01935 and 2021/43/D/NZ2/02461 given to SK.

P-32-067
The contribution of genetic factors to the process of prostate cancer in the Kazakh population

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Prostate cancer (PC) is the most common malignancy in men. More than 7% of cases are associated with hereditary risks. With the introduction of NGS, more opportunities have arisen to search for genetic polymorphisms that increase the risk of developing PC. However, such studies in the Kazakh population are currently insufficient. The research aims to determine the spectrum of pathogenic variants (PVs) associated with PC development. The study included 48 PC patients (the Gleason score (≥7) was used as the selection criteria) and 22 individuals with inflammatory prostate diseases and confirmed the absence of malignant neoplasms. NGS was performed using TruSight Rapid Capture kit on the Illumina MiSeq platform. The data were annotated by SnpEff and SnpSift. The study was funded by MSHE RK AP19680315, "Clinico-genetic aspects of prostate cancer development in the population of Kazakhstan based on targeted DNA typing and sequencing". In the results, we detected PV in the EHBP1 gene c.1185 + 30064G>A, rs721048 in 10 PC patients (9 heterozygous patients, one homozygous patient). According to ClinVar, Varsome, this mutation is pathogenic and increases the risk of developing PC. The proportion of patients with PVs was 21%. In the control group without PC, rs721048 was detected in 3 individuals, which accounted for 13%. The average age of patients with the detected heterozygous PV was 65 years (6074 years). The patient's age with the heterozygous PV was 55 years (Gleason score 10). The average age of patients without a PV was 68 years (6092 years). The average age in the control group is 66 years (5679 years). For the first time, this study demonstrates the frequency of occurrence of rs721048 in the Kazakh population, which corresponds to international databases. The identification of rs721048 among the male population of Kazakhstan will help form groups at increased risk of developing PC. *The authors marked with an asterisk equally contributed to the work.

P-32-068
How can an *Arabidopsis thaliana*-based model can help to better understand regulatory processes impaired in cancer?

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According to WHO survey, in 2022, there were an estimated 20 million new cancer cases and 9.7 million deaths. Due to this serious social problem, research on deciphering the mechanisms of tumor formation and progression, also using various model systems, is extremely important. *Arabidopsis thaliana* is a perfect model for studying important regulatory processes governing the cell and whole organism functioning including epigenetic control of gene expression. In our study we employ Arabidopsis and human cancer cell lines- based models to better understand the regulatory processes which are impaired in cancer or may lead to carcinogenesis. Malignant tumors are characterized by defects/impairments of various important regulatory pathways, e.g. related to the control of gene expression. Approx. 25% of all malignancies are characterized by mutations in genes encoding subunits of SWI/SNF chromatin remodeling complexes (CRCs), which represent evolutionarily conserved epigenetic regulators controlling gene expression through chromatin remodeling. Using viable Arabidopsis lines carrying mutations in genes encoding subunits of SWI/SNF chromatin remodeling complex we deciphered the not yet recognized interdependences between ATP-dependent chromatin remodeling and elements of various regulatory pathways including kinases controlling metabolism and plasma membrane receptors. We have subsequently shown that the human counterparts of these key factors, frequently related to cancer development and progression, undergo similar regulation indicative of it can be a general paradigm for the transcriptional control of gene expression maintained between plant and animal Kingdoms. Moreover, our findings executed in the direction from plants to human enabled us to propose numerous, new molecular targets, which may serve in the future as the basis for innovative cancer treatment basing e.g. on the synthetic lethality phenomenon. Funding: Narodowe Centrum Nauki: 2018/30/M/NZ1/00180

P-32-069
AREG regulates expression of nucleolar proteins and stimulates growth of renal cancer

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Clear cell renal cell cancer (ccRCC) is characterized by dysregulated nucleolar morphology that correlates with tumor malignancy. In our recent study we found that ccRCC cells of advanced tumors secrete amphiregulin (AREG) (Poplawski et al., 2023). Here, we hypothesized that AREG may contribute to the growth and nucleolar dysfunction of ccRCC tumors. ccRCC cell line 786-O was stably transduced with GFP plasmid expressing AREG or empty vector. AREG expression was verified using qPCR and ELISA. Proteomic analysis was performed using LC-MS/MS. The cells were subcutaneously inoculated into the flanks of immunodeficient nude mice (approved by the local Ethics committee). Tumor growth was monitored weekly for at least 5 weeks. Following mice sacrifice, tumors were weighed and pulmonary metastasis was monitored. ELISA and LC-MS/MS confirmed increased secretion of AREG in the transfected cells. Tumors resulting from 786-0-GFP-AREG cells were statistically significantly bigger when compared with control cells. Proteomic analysis revealed that AREG overexpression altered expression of 343 proteins. Gene Ontology analysis revealed that the top enriched cellular compartment was the nucleolus. 77 out of the 343 altered proteins were confirmed as associated with nucleolar morphology/function. AREG induces growth of ccRCC tumors *in vivo* and affects expression of nucleolar proteins. Financed by National Science Centre grants 2018/29/B/NZ5/01211 and 2019/35/B/NZ5/00695. Reference: Popławski P et al (2023) Stem Cell Res Ther 2023, 14(1):200.

P-32-070
HMG-CoA reductase deficiency results in disturbed fatty acid β-oxidation and accumulation of long-chain acylcarnitines in the mouse liver

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HMG-CoA reductase (HMGCR) is a pivotal enzyme in the cholesterol and isoprenoid biosynthesis pathway and is the target of statin drugs widely used for cholesterol-lowering therapy,

especially among the elderly in Western countries. Despite statin efficacy, 5-30% of patients experience side effects, leading to reduced adherence. The mechanisms behind these adverse reactions, particularly in the liver and muscles, remain elusive. To address this, we employed the Cre/loxP system to create a tamoxifen-inducible Hmgcr-knockout mouse model, allowing us to explore the impact of reduced HMGCR activity on liver mitochondrial and peroxisomal energy metabolism. Transcriptomic and metabolomic analyses, along with mitochondrial function assessment, unveiled time-dependent changes in metabolic functions. The Hmgcr-KO mice exhibited early-onset lethal liver failure, characterized by significantly elevated ALAT concentrations (600 U/L). High-resolution respirometry measurements revealed a marked decrease in mitochondrial respiration rates in liver tissues. The further progression of liver failure stemmed from peroxisome dysfunction, followed by impaired mitochondrial and peroxisomal fatty acid metabolism, resulting in heightened glucose utilization in the liver and subsequent whole-body hypoglycemia. At the study endpoint, the mitochondrial fatty acid oxidation rate decreased significantly by 2- to 3-fold. Dysfunctional mitochondrial and peroxisomal β-oxidation of long-chain fatty acids led to a remarkable up to 8-fold accumulation of long-chain acylcarnitines. Our findings underscore the crucial role of HMGCR in maintaining effective mitochondrial and peroxisomal β-oxidation in the liver, impacting whole-body energy homeostasis. Addressing the prevention of long-chain acylcarnitine accumulation emerges as a potential novel therapeutic strategy for metabolic liver diseases and statin intolerance.

P-32-071
Alterations of peripheral blood mononucleated cell metabolism in phosphomannomutase-2 deficiency

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Phosphomannomutase-2 (PMM2) deficiency represents the most common congenital disorder of glycosylation (CDG). To date, little is known about the potential alterations of cell metabolism characterizing these patients. Using a targeted metabolomic approach, we measured the concentrations of compounds connected to protein glycosylation (GDP-mannose and UDP-derivatives), energy metabolism (high-energy phosphates, nicotinic coenzymes, oxypurines), oxidative/nitrosative stress biomarkers (GSH, nitrite and nitrate) and free amino acids in peripheral blood mononucleated (PBMCs) cell extracts, obtained from a cohort of compound heterozygous PMM2-CDG patients, carrying the same R141H mutation on one allele. Results were compared with those recorded in PBMCs from control healthy donors. Besides showing a marked decrease of GDP-mannose, PBMCs of PMM2-CDG patients were characterized by higher values of UDP-glucose and UDP-galactose, lower values of ATP, GTP, UTP, altered values of ATP/ADP and NAD^+/NADH ratios, increased levels of xanthine, uric acid, biomarkers of oxidative/nitrosative stress and various free amino acids (asparagine, glutamine, serine, threonine, histidine, tryptophan and arginine), suggesting that an imbalance in cell energy metabolism is triggered by the PMM2 deficiency. These results may contribute to define a novel destiny of mannose 1-phopsphate accumulation in cell metabolism of PMM2-CDG patients, leading to the increase of cellular UDP-glucose, thus supplying a potential explanation for the anomalous liver glycogen accumulation of PMM2-CDG patients. Together with higher UDP-galactose levels, high cell UDP-glucose content may also uncouple the glutaminolysis-glycolysis axis, consequentially causing the incorrect utilization of nutrients to produce energy and activate substrates. Altogether, these results highlighted characteristics metabolism in PMM2-deficient cells, that may contribute to set up new therapies specifically targeted to PMM2-CDG patients.

P-32-072
Two novel biallelic missense variants in Spartin alter the mitochondrial protein import process leading to OXPHOS and Coenzyme Q10 biosynthesis impairment

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Troyer syndrome (TS) is an autosomal-recessive form of hereditary spastic paraplegia characterized by spasticity, weakness, cognitive impairment, and severe mitochondrial dysfunction. TS is caused by pathogenic variants in SPG20 gene which encodes for Spartin, a nuclear-encoded protein. It has been largely demonstrated that Spartin plays a pivotal role in several cell functions, such as the endosomal trafficking and mitochondrial membrane potential maintenance. The identification of two biallelic missense variants in SPG20 in a young patient with developmental delays and muscle weakness prompted further investigations. Firstly, we performed a bioenergetic characterization of the patient-derived fibroblasts which exhibited a decreased mitochondrial oxygen consumption rate, ATP/ADP ratio, and a rise in oxidative stress, as evidenced by increased ROS production, lipid peroxidation, and decreased levels of glutathione (1). Moreover, we found that Spartin mutation affected the expression of the proteins involved in the mitochondrial associated membranes assembly and nuclear-encoded protein import. Western blot analysis revealed a significant decrease in the levels of the NDUFA9 complex I subunit and the SDHA complex II subunit and, consequently, a decrease in the activity of the respective respiratory complexes. Additionally, patient's fibroblasts showed a decreased COQ7 and COQ9 expression levels, two enzymes involved in the last steps of CoenzymeQ10 (CoQ) biosynthesis, followed by a drop in the total cellular CoQ amount. Altogether, these data prompt us to speculate that Spartin is necessary for the mitochondrial functionality being involved in the protein import machinery. The low CoQ levels detected in TS patient cells may point to CoQ treatment as a potential therapeutic approach. Previously

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P-32-073
Biochemical characterization of ER stress mediators in SEPN1 and RYR1-related myopathy

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SEPN1 and RyR1-related myopathies belong to the common class of multi-mini core diseases and are caused by mutations in *SELENON* and *RYANODINE RECEPTOR type1* genes, encoding SEPN1 and RyR1 proteins, respectively. These myopathies are characterized by impaired redox and calcium homeostasis accompanied by endoplasmic reticulum stress (ER) at molecular levels. Here, we analyze the pathogenic role of the ER stress mediator ER oxidoreductin-1 (ERO1A) and of the ER stress-mediated defect in protein synthesis in these two diseases. SEPN1 is a type II protein of the ER involved in calcium homeostasis by regulating the calcium pump SERCA. ERO1A is upregulated in SEPN1-devoid preclinical models, suggesting that ERO1 might be a biomarker of SEPN1-RM. Furthermore, combined immunoprecipitation and mass spectrometric analysis identify a key interaction between SEPN1 and ERO1A. The two proteins interact in a redox-dependent manner and the redox inactive oligomeric SEPN1 levels depend on ERO1A, suggesting cross-regulation of their respective activities. *RYR1* encodes from the ryanodine receptor isoform 1, an ER calcium channel involved in calcium release from ER whose activity is critical for muscle contraction. I4895T is a calcium channel RyR1 mutation that causes excitation-contraction uncoupling, thus enfeebling muscle contraction. ER stress with augmented expression of GADD34, a phosphatase of the alpha-subunit of elongation eukaryotic factor (eif2alpha), is upregulated in skeletal muscles from RYR1I4895T knock-in mice, suggesting a defect in protein synthesis. We present preliminary data on the impaired protein synthesis associated with $RYR1^{I4895T}$ by a SUnSET method and a genetic and pharmacological approach to potentially rescue such a defect. This study sheds light on the pathogenic role of specific ER stress response mediators in SEPN1 and RyR1-related myopathies and it is pivotal for implementing a targeted therapy for such diseases.

P-32-074
Identification of a novel TUBB2B gene mutation associated with polymicrogyria

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Polymicrogyria (PMG) is a relatively common complex malformation in cortical development characterised by an exorbitant number of abnormally small gyri separated by shallow sulci due to a neuronal migration disorder. The genetic basis of PMG is emphasised by its occurrence in familial clusters and its association with chromosomal abnormalities and mutagenic events. Mutations associated with irregular patterns of neuronal migration and structural organisation include non-synonymous and splicing mutations in the genes encoding the α- and β-tubulin isotypes TUBA1A, TUBB2B, TUBB3 and TUBA8. In the TUBB2B gene, a total of eight missense mutations have been described that are associated with brain malformations. In this study, we reported a novel de novo heterozygous mutation c.751C>A in the 4 exon of the TUBB2B gene in a 4-year-old girl with bilateral perisylvian PMG, multifocal seizures, neurodevelopmental delay and autism. Neuroimaging revealed dysplastic changes in the sylvian fissures extending into the parietal region. Other dysmorphic manifestations included a dysplastic corpus callosum, dilated perivascular spaces, asymmetric lateral ventricles of the brain and pituitary gland, expansion of the subarachnoid space of the cerebral hemispheres in the anterolateral and mediobasal regions of the temporal lobes, and MRI evidence of asymmetry of the vertebral arteries. Whole-exome sequencing of the trion clarified the *de novo* origin of the detected mutation. It is a missense mutation leading to an arginine to serine substitution (Arg251Ser), which is predicted *in silico* to likely affect the structure and function of the protein. To our knowledge, this mutation has not yet been published as pathogenic. Our findings broaden the spectrum of cerebral malformations associated with mutations in the β-tubulin gene TUBB2B and highlight its important role in the processes of neuronal migration, cortical structural organisation and axonal pathfinding. *The authors marked with an asterisk equally contributed to the work.

P-32-075
Identification of the role of TG2 on the expression of TGFβ, TIMP1 and TIMP2 in aged skin

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Transglutaminase 2 (TG2) is a unique protein having enzymatic and nonenzymatic functions that have been implicated in various biological and pathological processes such as cell survival and apoptosis, cell signaling, differentiation, adhesion and migration, wound healing and inflammation. As reported in previous studies, TG2 expression and activity increase by age suggesting that TG2 possibly has roles in cellular aging process. In this study, we aimed to explore the role of TG2 in chronological skin aging through its impact on the expression of some important extracelular matrix (ECM) proteins including TGF-β, TIMP-1 and TIMP-2. We have compared TG2 expression and activity in young and *in vitro* chronologically aged human dermal fibroblasts via western blot and *in situ* TG2 activity assays. Afterwards, we inhibited TG2 expression via siRNA transfection and activity via active site inhibitor of TG2 separately in aged dermal fibroblasts and monitored the expression levels of TGF-β, TIMP-1 and TIMP-2 in these cells by western blot and compared to that of untreated control cells. We obtained evidence that both TG2 expression and activity increase in aged cells. However, protein levels of TGF-β, TIMP-1 and TIMP-2 do not exhibit any significant difference in TG2 downregulated or TG2 activity inhibited aged cells compared to control cells. Our results indicate that changes in the expression and activity of TG2 in (*in vitro*) chronologically aged human dermal fibroblasts do not impact the expression patterns of TGF-β, TIMP-1 and TIMP-2 proteins.

Biosensors

P-33-001
Electrochemical sensor systems for detecting cell culture contaminants

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Cell cultures are pivotal in biomedical research, yet contamination poses a significant challenge. Microbiological organisms and cross-contamination are key concerns. We focused on microbiological contamination, particularly *Mycoplasma fermentas*, and developed two biosensor systems. Biosensors utilize biological elements like enzymes, antibodies, or peptides to detect analytes. The first system employed surface-printed gold electrodes (AuSCE) and peptides. Characterized by CV and EIS, it achieved a LOD of 8.85 CFU/ml. The second system aimed at a CRISPR/cas9-based biosensor for *Mycoplasma* detection. This adaptable system, using AuSCE electrodes, allowed DNA-dependent measurements for specific identification and precautionary measures. Binding of AuSCE/dCas9-sgRNA was facilitated by cysteamine modification. CV and EIS confirmed its efficacy with a LOD of 8.85 CFU/ml. Selectivity and repeatability studies were carried out, and the recovery was higher than 95%. Validation studies included *Mycoplasma fermentas* DNA banding pattern analysis via agarose gel electrophoresis. In summary, both systems offer direct *Mycoplasma fermentas* detection and show potential for adaptation to other microorganisms.

P-33-002
Tuning the spectral properties of luciferases using small chemogenetic fluorescent reporters

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The emission maximum of the luciferase Nanoluc has been pushed to higher wavelength more suitable for biological imaging by fusion to fluorescent proteins or self-labeling tags (e.g. HaloTag) enabling bioluminescence resonance energy transfer (BRET), resulting however in a significant increase of its size. Here, we create fusions of Nanoluc with pFAST, a small protein tag(14kDa) that can form tunable fluorescent assemblies with fluorogenic chromophores (Benaissa et al. Nat.Commun. 2021, 12(1):6989). This approach allowed us to push the spectral properties of Nanoluc to higher wavelength while keeping the overall size of the luciferase reasonable. The ability to tune the spectral properties of pFAST changing the chromophore used allowed us to optimize the BRET efficiency and generate chimeric luciferase with tunable emission from green to red, opening great prospect for bioimaging and the design of new biosensors.

P-33-003
Kinases in motion: impact of protein and small molecule interactions on kinase conformations

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Protein kinases act as central molecular switches in the control of cellular functions. Alterations in the regulation and function of protein kinases may provoke diseases including cancer. In this study we investigate the conformational states of such disease-associated kinases using the high sensitivity of the kinase conformation (KinCon)-reporter system. We first tracked BRAF-kinase activity conformation changes upon melanoma drug binding. Second, we also use the KinCon reporter technology to examine the impact of regulatory protein interactions on LKB1-kinase tumor suppressor functions. Third, we explore the conformational dynamics of RIP-kinases in response to TNF-pathway activation and small molecule interactions. Finally, we show that CDK4/6 interactions with regulatory proteins alter conformations which remain unaffected in the presence of clinically applied inhibitors. Apart from its predictive value, the KinCon technology helps identify cellular factors that impact drug efficacies. The understanding of the dynamics of full-length protein kinases when interacting with small molecule inhibitors or regulatory proteins is crucial for designing more effective therapeutic strategies. *The authors marked with an asterisk equally contributed to the work.

P-33-004

Abstract withdrawn.

P-33-005
Development of a machine learning-enhanced rapid diagnostic kit for assessing ovarian reserve using AMH Levels with aptamer-based biorecognition with smartphone integration

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Infertility poses a significant public health challenge globally, impacting individuals on multiple levels including psychological, physical, mental, spiritual, and medical. Ovarian reserve refers to the pool of oocytes within the ovaries, with variations in number and/or quality significantly affecting fertility potential. Traditional methods for evaluating ovarian reserve, such as ultrasound egg counting and day-3 follicle-stimulating hormone (FSH) measurements, have limitations in accuracy and predictive value. Consequently, Anti-Müllerian Hormone (AMH) levels have emerged as a critical biomarker, offering insights into the primordial follicle pool and representing a constant, cycle-independent indicator of ovarian health. This project introduces a novel

approach to fertility diagnostics by developing an affordable, reliable, and rapid diagnostic kit aimed at measuring AMH levels to assess ovarian reserve. This kit incorporates lateral flow assay technology with a significant innovation: the use of aptamers as biorecognition elements. Aptamers are synthetic molecules that bind specific targets with high affinity and specificity, offering a robust alternative to traditional antibodies. Their advantages include greater stability, lower cost, and the ability to be easily modified, making them ideal for sensitive and selective detection of AMH levels. AMH levels were calculated by Python programming to investigate the lateral flow test by imageJ. Aptamers are conjugated bu gold nanoparticles and embedded in glass paper for AMH detection. The detection levels were 0.05 ng/ml and 10 ng/ml. R2 was calculated as 0.9981 and detection time was obtained in 4 min. The project uniquely integrates machine learning algorithms with lateral flow assays, enhancing the kit's ability to provide accurate and quick analyses through smartphone integration.

P-33-006
A novel fluorescent Schiff base grafted on dialdehyde nanocellulose for the selective detection of Fe^{2+} and Fe^{3+} in environmental and biochemical applications

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In biological systems, metals and transition metals have fundamental roles, explain and supporting different proteins and enzymes. Variation in their amounts and in their oxidation states can remarkably modify the steady states of living organisms and of consequent biological functions. Starting from these points of view, we have tried to synthetize a new biological sensor, based on fluorescent Schiff base grafted on dialdehyde nanocellulose (Py-PDA-DANC) fluorescent chemo-sensor, giving rise to the new blue-fluorescent complex. All the synthesized products were characterized using by different spectroscopic techniques (such us FTIR, XRD, EDX and UV-VIS spectroscopy) analyzed for their biocompatibility and their ability to interact with various metal ions (Co^{2+}, Cr^{3+}, Cu^{2+}, Fe^{2+}, Fe^{3+}, Hg^{2+}, K^{+}, Mg^{2+}, Mn^{2+}, Na^{+}, Ni^{2+}, Pb^{2+} and Zn^{2+}). The fluorescent sensor showed high and selective quenching response to both Fe^{2+} and Fe^{3+} ions with low limit of detection (LOD), resulting promising for the determination of these pollutant heavy metal ions in biological environments. Py-PDA-DANC was also successfully applied for sensing of heme-iron coordinated to hemoglobin and methemoglobin, displaying a LOD of 28.45 nM and 55.18 nM, respectively. Subsequently, it was applied for the analysis of hemoglobin and methemoglobin and the corresponding amounts of Fe^{2+} and Fe^{3+} amount in biological samples with recoveries and sensibility comparable or superior to the one present in the commerce. This work has been partially funded by the European Union (Next Generation EU), through the MUR-PNRR project SAMOTHRACE (ECS00000022).

P-33-007
Generation of an innovative dual-display phages for *in vitro* diagnosis

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Phage display is a technique based on the genetic addition of nucleotide sequences in-frame at one of the genes coding for a capsid protein of a specific bacteriophage. It is mainly used for the discovery of biomarkers, the development of therapeutic antibodies, and the investigation of protein-protein interactions. A challenge in using phages as bio-probes in diagnostic and therapeutic monitoring assays is the need for a detection system that reveals the interaction between the peptide exposed on the phage capsid and the target of interest. Here, we generated a dual-display phage capable of detecting and quantifying the interaction phage/target. We manipulated the M13K07 helper phage by adding the genes coding for green fluorescent protein (sfGFP) or Avi-tag peptide in-frame to gene 3 of the M13 phage. Then, we developed a dual-display phage by combining our newly established pIII-engineered phage helper vector with a phagemid vector harboring the engineered pVIII with a peptide probe. Thereby, the sfGFP or Avi-tag and peptide-probe are displayed on the same phage fused to the pIII and pVIII, respectively. To create a biotin-tagged phage engineered with Avi-tag, we further constructed a plasmid carrying the BirA gene capable of functioning in *E. coli* and hosting the phage and/or phagemid vectors. Remarkably, our dual display phage can be visualized without signal amplification. Our system allows for easy exchange of the displayed peptide probe on the pVIII to achieve the desired selectivity while maintaining the pIII-engineered gene. To illustrate its effectiveness, we performed proof-of-concept studies on *P. aeruginosa*, *E. coli*, and the SH-SY5Y cell line, demonstrating the capability to detect and quantify the reaction using a plate reader or fluorescence microscopy. We expect that multifunctional phages will increase the development of simple detection systems for in-vitro diagnosis, reducing time and costs compared to current detection systems.

P-33-008
Synergistic screening of valency and affinity for enhanced target recognition sensitivity and selectivity in multivalent interactions

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Multivalent interactions, with multiple binding sites on molecules or particles, play a crucial role in various biological processes, enhancing overall interactions through weak binding. This utility extends to materials and nanoparticles in vaccine development,

drug delivery, and sensing. However, concerns arise in biomedical applications due to increased nonspecific binding with elevated valency, leading to reduced selectivity. To apply multivalent interactions, strategies must be devised to decrease non-specific interactions. Lee et al. demonstrated that nonspecific binding increased with valency, necessitating the introduction of negative charge to mitigate unwanted interactions. Another approach, 'superselectivity', emphasizes discrimination based on relative surface densities of a receptor, enhancing selectivity without relying on distinct receptor types. Experimental validation using short DNA-coated magnetic nanoparticles confirmed the principle that many weak bonds are more selective than a single strong bond. Optimal selectivity and sensitivity in biomedical applications require a precise understanding of the correlation between valency, affinity, and signal. Using oHelix, coiled-coil protein with discrete valency changes, and ssDNA, whose affinity can be finely controlled, we quantitatively evaluated multivalent interactions via surface plasmon resonance (SPR) analysis. Biotin–avidin conjugation facilitated modular coupling between DNA and protein. Evaluation of multimerized ssDNA interactions with surface-immobilized ssDNA was also performed using ELISA and dot blot techniques, commonly employed in biosensing applications. In conclusion, this comprehensive exploration underscores the critical balance between valency and affinity in multivalent interactions, offering practical insights for enhancing selectivity and sensitivity in various biomedical applications. Previously published in: Lee et al. (2017) Angew. Chem. Int. Ed. 56, 15998.

P-33-009
Solvatochromism: a revolutionary tool for precise monitoring of polarity in delivery nanoplatforms for potential biological applications

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The modern world confronts us with numerous challenges that can only be effectively addressed by implementing modern, interdisciplinary approaches. As civilization advances, human life expectancy lengthens, yet the problem of aging remains continuously present. Understanding the profound biochemical mechanisms and complex metabolic processes thus becomes crucial for developing effective therapeutic strategies. Biosensors enable precise and rapid detection of chemicals, and they are widely applied in various fields of science, technology, and everyday life. Solvatochromic dyes, as molecular probes, can be utilized to monitor the local micropolarity of nanocarriers used in the pharmaceutical and medical industry, thereby facilitating a better understanding of the solubilization process of active substances within the interior. In this study, nano and microemulsion systems were designed and optimized to achieve delivery nanoplatforms with high stability and morphological uniformity. The results were confirmed using dynamic and electrophoretic light scattering techniques. The same surfactant and oil phase were utilized in both cases, with only changes in the proportions of the components to create the desired type of nanoplatform. Differences in ambient micropolarity were monitored using a UV-Vis spectrophotometer and a solvatochromic probe. Variations in micropolarity between the systems were observed, with the UV-Vis spectra of the microemulsions displaying a bathochromic shift compared to the nanoemulsions, indicating slightly different solubilization patterns. These observations could lead to breakthroughs in the field of drug delivery systems. Manipulation of the composition and formulation of the nanoplatform can effectively enhance solubilization, and stability, and ensure high bioavailability of the active compound, consequently improving therapeutic efficacy. Research supported by WUST Department of Physical & Quantum Chem. with thanks to FEBS for bursary program

P-33-010
Exploring upper reference limits (URLs) for two immunoassays for galectin-3 serum concentration measurement

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Galectin-3 (Gal-3) is a β-galactoside binding lectin known to play a pivotal role in the development and progression of heart failure (HF). While the current HF biomarker, NT-proBNP, is released after the onset of the pathophysiological process, Gal-3 holds promise as an early indicator of cardiac muscle remodelling that occurs in HF. This study aimed to compare the results and establish an upper reference limit (URL) for a healthy population using two immunoassays for Gal-3 determination: chemiluminescence micro-particles immunoassay (CMIA) on the Abbott Alinity-i automatic analyser, and R&D ELISA assay in 96-well plate. The study involved 150 volunteers (mean age 59 years, age range: 22–86 years) without any cardiac pathological changes (84 female and 66 male). Results indicated that ELISA and CMIA methods cannot be used interchangeably due to the utilization of different antibodies. The established URLs for ELISA and CMIA methods were 14.09 ng/mL (90% CI: 10.7019.80) and 25.05 ng/mL (90% CI: 22.2028.40) respectively. Gender had no significant impact on URLs in both methods. However, results obtained with CMIA method showed statistically significantly age-dependent differences (cut-off age: 60 years; URL < 60 years: 24.33 ng/mL (90% CI: 16.8026.70 ng/mL), URL ≥60 years: 25.43 ng/mL (90% CI: 22.2029.30)) ($p = 0.001$) and estimated glomerular filtration rate (eGFR)-dependent (cut-off eGFR: 90 mL/min/1.73 m^2; URL eGFR < 90: 26.96 ng/mL; 90% CI: 24.70–29.30, URL eGFR ≥90: 19.95 ng/mL (90%CI: 19.50–26.70)) ($p = 0.002$) URLs. These findings suggest that when the CMIA method is used for Gal-3 measurement, different URLs should be applied for individuals older than 60 years and those with impaired renal function (eGFR <90 mL/min/1.73 m^2). In conclusion, caution should be exercised when using CMIA for Gal-3 measurement on the Abbott Alinity-i automatic analyser in routine clinical practice, particularly concerning age and impaired renal function.

P-33-011
Ultrasensitive *in vitro* diagnostics using luminescent lanthanide-ion based nanoparticles

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Swift identification of pathogens (viruses, bacteria, toxins, or parasites) and biomarkers in diverse environments (bioaerosols, water, food matrices, etc.) or biological samples (blood, serum, urine) is vital for preventative, diagnostic, and therapeutic actions. Current protein detection methods either boast ultrahigh sensitivity (~fM), with associated high costs and implementation challenges, or lack the sensitivity needed for early detection of specific biomarkers like p24 or botulinum toxin. Our research demonstrates that the application of luminescent YVO4:Eu nanoparticles effectively lowers the limit of detection (LOD) for multiple targets, employing an approach akin to conventional detection methods such as ELISA, leveraging the unique optical properties of these nanoparticles. Rare-earth doped vanadate nanoparticles exhibit broad-band, strong absorption of the vanadate matrix around 280 nm, followed by energy transfer to several lanthanide ions leading to emission with large Stokes shifts and narrow emission bands. These latter characteristics facilitate the efficient rejection of unwanted signals. These nanoparticles have enabled ultrasensitive detection using a simple ELISA-like protocol and an economical, portable, homemade microplate reader. Specifically, the detection sensitivity for insulin (utilized as a proof-of-concept analyte), interferon-γ, and HIV p24 was 10,000×, 100×, and 40× better, respectively, compared to a commercial ELISA test employing the same antibodies [1]. Moreover, at a low cost and high sensitivity, we've expanded this technology to nucleic acid detection, successfully detecting the n1 gene of SARS-CoV-2 at concentrations similar to those accessible with standard PCR Reference: [1] R. Kuhner, C. et al. Procédé de détection ultra-sensible à l'aide de particules photoluminescentes, FR2308669 11/08/2023

P-33-012
Optimization of LAMP-based detection of GMO in agriculture

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The range of genetically modified organisms (GMOs) and their diversity is continually growing, which makes their detection harder than ever before and significantly hinders food traceability, safety control and monitoring. Current research indicates that GM crop technology can pose risks to human health and environment. The aim of this study was development of an experimentally supported conceptual framework for the design of a LAMP-based GMO detection device. The first step was to optimize a simple and fast DNA extraction methodology from soybean seed and leaf using a Chelex-based method. Second, we optimized LAMP amplification of P-35S, P-FMV and T-NOS regions (three elements that are most often present in genetically modified plants as part of transgenic construct) using gBlocks, synthetic dsDNA fragments that have an identical DNA sequence to that of the targeted part of a transgenic construct. Third, we tested the detection limit of LAMP using DNA samples from soybean seed and leaf spiked with gBlocks, simulating real GMO samples. Last, we worked on optimization of an electrochemical DNA sensor based on detection of LAMP products, using single-stranded DNA probes that contain methylene blue as a redox probe and that are complementary to the target DNA. Our results on LAMP reaction optimization confirmed successful detection of GMO for the threshold of 0.001 ng/μL of GM DNA in 1 ng/μL of total gDNA. Additionally, electrochemical detection based on alternating current voltammetry resulted in signal decrease as a consequence of conformational changes of the probe upon attachment of the target DNA. These results hold promise for development of a device that will enable a specific, sensitive, fast, cost-effective, and precise in-field detection system for routine detection of GMOs. Acknowledgement: The Science Fund of the Republic of Serbia – LABOUR project (GA 6710) and The Ministry of Science, Technological Development and Innovation of the Republic of Serbia (451-03-66/2024-03/200358).

P-33-013
Ultra-sensitive multiplexed detection of protein cancer biomarkers with signal amplification strategy

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Cancer diagnostics based on protein biomarkers in the blood hold promising potential for early disease screening and monitoring. Proteins can enter the blood circulation through active secretion or cellular leakage, thereby providing comprehensive information about the current state of health. However, the simultaneous detection of multiple protein targets at extremely low concentrations often presents challenges when using standard methods, such as ELISA. Therefore, to increase the reliability of detection and improve sensitivity, new developments are needed that minimize background noise and allow for the miniaturization of assays. Here, we present recent advances in the multiplexed, selective, and sensitive detection of cancer protein biomarkers by combining target immunorecognition with nucleic acid signal amplification techniques, such as rolling circle amplification (RCA) and exponential DNA amplification. More specifically, we have designed an advanced assay for the simultaneous quantification of prostate-specific antigen (PSA), α-fetoprotein (AFP), and carcinoembryonic antigen (CEA), as these can be found in liquid biopsies and are important in a clinical context. In conclusion, this study conducted a comparative analysis of direct (not-amplified) and isothermally amplified approaches for the sensitive detection of proteins. The direct labeling assays, as presented in this work, emerge as an excellent alternative to the conventional ELISA method. Our amplification-based immunoassays achieve a low femtomolar level of detection (LOD) for selected biomarkers, offering substantial potential for early-stage diagnostics. This work was supported by Polish Academy of Sciences and the European Commission (H2020-MSCA-COFUND-2018) under the Marie Skłodowska-Curie grant agreement No 847639 (PASIFIC).

P-33-014
Aptamers as molecular tools to study ribosomal assembly in *Staphylococcus aureus*

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Ribosomal assembly is a multistep process required to ensure that a large ribonucleoprotein complex acquires a functional structure. The specific involvement of ribosome-associated GTPases (RA-GTPases), and their role in driving the accuracy of ribosome assembly, remain largely to be elucidated. This research harnesses aptamers, in vitro derived synthetic molecules, which can inhibit the ribosome assembly factors RbgA, Era, and HflX in *S. aureus*. Through the systematic evolution of ligands by exponential enrichment (SELEX), followed by comprehensive sequencing and bioinformatics analysis, we identified aptamers that bind the GTPase Era with high affinity and specificity. Computational modeling suggests that Apt T3_6 interacts with the KH domain of Era, likely precluding GTP binding or GTPase activity. In fact, Apt T3_6-Era interaction leads to a significant reduction of GTP hydrolysis, indicative of inhibition of an essential property of this RA-GTPase. ELONA and thermophoresis assays further confirmed that Apt T3_6 interaction with Era is in the 200 nM range of affinity and displays a high level of specificity. Thus, Apt T3_6 is a potent binder for Era that results in GTPase activity inhibition. The application of aptamers provides a versatile tool for elucidating the complex processes of ribosome biogenesis, offering insights into bacterial protein synthesis mechanisms. This approach not only contributes to our understanding of bacterial biology but also opens venues for developing novel antimicrobial strategies, underscoring the value of aptamers in investigating essential biological processes.

P-33-015
Impedimetric single carbon fiber electrode for ultrasensitive detection of *Staphylococcus aureus* pathogen DNAs in breast milk by CRISPR technology

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This study introduces a novel biosensing approach for the detection of pathogen DNA in breast milk, utilizing single carbon fiber electrodes (SCFE) enhanced with MXene nanomaterial layers. The primary innovation lies in the modification of SCFE with MXenes to increase the electrode's surface area, followed by surface activation for the immobilization of dCas9-sgRNA complexes. This modification aims to leverage the unique properties of MXenes and the selective binding capability of CRISPR technology for efficient and specific pathogen detection. Electrochemical impedance spectroscopy (EIS) and scanning electron microscopy (SEM) analyses were employed to characterize the electrode modifications and the immobilization process, demonstrating successful enhancement of biosensor performance. The study further optimized the chronoimpedimetric detection method to achieve rapid, sensitive, and selective detection of *Staphylococcus aureus* (SAu) DNA in breast milk, with a notable detection time of 60 seconds in real samples. The biosensor demonstrated high selectivity and sensitivity, with a linear detection range between 50–6000 fM and a limit of detection (LOD) at 14.5 fM. The reproducibility and stability of the biosensor were also confirmed through multiple tests, showing promising potential for clinical and public health applications

Biochemistry for Drug Repurposing

P-34-001
Bacterial DNA replication initiation: structural and kinetic insights into the replicative helicase loading mechanism in *Mycobacterium tuberculosis* and *Vibrio cholerae*

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The DNA replication is an essential step for the survival of every organism, and the biochemical processes underlying this event are strictly regulated. In bacteria, the allocation of the replicative helicase DnaB onto the origin of replication requires fundamental actors named helicase loading factors, which are active throughout the DNA replication initiation process. Most pathogens, including *Mycobacterium tuberculosis* (*Mt*) and *Vibrio cholerae* (*Vc*), possess the ancestral replicative helicase loader DciA, which displays key features of the replicative helicase-operating proteins associated with the DNA replication initiation. Even though the loading of the replicative helicase onto the DNA is a key step of the DNA synthesis, structural and kinetic details of this process in *Mt* and *Vc* still need to be further investigated. As DciA was described to form a stable complex with the replicative helicase DnaB, promoting its loading onto ssDNA (1), we proved this interaction via surface plasmon resonance, establishing the fundamental role of DciA's lasso/grappling hook-like domain (2) in liaising with the linker helix-docking helix module of DnaB, making it a druggable target. Furthermore, the loader should repress the helicase activity to prevent the initiation of unforeseen replication. Thus, we showcased how DciA can repress DnaB's ATPase activity in both organisms, evaluating also the contribution of both loader's domains. Next, as DciA should sustain the loading of the replicative helicase onto the replication fork, here we present how the loader can stimulate the DnaB's ATP-dependent dsDNA unwinding activity thanks to a reliable fluorescence-based assay. Finally, here we show a new cryo-EM 3D structure of *Vc*DnaB in complex with ssDNA, unveiling new details on the helicase-DNA interaction. Previously published in: (1) Mann, Katherine M et al. (2017), PLoS Genet 13(11):e1007115. (2) Chase, Jillian et al. (2022), Trends Biochem Sci 47(7):620–630. *The authors marked with an asterisk equally contributed to the work.

P-34-002
In-vitro apoptotic and metastatic effect of ibuprofen on hepatocellular carcinoma cells

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Ibuprofen (2RS)-1 [4-(2-methylpropyl)phenyl] propionic acid is the first derivative of propionic acid developed in 1969 as an alternative to aspirin. In the literature, it has been determined that ibuprofen has a favorable effect on long-term use of small doses of neuro-degenerative diseases such as Alzheimer's and Parkinson's disease, and at the same time, daily intake of ibuprofen produces risk reductions for colon, breast, lung, and prostate cancer. However, the metastatic and apoptotic effects of ibuprofen on human hepatocellular carcinoma cells (Hep3B) have not been studied yet. Therefore, in this study, we aimed to investigate the metastatic and apoptotic properties of ibuprofen in Hep3B cells. For this purpose, ibuprofen was exposed to Hep3B cells with different doses (150 μg/mL, 75 μg/mL, 39 μg/mL, 18 μg/mL, and 9 μg/mL) at different time points (24 h and 48 h). Cell proliferation was determined with the MTT assay. Apoptotic effect of ibuprofen was analyzed with the real-time PCR method at the mRNA level of Bcl-2, Bcl-XL and Bax expressions. A scratch assay was performed to investigate the metastatic effect of ibuprofen (150 μg/mL and 9 μg/mL) on Hep3B cells. As a result, ibuprofen inhibited cell proliferation in a dose- and time-dependent manner. The IC50 values were 75 μg/mL for 24 h and 32.5 μg/mL for 48 h. In addition, Bax expression was upregulated as a result of the administration of a high dose of ibuprofen and a decreased level of Bcl-2 and Bcl-XL mRNA expression. Consistent with these results, the cell migration capability of Hep3B cells was inhibited at 150 μg/mL of ibuprofen doses. These findings suggest that ibuprofen has an anti-cancer effect on Hep3B cells at the highest doses.

P-34-003
Structural determinants of Ivabradine block of HCN4 pacemaker channels

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HCN1-4 channels are the molecular determinants of I_f/I_h current that crucially regulates cardiac and neuronal cell excitability. HCN dysfunctions lead to sinoatrial block (HCN4), epilepsy (HCN1) and chronic pain (HCN2), widespread medical conditions awaiting subtype-specific treatments. We have addressed the problem by solving the cryoEM structure of HCN4 in complex with Ivabradine, to date the only HCN-specific drug on the market. Our data show Ivabradine bound inside the open pore at 3Å resolution. The structure unambiguously proves that Y507 and I511 on S6 are the molecular determinants of Ivabradine binding to the inner cavity while F510, pointing outside the pore, indirectly contributes to the block by controlling Y507. Cysteine 479, unique to the HCN selectivity filter, accelerates the kinetics of block and underlies Ivabradine selectivity for HCN channels. MD simulations further reveal that Ivabradine blocks the permeating ion inside the selectivity filter by electrostatic repulsion, a mechanism already proposed for quaternary ammonium ions.

P-34-004
Evaluation of anticholinesterase activity for the fungicides mefentrifluconazole and pyraclostrobin

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Triazoles are compounds with various biological activities, including fungicidal action. They became popular through cholinesterase studies after the successful synthesis of dual binding femtomolar triazole inhibitor of acetylcholinesterase (AChE, EC 3.1.1.7) by K. B. Sharpless via *in situ* click chemistry. Here we evaluate the anticholinesterase effect of the first isopropanol triazole fungicide mefentrifluconazole (Ravystar®), developed to overcome fungus resistance in plant disease management. Mefentrifluconazole is commercially available individually or in a binary fungicidal mixture with i.e. pyraclostrobin (Ravycare®). Pyraclostrobin is a carbamate that contains a pyrrole ring. Moreover, carbamates are known inhibitors of cholinesterases and carbamate rivastigmine is used for the treatment of Alzheimer's disease. We tested the type and potency of anticholinesterase activity of mefentrifluconazole and pyraclostrobin. Mefentrifluconazole reversibly inhibited human AChE and BChE with a 7-fold higher potency toward AChE (K_I = 101 ± 19 μM). Pyraclostrobin (50 μM) inhibited AChE and BChE progressively with a rate constant ($t_{1/2}$ = 2.1 min; $k_i = 6.6 \cdot 10^3$ M^{-1} min^{-1}) and ($t_{1/2}$ = 1.5 min; $k_i = 9.2 \cdot 10^3$ M^{-1} min^{-1}), respectively. A molecular docking study indicated key interactions between the tested fungicides and residues of the lipophilic active site of AChE and BChE. Additionally, the physicochemical properties of the tested fungicides were compared to values for CNS active drugs to estimate the blood-brain barrier permeability. Our results can be applied in the design of new molecules with a lesser impact on humans and the environment.

P-34-005
Modulation of health span with repurposed drugs in aging nematodes

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Aging is the progressive decline of many physiological and molecular functions. Health span deteriorates during aging, leading to a poor quality of life. This study focuses on the dynamics of aging in *Caenorhabditis elegans*, with a particular emphasis on health span modulation through drug repurposing. The objective is to dissect the gradual decline in physiological and molecular functions associated with aging, which culminates in compromised health span and reduced quality of life. Pharmaceutical agents with different functions are combined to address a spectrum of molecular processes. To systematically evaluate health span parameters in aging *C. elegans*, I use techniques such as automated movement tracking and microscopy. Single and combinatorial drug treatments are administered to determine

potential improvements in health span. This offers a targeted strategy for rejuvenation. Our project aims to improve the health span in the aging *C. elegans* population. We achieve this by deliberately repurposing compounds and emphasizing late-stage interventions. This study provides valuable insights into health span modulation in the aging paradigm.

P-34-006
Evaluation of the effectiveness of some benzoxazole derivatives in the experimental Alzheimer's models *in vitro*

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Alzheimer's disease (AD) is a common primary health problem, especially in the aging population. AD is a neurodegenerative disease characterized by loss of neurons and synapses, with extracellular plaques containing Aβ and intracellular neurofibrillary tangles containing tau. In this study, we were evaluated the preventative effect of the Aβ aggregation in the SH-SY5Y cells of the three different benzoxazole derivative compounds, which were called compounds 1, 2, and 3. After 24 hours of 40μM Aβ incubation, SH-SY5Y cells were treated for 24 and 48 hours with the three different benzoxazole derivative compounds separately and the proliferation of SHSY-5Y cells were assessed using the MTT assay. We observed significant neurite inhibition with moderate damage by the NST at IC50 dilutions of the compounds. Then AD's disease related Aβ plaque analyzed by microscopic and FOXA2 and PEN2 markers *in vitro* by ELİSA methods. At the end of the 24 and 48 hours, compound 2 and 3 treated cells were shown reduced Aβ plaque formation, while compound 1 treated cells were shown the same Aβ plaque formation manner according to the non-treated Aβ control group. However, compound 3 treated cells showed a higher reduction rate on the Aβ plaque formation in 40μM Aβ treated cells. Also, FOXA2 cytokine level was found to significantly decrease in the compound 2 treated cells. In contrast, FOXA2 cytokine level significantly increased in the compounds 1 and 3 treated cells according to the non-treated Aβ control group. On the other hand, the PEN2 cytokine level was significantly decreased in the compound 1, 2, and 3 treated cells. Additionally, neurite extension assays revealed that the compounds 3 treated cells were shown higher neurite extension rates. Thus, among the heterocyclic compounds we used, the compound 3 significantly prolongs the neuritis and may have a therapeutic effect while reducing beta-amyloid accumulation in AD's patients.

P-34-007
Metal complexes with diflunisal as potential anticancer drugs

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Although diflunisal is a well-known and commercially available non-steroidal anti-inflammatory drug (NSAID), recent studies have also focused on the repurposing of this therapeutic agent for the treatment of cancer. To attempt to contribute to the investigation of metal complexes with NSAIDs, three novel complexes containing diflunisal (Hdif) and neocuproine (neo) were designed, prepared, and spectroscopically and structurally characterized. Furthermore, their biological activity was investigated in terms of DNA interaction as well as antiproliferative activity against prostate (PC-3), colon (HCT116) and breast (MDA-MB-468) cancer cell lines. Although molecules have similar composition of [MCl(dif)(neo)], where M is Zn(II), Co(II), and Cu(II), respectively, the copper complex significantly differs in molecular and supramolecular structure with diflunisalato ligand (dif) coordinated in bidentate chelate mode, while zinc and cobalt complexes are isostructural with dif bound in monodentate manner. All three complexes showed exceptional cytotoxicity against the tested cancer cell lines with IC_{50} values in nanomolar concentration range, while the free diflunisal was found to be inactive (IC_{50} >100 μM). Even though the complexes have similar composition, differences in their cytotoxic effects have been observed, with the Cu(II) being the most active. Cu(II) complex induces caspase-independent apoptosis in PC-3 cell line. Interestingly, the binding affinity of the complexes to the genomic DNA samples isolated from the respective cell lines shows the same trend as the cytotoxicity. Since the obtained results indicate that the interaction of the complexes with DNA might be directly involved in the mechanism of action of the studied compounds on the cellular level, further studies of their effects on the DNA replication and transcription can be performed. This work was supported by VEGA project 1/0126/23.

P-34-008
Development of new anti-lung cancer drugs from an antipsychotic aripiprazole

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Despite remarkable advances in diagnostic technology and medicine, lung cancer still ranks first among the causes of cancer-related deaths worldwide. Recent anticancer drug research is mainly focused on immunotherapy drugs, but due to problems such as low application rate and high cost, the development of efficient targeted anticancer drugs is more urgent for many lung cancer patients. However, since the development of general anticancer drugs requires enormous costs and time, it is considered important to develop anticancer drugs based on drug

repositioning. Antipsychotics are mainly prescribed to patients with schizophrenia. Recently, there have been research results showing that various antipsychotics have anti-cancer effects. This study screened drugs with lung cancer suppressing effects among second generation antipsychotics and obtained the results that aripiprazole was the most effective in suppressing lung cancer. Next, we synthesized aripiprazole derivatives and evaluated their efficacy to alleviate the side effects of antipsychotics and maximize their anticancer efficacy. The derivative selected as having the highest efficacy was treated with H1299 cell line and RNA-seq analysis was performed. Approximately 200 genes were found to be increased or decreased. We plan to establish a system to verify the efficacy of the drug by analyzing the differences and commonalities between aripiprazole and its derivatives and investigate the possibility of clinical application through additional experiments.

P-34-009
Zn(II) and Mn(II) flurbiprofenato complexes and their effects on endometrial, uterine and ovarian cell lines

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Flurbiprofen ((RS)-2-(2-fluorobiphenyl-4-yl)propanoic acid) is a conventional non-steroidal anti-inflammatory drug (NSAID) used for suppressing inflammation and pain relief in treatment of various inflammatory diseases including endometriosis. Since numerous studies have recently demonstrated that metal complexes with NSAIDs often exceed the free drugs in terms of activity, a rational design of novel coordination compounds containing flurbiprofenato ligands (flur) and investigation of their effects on inflammatory as well as cancer cell lines can lead to development of highly efficient therapeutics. Based on this premise, two flurbiprofenato zinc(II) and manganese(II) complexes containing neocuproine (neo) have been synthesized in order to study their effects on endometriotic (12Z), uterine carcinoma (SK-UT-1), ovarian carcinoma (A2780) and healthy control epithelial cell line (HME1). The single-crystal X-ray diffraction analysis affirmed that the two complexes are isostructural and form analogous molecules with a composition of $[Zn(flur)_2(neo)]$ and $[Mn(flur)_2(neo)]$ where flurbiprofenato ligands bind to the metal centers through the both oxygen atoms of the carboxylate group in a bidentate chelate manner while the chelate-bonded neo ligand plays a role of a supporting ligand. It is to note that the prepared complexes also exhibit characteristic fluorescence that can be potentially used in their tracking on the cellular level. Despite their structural similarity, cytotoxic studies revealed differences in the activity of the two complexes which are further supported by binding studies performed on the samples of genomic DNA isolated from the respective cell lines. In addition, the impact of the complexes on the gene expression of selected miRNAs associated with inflammation and angiogenesis in the studied cell lines was determined by qRT-PCR. This work was supported by Slovak grant agency project VEGA 1/0435/23.

P-34-010
NSAIDs from anti-inflammatory to PAF AH potential inhibitor, far beyond inhibition of COX to potential anti-tumor agent

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Our *in silico* research has uncovered a novel interaction between specific non-steroidal anti-inflammatory drugs (NSAIDs) sulindac, celecoxib, diclofenac, and nimesulide – and the enzyme platelet-activating factor acetylhydrolase (PAF-AH). This enzyme plays a vital role in the hydrolysis of phospholipids and the removal of oxidized fatty acids, thereby being crucial for maintaining the fluidity and functionality of cell membranes, particularly in tumor environments characterized by inflammation and oxidative stress. The discovery that these NSAIDs can bind to the enzymatic cavity of PAF-AH suggests they might modulate its activity, thereby influencing tumor behavior. Such modulation could lead to significant antitumor effects, including the reduction of inflammation, inhibition of angiogenesis, and suppression of metastasis, notably in breast, colon, prostate, and lung tumors. However, in certain contexts, this interaction might also promote tumor growth. This new insight opens avenues for developing therapeutic strategies based on the modulation of PAF-AH, highlighting the importance of considering the tumor microenvironment in assessing the potential effects of NSAIDs. Moreover, this finding could guide the selection of NSAIDs in the anti-inflammatory treatment of patients with tumors, avoiding those that may promote tumor progression, and indicating the potential for reallocating certain NSAIDs in tumor treatment, thereby contributing to more precise and personalized therapeutic approaches. Funding: a Doctoral Fellow 2023.00566 FCT-PT; b Master Fellow FAPESP 2023/01973-4 and 2022/04772-7; c Financial Support 442831/2023-4, 309271/2022-3, CNPq.

P-34-011
Increasing treatment efficacy by drug repositioning in acute lymphoblastic leukemia

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Acute lymphoblastic leukemia (ALL) is acknowledged for its intrinsic heterogeneity and the presence of diverse genetic abnormalities influencing disease progression. The predominant subtype, characterized by high-risk and aggressive Philadelphia positive ALL (Ph(+) ALL) with BCR/ABL translocation, has been effectively treated with Imatinib mesylate, a tyrosine kinase inhibitor (TKI). However, the challenge of achieving sustainable therapeutic success arises due to the emergence of TKI resistance.

Consequently, there is an urgent need to identify alternative targets for ALL treatments. This research endeavors to propose an innovative treatment approach through drug repositioning, employing a comprehensive analysis of transcriptome datasets for ALL and Ph(+) ALL to identify Differentially Expressed Genes (DEGs) associated with disease progression. The study highlights Maytansine and Isoprenaline as potential interventions for ALL, and Glipizide and Desipramine for Ph(+) ALL. Validation through MTT and Trypan blue assays confirmed the cytotoxic effects of these drugs on both ALL (Jurkat) and Ph(+) ALL (SUP-B15) cell lines. Additionally, Annexin/FITC dual staining was utilized to assess apoptotic effects. Further examinations involved tests on Imatinib-resistant SUP-B15/R cells to evaluate the impact of Imatinib resistance on the cytotoxic and apoptotic effects of Desipramine and Glipizide. Results indicated significant cytotoxic and apoptotic effects for all drugs on the cells. Moreover, synergistic doses with Imatinib were identified in both SUP-B15 and Imatinib-resistant SUP-B15/R cells using the CompuSyn tool. Consequently, these repositioned drugs, exhibiting promising cytotoxic and apoptotic effects, have the potential to enhance treatment effectiveness, thereby increasing the survival rate in both Ph(-) ALL and Ph(+) ALL patients. Acknowledgments: This work was supported by the Scientific and Technological Research Council of Türkiye (TUBITAK 121Z765)

P-34-012
Exploring the polyamine pathway in human enteric protozoan parasite *Entamoeba histolytica*

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Amebiasis is a disease caused by a unicellular protozoan parasite *Entamoeba histolytica*. The main treatment is metronidazole, but it's not always effective, especially for people without symptoms or in some cases of treatment failure. The parasite can also become resistant to metronidazole *in vitro*. Therefore, the development of a novel prophylactics and chemotherapeutics to control amebic infections is required and, to this end, identification of novel essential pathways and enzymes is particularly needed. Metabolic pathways that are essential for the survival of parasite offer excellent intervention candidates for therapeutic and prophylactic chemotherapy. One such pathway that is essential for parasites survival is the polyamine biosynthesis pathway. Genome database revealed that *E. histolytica* lacks the conventional enzymes involved in polyamine biosynthesis and it was presumed that this parasite lacks polyamine metabolism. However, our previous metabolomic study suggests that the ameba is capable of synthesizing various polyamines. In this study, we have conducted polyamine analysis in trophozoites and in the BI-S-33 culture medium before and after culturing the trophozoites for 24 hours. Our data revealed that the levels of various polyamines, including spermidine and putrescine, increase in the trophozoites and the spent media, suggesting that the ameba can synthesize these polyamines through as-yet-unidentified pathway(s). Additionally, we have identified novel metabolites, such as norspermidine, suggesting an alternative polyamine biosynthetic pathway, as reported in some organisms lacking conventional polyamine pathway enzymes. Our discovery opens up new possibilities for understanding and targeting the parasite's biology to develop better treatments for amebiasis.

P-34-013
Metabolic imprint of SARS-CoV-2 and influenza viruses on host cell metabolism

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Interference of DNA and RNA viruses with host cell metabolism is one of the key factors of their pathogenesis, as these changes can promote inflammation and/or contribute to carcinogenesis. In addition, some of them are used by infections to ensure efficient replication. However, such studies are often conducted in non-physiological settings due to usage of culture medium that itself rewires cell metabolism. Here we analysed how SARS-CoV-2 or influenza A virus (IAV) infections interfere with metabolic landscape of cells cultivated in classical or plasma-like Plasmax media. Using RNA-seq transcriptome analysis, GC-MS metabolome quantification and measurement of glycolysis and mitochondria respiration rates by Seahorse technology we show that the imprint of both infections is dependent on culture medium and cell line used. Both SARS-CoV-2 and IAV suppress respiratory activity of mitochondria without pronounced effect on glycolytic activity of cells, according to Seahorse data. RNA-seq analysis revealed down-regulation of amino acid metabolism in infected cells in classical media and up-regulation in Plasmax medium. Metabolome analysis supported these conclusions by revealing suppressed de novo Ser/Gly biosynthesis and glutaminolysis pathways as well as decreased levels of Asn/Asp and sulfur-containing amino acids in cells maintained in DMEM. Noteworthy that in Plasmax the viruses did not affect these pathways with the exception of moderate inhibition of cysteine biosynthesis. In Plasmax medium these infections also caused a more pronounced oxidative stress. SARS-CoV-2 also suppressed biosynthesis of biogenic polyamines in classical media but activated in Plasmax. Importantly, the inhibitors of polyamine-metabolizing enzymes including FDA-approved drugs exhibited specific antiviral activity. Thus, the drugs that target metabolic enzymes could be repurposed against infections caused by RNA viruses. This work was supported by RSF (grant #24-14-00410). *The authors marked with an asterisk equally contributed to the work.

P-34-014
Study of the photodynamic inactivation efectivenness on rickettsial infections

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Rickettsia (*Alphaproteobacteria*; *Rickettsiales*, *Rickettsiaceae*) are small (0.3–0.5 by 0.8–2.0 μm) Gram-negative obligate intracellular bacteria that target vascular endothelial cells and grow within the cytoplasm of eukaryotic host cells. Arthropods such as the ticks *Ixodes ricinus*, *Dermacentor reticulatus* and *D. marginatus* are vectors for rickettsioses causing spotted fever and the typhus groups of diseases in humans [Sekeyová, Z., (2013) Acta Virol 57 (2):180-99]. CQDs are a new type of 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, K., (2019) Polymers 11(10):1670]. The present study revealed a lower rate of *R. conorii* infected VERO cells after PDT. By using RT-PCR, it was possible to demonstrate that the treatment also successfully lowered the *R. conorii* replication rate inside the cells. A preliminary study of the protein differential expression of *R. conorii* infected VERO cells, performed using a Waters Synapt system and the ProGenesis QI software, showed increased expression of proteins involved in the rickettsial lipopolysaccharide biosynthesis and lower expression of proteins involved in ATP synthesis and DNA repair as consequence of the PDT. In VERO cells, among the most affected cell functions were detoxification and metabolism. Monitoring the above-mentioned changes is interesting, since it unveils the mechanisms by which intracellular bacteria contends with cellular damage from PDT and how the host cells can take advantage of a treatment to overcome an infection, can help to develop improved antimicrobial treatments to fight antibiotic resistance.

P-34-015
SARS-CoV-2 proofreading exoribonuclease as a promising target for antiviral drugs

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A key factor in maintaining the fidelity of SARS-CoV-2 RNA replication is the proofreading exoribonuclease (ExoN) complex nsp14:nsp10. In addition, ExoN repair activity is often linked with resistance to nucleoside analogs such as Remdesivir. Therefore, targeting this enzyme is a promising way to prevent viral replication directly or by increasing the efficacy of drugs already in use. In this study, we established a procedure to produce both ExoN subunits by co-expression and separate expression in bacterial cells. Although the individually expressed proteins exhibited non-stoichiometric binding, the nuclease activity of the complex was retained. We then established an enzymatic assay to evaluate ExoN inhibition. To analyze the binding of small-molecule inhibitors, we optimized a nano-differential scanning fluorimetry (nanoDSF) assay. We first verified the applicability of these methods for inhibitor assessment by analyzing aurin tricarboxylic acid, which was previously reported to inhibit ExoN activity. Finally, we tested a series of phytochemical compounds and their precursors for binding and inhibition. Some of the analyzed compounds were promising in ExoN inhibition, suggesting their potential involvement in further studies of COVID-19 treatment. This project was supported by The Czech Science Foundation 22-17118S and by the grant of Specific university research A2_FPBT_2024_019.

P-34-016
Exploring the efficacy of WY-14643 and serum deprivation on lipolysis in fibroblasts of patients affected by neutral lipid storage disease with myopathy

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Mutations in the PNPLA2 gene, which encodes adipose triglyceride lipase (ATGL), lead to neutral lipid storage disease with myopathy (NLSDM), a rare disease mainly characterized by myopathy, cardiomyopathy, and abnormal increase in intracellular lipid droplets (LDs). After obtaining skin biopsies from NLSDM patients and establishing primary skin fibroblasts, we assessed the ability of WY-14643, Clenbuterol and Salmeterol to enhance triglyceride (TAG) breakdown *in vitro*. We selected two lines of fibroblasts in which PNPLA2 mutations caused a complete loss of ATGL function (Group A) and two with reduced lipase activity due to PNPLA2 missense variations (Group B). The LD number and size, as well as intracellular TAGs in NLSDM fibroblasts cultured in Earle's MEM medium with either 3% or 10% FBS before and after treatments with 70, 100, and 200 μM WY-14643 for 96 hours, were quantified. The analysis revealed that 200 μM WY-14643 was highly toxic with both 3% and 10% FBS, and 100 μM showed toxicity in 3% FBS for most fibroblast lines. Instead, 70 μM WY-14643 in 3% FBS notably reduced intracellular TAG content ($p < 0.05$), LD area ($p < 0.05$) and number ($p < 0.05$) in Group B, but not in Group A fibroblasts. Additionally, experiments with Salmeterol (100 nM) and Clenbuterol (1 μM) in 10% FBS medium for one week showed that Clenbuterol decreased TAG content, while Salmeterol had no effect, suggesting that further tests with different concentrations are necessary to explore their potential in activating intracellular lipolysis. The findings of this investigation indicate that fibroblasts from NLSDM patients can be considered a viable model for assessing drug treatments. Specifically, WY-14643, a PPARα agonist, appears to facilitate the mobilization of TAG in these fibroblasts when cultured in a medium with low serum concentrations. Funding: This work was supported by European Union – Next Generation EU, PRIN 2022 PNRR (P2022AWA84). *The authors marked with an asterisk equally contributed to the work.

P-34-017
The effect of disulfiram and its metabolite N, N-diethyldithiocarbamate combined with copper ions in renal cell carcinoma

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Disulfiram (tetraethylthiuram disulfide, DSF) is an old alcohol-aversion drug that has been shown to be effective against some

types of cancer including lung, liver, breast, prostate and melanoma [1]. DSF is *in vivo* broken into two molecules of N,N-diethyldithiocarbamate (DDC) which is probably responsible for pharmacological effect of DSF. Some studies suggest that the presence of Cu (II) ions significantly potentiates the anticancer efficacy of DSF [2]. Renal cell carcinoma (RCC) is one of the most frequently diagnosed cancers in the world. RCC is characterized by high invasiveness and metastatic potential and new drugs are needed for its treatment. So far, the anti-cancer potential of DSF in RCC cells has not been investigated. The aim of our research was to examine the effect of DSF and DDC combined with Cu in Caki-2 cells which are RCC cells. The obtained results indicate that both DSF and DDC combined with Cu in a concentration-dependent manner are able to suppress viability and metabolic activity of Caki-2 cells as shown by the crystal violet and MTT test. The significant effects were observed in the DSF and DDC concentration range of 1–10 μM. DSF and DDC tested alone (without combination with Cu) as well as Cu alone did not affect the viability and metabolic activity of Caki-2 cells. We also observed that DDC combined with Cu ions was more active in suppressing the viability of Caki-2 cells than DSF with Cu ions administered in the same concentration. Our results demonstrate that DSF, a novel repurposed drug for cancer therapy combined with Cu ions has properties that limit the viability and metabolic activity of Caki-2 cells what provides a promising strategy for cell renal carcinoma chemotherapy. This work was supported by a grant from the National Science Centre of Poland, based on decision no. DEC-2023/07/X/NZ7/00757. References: 1. Kannappan et al. (2021) Front Mol Biosci 8, 741316. 2. Kang et al. (2023) Pharmaceutics 15, 1567.

P-34-018
Light and pH effects on Ipilimumab conformational stability

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Monoclonal antibodies (mAbs) are an essential class of therapeutic proteins for the treatment of cancer, auto immune and rare diseases. Throughout their production, storage, and administration processes, these proteins encounter various stressors. Viral inactivation is a key step in downstream processes, and it is achievable through the titration of the protein at low pH. This procedure involves the use of acidic substances to temporarily reduce the pH of protein solutions, followed by subsequent neutralization. pH alterations pose a significant risk to proteins due to the unfolding and subsequent aggregation, thereby reducing the availability of the therapeutic protein. Additionally, unexpected exposure to light during the viral inactivation process can further impact the structural integrity of mAbs. This study aims to investigate the nature of the mAb modifications in regard to pH and light exposure. Moreover, a specific purpose of this work is to establish a correlation between structural features and the susceptibility of a specific mAb, Ipilimumab, to these stressors. The characterization of stressed Ipilimumab through biophysical and biochemical analyses reveals that pH variation is a considerable risk, with irreversible unfolding observed at pH 2. The reversible conformational changes observed at pH 3 upon neutralization suggest potential applications for pH-responsive drug delivery systems. Light exacerbates some local and global effects making pH-induced exposed regions more vulnerable to structural and chemical changes. Our findings underscore the critical role of pH optimization in preserving the structural integrity and therapeutic efficacy of proteins such as Ipilimumab, particularly within the context of pH-sensitive drug formulations.

P-34-019
In vitro exploration of small molecule modulators of carnitine O-acyltransferase (CRAT) activity with therapeutic potential

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The impairment of the catalytic activity of carnitine O-acyltransferase (CRAT) enzyme has recently been proposed as an additional cause for the onset of Leigh syndrome, or subacute necrotizing encephalomyelopathy. This syndrome represents one of the most severe pediatric disorders caused by mitochondrial dysfunction. The CRAT enzyme is responsible for the reversible transfer of acyl groups from acetyl-coenzyme A to carnitine. A missense mutation (p.Tyr110Cys) was recently identified as the cause of severe impairment of the CRAT enzymatic function in a pediatric patient exhibiting Leigh-like syndrome symptoms [Previously published in: Laera L *et al*. (2019) Hum Mutat 41(1):110-114]. This study aimed to identify small molecules, able to modulate CRAT activity, by selecting them from a drug-library, for therapeutic scopes, in the context of the "drug repurposing strategies." A group of drugs, chosen by virtual screening, was tested on both recombinant wild-type (WT) protein and the p.Tyr110Cys CRAT mutant. The specific activities of both the WT and the mutant CRAT proteins were calculated from experiments conducted in the presence or absence of artemisinin, glimepiride, dorzolamide and suramin. While glimepiride and dorzolamide did not exhibit a statistical difference in terms of enzyme activity in the mutant compared to the control, artemisinin acted as a selective activator of the mutant variant, showing an approximately 15% increase ($p < 0.05$) in mutant activity. Conversely, suramin led to almost complete inhibition of both WT and mutant proteins. These preliminary analyses demonstrate a significant drug-induced activation of the p.Tyr110Cys CRAT variant by artemisinin, suggesting it as a promising scaffold candidate for developing new drugs for the treatment of CRAT deficiency.

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P-34-020
Investigation on the mechanism of action and translational potential of desogestrel in congenital central hypoventilation syndrome (CCHS)

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Congenital central hypoventilation syndrome (CCHS) is a rare genetic disorder affecting the autonomic nervous system and central chemosensitivity. It is caused by heterozygous mutations of the transcription factor PHOX2B, which is essential for the development of several classes of autonomic and brainstem neurons. CCHS patients display hypoventilation and severe impairment of the central chemoreflex, especially during sleep, that may be fatal. No pharmacological intervention is currently available for treating the disease or its symptoms. It has been proposed, by serendipitous observation in three CCHS patients, that hormonal treatment (desogestrel, ETO; levonorgestrel) may provide partial recovery of chemoreflex impairment, therefore opening the possibility for a relief of the respiratory symptoms and a reduction of risks of death during sleep in CCHS patients. Systemic ETO treatment rescues the chemoreflex response in an animal model in which central chemosensitivity is impaired in adulthood. *In vitro* data [Previously published in Cardani et al. (2018) Exp. Cell Res. 370(2): 671-679] showed that desogestrel down-regulates the expression of PHOX2B, by a post-transcriptional mechanism, and some of its target genes, a mechanism shared by other progestins, and occurring also *in vivo*. ChIP-seq analysis in a neuroblastoma cell line identified many PHOX2B target gene candidates, among which ion channels, a key class of genes for the chemoreflex response. Using a custom panel of ion channels genes (RT^2 Profiler PCR Arrays, Qiagen), chosen according to the ChIP-seq analysis, we show that the progestin desogestrel regulates the expression of ion channels that are targets of PHOX2B, and whose expression is affected by the presence of mutant PHOX2B. The modulation of ion channels genes by desogestrel is indicative of the role of these genes in the recovery of chemosensitivity and reinforces the role that PHOX2B has in the pathogenesis of CCHS and in therapy response.

P-34-021
Targeting dual sites of DKK1 protein for therapeutic intervention: modulating Wnt signaling pathway by disrupting LRP5/6 and Kremen interface

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The Wnt signal pathway is a critical pathway in cellular signal transmission. Dickkopf1 (DKK1) is one of the natural antagonists for Wnt signaling. It inhibits the Wnt signal by binding to LRP5/6 receptors, and its binding affinity to LRP5/6 is increased by its interaction with Kremen. As inhibition of Wnt signaling by DKK1 is associated with many pathological conditions such as baldness, cancer, cardiovascular system, and neurodegenerative disease, modulation of the DKK-1 activity by the antagonists seems a good candidate for therapeutic purposes, especially for small molecules. In our previous study, which screened PubMed compounds using virtual screening and molecular dynamic simulations (MD), 657042 has been identified as a joint candidate antagonist for both the LRP5/6 binding site and the Kremen binding site of DKK1. Therefore, compounds derived for 657042 have been obtained using combined ligand-screening methods from the ZINC database, and their binding affinity to the DKK1 domain has been investigated. Calculations showed that ZINC000005160612, ZINC000038352321, ZINC000004777583, ZINC000003878464, and ZINC000003878471 have significant binding affinity to the DKK1 (−51.98 to −49.53 kcal/mol). In summary, the compounds identified as hits have standard heterocyclic rings. As a result, compounds featuring aromatic structures can be viewed as promising candidates for blocking the DKK1, and these compounds need to be investigated further by *in-vivo* and *in-vitro* studies.

P-34-022
Focused protein arrays for parallel phage display library screening

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Phage display libraries brought a revolution in the design of protein therapeutics as they offer the possibility of testing billions of sequences in the search for desirable effects. The limiting factor is the screening of libraries in search of specific binders to target molecules. Here we explored screening options for our previously designed phage display libraries based on the calbindin scaffold with (1) 6 randomized positions on the side of the EF1 domain positioned nearby in 3D structure, (2) a loop containing 7, and (3) 5 variable residues in both cases bridged by three glycine residues to EF1 and EF2 hand, called side and loop libraries, as well as Thomlinson library. Our approach was to use proteins from our lab collection (commercial or expressed and purified in our group, including various amyloid proteins) for building homemade arrays that would serve for fast, simple, and cost-efficient testing of specific binding with library members. We printed 56 proteins on 16 × 4 field ONCYTE porous nitrocellulose film

slides and incubated them with phage libraries using helper phages as a control to avoid non-specific binding. For detection purposes, we used amine active IR-dye 680 for phage labeling and imaged the arrays using an IR scanner. We did three rounds of selection toward all 56 proteins to enrich the population of high-affinity binders. In the first two rounds, we detected a significant increase in the number of binders and reached saturation in third based on the similar number of colonies obtained. After the third round, we did a specific selection using the same approach with nitrocellulose surface, but towards single proteins, namely Aβ40, Aβ42, α-synuclein, IAPP, and tau in both, monomeric and end-fibril states. Specific binders were subjected to next-generation sequencing to explore homology groupings among binding sequences. Our results suggest that the method developed is suitable for both, group and specific selection and screening of sequences.

P-34-023
Repurposing of antipsychotic nefazodone for cancer therapy targeting mitochondrial metabolism

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Cancer represents a major public health problem with total therapy that is still elusive for many cancers. Despite progress in precision medicine, resistance to chemotherapy in patients with recurrent and advanced disease raised concerns over the progress of cancer therapy, making it necessary to change the paradigm in the search for more effective therapeutic options. In this context, repurposing of existing drugs became an alternative strategy to *de novo* drug development. In our laboratory, we screened psychotropic drugs finding nefazodone as a promising candidate for oncological drug repurposing since it displayed significant toxicity followed by strong metabolic effects. The aim of our study was to investigate the mechanism of nefazodone mediated metabolic impairment to develop new therapeutic strategies targeting cancer metabolism. Our results showed that nefazodone treatment induces strong mitochondrial depolarization in MCF7, while a milder but still significant depolarization was evaluated on U87 and in HCT. Nefazodone treatment was able to alter mitochondrial network, number and ETC complexes expression in all cell line tested. OCR analysis displayed a strong reduction of mitochondrial respiration, as well as ATP production. Lastly, cotreating cells with nefazodone and glycolysis inhibitor 2-deoxygluose resulted in increased cell death compared to the single treatments alone in 2D grown cells and in 3D spheroids. In conclusion, our data demonstrate that nefazodone strongly affect mitochondrial metabolism acting directly on ETC and mitochondrial respiration. The dual targeting of mitochondrial and glycolytic metabolism strongly reduces cancer cell viability and spheroid formation. Although more evidence needs to be produced to evaluate the efficacy on tumor with different metabolic profiles and on chemoresistance, targeting with more specific and precise drugs metabolic pathways in cancer could represent a valid approach in treating different tumor types.

P-34-024
The reactivity of cysteines of 3CLpro affects the oligomerization state of the Sars-CoV-2 protease

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Drug research makes daily advancements in the discovery of compounds that can enhance human life, seeking effective treatments for diseases of various origins. To follow this purpose, the first step is to understand and characterize the interaction between target proteins and molecules with potential pharmacologic activity through *in vitro* studies. This project, co-funded by Dompé Farmaceutici S.p.A., is placed in this context. The main focus of the project is Covid-19, and, more specifically, the interaction of 3C-like protease (3CLpro, also known as Mpro), the main protease of the SARS-Cov-2 virus, with three different molecules was characterized. 3CLpro is a cysteinyl protease, playing a pivotal role in the replication as well as transcription of the virus. This protease has 12 cysteines residues in reduced form, and it is known to be active as a homodimer. Among several Mpro molecules indicated as potential inhibitors from the Exscalate 4Cov project (https://www.exscalate4cov.eu/), this research has been focused on three drugs: two proposed as non-covalent binders, and a third as covalent inhibitor, with a mechanism involving the modification of the catalytic cysteine. Proteomic and mass spectrometry analysis have been carried out to elucidate the mechanism of action of the covalent inhibitor. Surprisingly, already at low concentrations of inhibitor, we found also other cysteines residues modified. By using native electrophoresis, we highlighted different oligomerization state of the protease, according to the modified residues, which were characterized by a modified proteomics protocol relying on pepsin hydrolysis in gel.

P-34-025
The human protein disulfide isomerase isoform A3 (PDIA3) as an innovative target for the development of new broad-spectrum antivirals agents

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The SARS-CoV-2 pandemic has underscored global weaknesses in responding to viral outbreaks. RNA viruses, being zoonotic and highly mutable, pose challenges for immunity and vaccine effectiveness. While vaccines are critical, the fact that they must be administered prior to the infection and that they are single-antigen specific, highlight the need for secondary antiviral defence against ongoing infections. Traditional antiviral research

[1] focused on direct acting antivirals, but host-targeting antivirals (HTAs) are gaining attention for their potential as broad-spectrum antivirals, less prone to resistance and effective against various viruses, including emerging ones. The host translation machinery and also the host protein chaperones are required for the proliferation of RNA viruses. The thiol oxidoreductase protein disulphide isomerase A3 (PDIA3), acting as protein chaperone, has been observed to be necessary to refine the folding of viral glycoproteins [2], including the spike proteins of Coronaviruses. PDIA3 (505 aa) is a U-shaped protein containing four thioredoxin-like domains. The two active domains (a, a') are located at the N- and C-termini, which contain a redox-active CGHC catalytic sequence, and in the inner part of the protein the two inactive domains (b, b') bind to calreticulin and calnexin [3], assisting the folding of glycoproteins. In our work, we expressed and purified the recombinant PDIA3 in *E. coli* and tested the inhibitory activity of compounds derived from a known PDI family inhibitor. Crystals of PDIA3 in the ligand-free form were obtained and reproduced, and diffraction analysis is pending. We aim to obtain the crystal structure of PDIA3 alone and in complex with the most effective compounds in the context of innovative HTAs drug discovery. Previously published in: 1. Chitalia, V.C. et al. (2020) J Transl Med 18, 390. 2. Mahmood F. et al. (2021) Biomed Pharmacother 143, 112110. 3. Tian G. et al. (2006) Cell 124, 61-73.

P-34-026
Repurposing cyclosporin A analogues for targeting *Toxoplasma gondii* cyclophilins: insights from structural studies

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Cyclosporin A (CsA) is a potent immunosuppressant widely used to prevent organ rejection post-transplantation. Its mechanism of action involves binding to intracellular receptors called cyclophilins (Cyps), which play vital roles in cellular processes. In addition to an immunosuppressive effect, CsA has demonstrated efficacy against a broad spectrum of parasites, including *Toxoplasma gondii*. Although the antiparasitic mechanism of action of CsA has not been fully understood, parasite Cyps represent promising drug targets. We previously identified a novel *T. gondii* Cyp, named TgCyp23, as a high-affinity CsA target, suggesting therapeutic potential [Previously published in: Favretto F et al. (2023) ACS Infect Dis 9(2), 365–377]. However, the immunosuppressive nature of CsA limits its parasiticidal use necessitating exploration of non-immunosuppressive alternatives. In this study, we conducted a drug repurposing investigation using three CsA analogs devoid of immunosuppressive effects against TgCyp23. Our findings revealed nanomolar affinity binding and significant enzymatic inhibition by these analogues. The determination of the crystal structure of the complexes at high resolution, along with nuclear magnetic resonance spectroscopy (NMR) experiments, elucidated the exact binding mechanism of the analogues to TgCyp23, allowing us to uncover the determinants of the interaction. In conclusion, our study provides valuable insights into how CsA analogues can be leveraged against *T. gondii* infections through the inhibition of parasite Cyps, emphasizing the significance of drug repurposing strategies in combating infectious diseases. *The authors marked with an asterisk equally contributed to the work.

P-34-027
Harnessing plant extracts to overcome antibiotic resistance: leaf extracts of *Ribes nigrum, Ficus carica,* and *Vitis vinifera* as resistance modifiers of *E. coli* strains

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The emergence of bacterial resistance to antibiotics is the increasing global health issue. It continues to escalate due to the rapid development of resistance against newly introduced antibiotics and the decrease in the discovery of new preparations. This research aims to investigate the antibiotic modulatory potential of leaf extracts from *Ribes nigrum, Ficus carica,* and *Vitis vinifera* plants, and their effects on susceptibility of ampicillin and kanamycin-resistant *E. coli* strains toward antibiotics. The study included the extraction of plant leaves using a maceration technique and subsequent metabolomic characterization using LC-Q-Orbitrap HRMS technique. Antibiotic-resistant *E. coli* strains were used to assess the resistance modifying properties of the test extracts. Broth microdilution assay was applied to determine the minimal inhibitory concentrations of antibiotics and plant extracts, both individually and in combination. Additionally, measurements of H^+-fluxes across bacterial membranes were conducted to assess possible mechanisms behind the resistance-modifying effects of the extracts. The obtained data revealed that while the extracts exhibited no direct antibacterial activity, *R. nigrum* and *F. carica* demonstrated significant modulatory effects on antibiotics, enhancing the efficacy of ampicillin and kanamycin. The study also found that all plant extracts enhanced H^+-fluxes and promoted ATPase activity in kanamycin-resistant *E. coli* strain, suggesting the potential role of these plant preparations in altering bacterial membrane integrity as resistance modulation. The findings show that leaf extracts from *R. nigrum, F. carica,* and *V. vinifera* may be considered as the natural modulators of antibiotic resistance. These extracts can increase the efficacy of antibiotics against resistant bacterial strains, thereby offering a promising avenue for developing new strategies to combat antibiotic resistance.

Other Topics

P-35-001
Immunological changes in the spleen tissues of diabetic rats following gastric administration of emodin and metformin

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Diabetes is becoming an increasingly significant global health concern, with its rising prevalence across the world. Elevated glucose levels, the central feature of diabetes, are hypothesized to induce impairment in immune response functionality, affecting its ability to successfully combat infections. This study investigates effects of emodin and metformin on the immune system in rats with experimentally induced diabetes using streptozotocin (STZ). adenosine deaminase (ADA) and myeloperoxidase (MPO) enzyme activities in the spleen tissues of diabetic rats were determined. The serum levels of immunoglobulins (IgG and IgA) and the expression of genes such as Tnf-α (tumor necrosis factor-alpha), IL-1β (interleukin 1 beta), IL-6 (interleukin 6), and INF-γ (interferon gamma) in the spleen tissues of diabetic rats were analyzed. According to the results, the observed increase in MPO and ADA activities in the spleen of diabetic rats indicated that diabetes increased oxidative stress. Emodin and metformin individually decreased MPO activity in the spleen compared to the diabetic control (DK) group. However, co-administration of both compounds resulted in an increase in MPO activity compared to the DK group. No statistically significant variances were noted in ADA activity between the DK group and the groups administered with emodin and metformin. Diabetes led to an increase in serum immunoglobulin levels, whereas both emodin and metformin treatments resulted in a reduction of analyzed immunoglobulins. Furthermore, the expressions of inflammatory cytokines, including IL-1β, IL-6, TNF-α and IFN-γ, exhibited an increase in the spleen across all diabetic groups. Particularly, the DK group showed elevated expressions of IL-1β and IL-6, aligning with the observed levels of serum immunoglobulins. In conclusion, the treatment of diabetic rats with emodin and metformin exhibited positive influences on both MPO activity and immunoglobulin levels. *The authors marked with an asterisk equally contributed to the work.

P-35-002
Histopathological alterations induced by *Macrovipera lebetina obtusa* venom on hepatic, renal and cardiac tissues of mice

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Snakebites are a global health concern, with up to 2.7 million poisonings occurring annually, leading to a substantial number of fatalities, amputations, and disabilities. Among the venomous species of the South European region, *Macrovipera lebetina obtusa* (MLO) stands out for its potent venom, known to induce a wide array of harmful effects in envenomated organisms. The study was carried out on mice, divided into two groups, (a) healthy and (b) mice which were injected intraperitoneally with freshly diluted MLO venom at a lethal lose – $5LD_{50}$ (5×18.4 μg). The organs underwent histopathological examination to evaluate their condition. All organs in the control group exhibited a normal histological appearance. While in the venom group, we observed myocardial vessel hyperemia with erythrostasis in small vessels and the presence of fibrin clots, primarily in veins. Myocytolysis, extravasation of erythrocytes, and the formation of hemorrhage are evident in other myocardial foci. The hepatic architecture exhibits disruption in both lobular and tubular structures. There is a diffuse widening of capillaries, with sinusoids being engorged with erythrocytes leading to stasis and sedimentation. There are foci of intercellular membranes disappearence, where nuclei are absent, and tissue imbibed with erythrocytes – necrosis foci and hemorrhages. In the kidneys, there are large vessels that are mostly empty. There is an infiltration of lymphocytes and polymorphonuclear leukocytes in the stroma of particular renal papillae, where focal hemorrhages were observed. In this study, we demonstrated that MLO envenoming causes profound histopathological changes in the heart, liver and kidney tissues. Further physiological determinations and investigations of anti-venom may enhance our comprehension and improve the management of envenoming. The study was performed in the frames of project #23AA-1F008 supported by the Science Committee of MESCS RA and by the NAS RA under the code 22YSSPD-003.

P-35-003
Boosting expression of collagen in mammalian cells and enhanced bone regeneration via modulation of intracellular protein–protein interactions

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Collagen is the most abundant protein in mammalian tissues and is imperative in numerous biotechnological and clinical fields, from cultivated meat to tissue and bone regeneration. Despite its significance, high-yield production of natural collagen in mammalian cells remains a formidable challenge, resulting in reliance on animal-derived collagen. To enable mammalian cell production of collagen, efforts have primarily focused on optimizing cell culture conditions, such as increasing cell density and refining growth media. We recently showed that intracellular protein

modulation of the hypoxia pathway is a vital approach to boosting the expression of collagen[I]. By application of a small molecule known to upregulate the activity of the transcription factor hypoxia-induction factor (HIF), 3-fold higher collagen was measured in fibroblast and osteoblast cells. Based on intracellular protein modulation, our research explores additional signaling pathways via the application of small molecules and peptides, achieving even higher collagen yields. Importantly, since collagen is a primary organic component of bones, our method not only accelerates collagen production but also enhances mineralization in osteoblast cells and bone regeneration *in vivo*. Our experimental results demonstrate, for the first time, the potential of externally modulating the protein interaction network to boost collagen synthesis in mammalian cells. Our findings mark a significant stride towards enhancing natural collagen production by altering cellular signaling pathways, offering a promising avenue for numerous biomedical applications.

P-35-004
Hepatitis C virus envelope protein E2 antigen design and characterization for vaccine development

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With over 58 million global cases of hepatitis C virus (HCV) infection and the economic burden of antiviral treatments, the urgency for a vaccine is paramount. A key target in vaccine development is the viral envelope protein E2, specifically the 412-423 epitope, responsible for binding to the CD81 receptor, a critical entry factor for HCV. This epitope is recognized by broadly neutralizing antibodies (nAbs), making it a focal point for vaccine design. The 412-423 epitope exhibits multiple conformations, including a beta hairpin, shown by co-crystallization with nAbs. Our study aims to construct and characterize novel E2-derived antigens that induce a potent neutralizing humoral response by stabilizing the 412-423 epitope in a beta hairpin conformation. Advanced bioinformatics tools predicted mutations to stabilize the epitope. A conformational ensemble was generated using Hamiltonian Monte Carlo and Gibbs Sampling, followed by molecular dynamics simulations to assess mutant stability. Selected mutants were expressed in HEK 293T cells, and biochemical and antigenic properties were analyzed through western blot, ELISA, and glycan digestion. A vaccine candidate, expressed in Expi293 cells, was purified and further characterized. BALB-C and CD1 mice were immunized, and sera were used to neutralize HCV pseudoparticles (HCVpp) infection. Screening identified a candidate with preferential binding to an antibody recognizing the beta hairpin structure. The antigen, successfully purified in monomeric form, demonstrated altered CD81 binding. Preliminary immunization studies revealed a higher immunogenicity than WT E2, with indications of potentially enhanced neutralization capacity in the conformationally stabilized antigen using the HCVpp system. Our findings propose that *de novo* antigen design can stabilize the 412-423 epitope in a desired conformation, potentially influencing its immunogenicity. This approach holds promise for advancing the development of an HCV vaccine.

P-35-005
Inhibitory effect of oligoribonucleotides on interaction of SARS-CoV-2 Spike pseudotyped lentiviral particles with host cells

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Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) causes a respiratory illness coronavirus disease 2019 (COVID-19) that led to numerous deaths. SARS-CoV-2 enters cells using its Spike protein, which is also a prospective target for the development of new anti-COVID-19 drugs. Oligoribonucleotides (ORNs), fragments of RNA extracted from yeast, have antiviral activity by inhibiting the activity of surface viral proteins. We supposed that the ORNs can inhibit SARS-CoV-2 Spike activity. Because SARS-CoV-2 is a biosafety-level-3 virus, one way to simplify such assays is to pseudotype biosafety-level-2 viral particles with Spike. Therefore, in this study, we investigated the ORNs efficiency against pseudotyped lentiviral particles with SARS-CoV-2 Spike (Spike-pseudovirus). We generated the Spike-pseudovirus by transfecting the 293T cells with a plasmid complex (lentiviral Luciferase-IRES-ZsGreen-backbone, SARS-CoV-2 Spike and plasmids expressing the other HIV proteins needed for virion formation (Tat, Gag-Pol, and Rev)). Fluorescent microscope images showed the ZsGreen expression in the infected 293T-ACE2 cells (transformed 293T cells constitutively expressing the Spike's ACE2 receptor) with Spike-pseudovirus. Low ZsGreen fluorescence was observed in the Spike-pseudoviruses-infected 293T-ACE2 cells that were pre-incubated with the ORNs compared to the Spike-pseudovirus control. The luciferase activity was indicated in the infected 293T-ACE2 cells with Spike-pseudovirus. Preincubation of the Spike-pseudovirus with the ORNs reduced luciferase activity significantly compared to the Spike-pseudovirus control. Decreased expression of the ZsGreen and luciferase, marker proteins of cell infection with Spike-pseudovirus, indicates the ORNs suppress Spike-pseudovirus infectivity by inhibiting the interaction of Spike-pseudovirus with host cells. The results show that by suppressing the Spike-pseudovirus infection the ORNs can have an anti-COVID-19 activity.

P-35-006
Sensitizing cancer cells to the FAS ligand through strong activation of the p53 protein may prove to be a spectacular solution in anticancer therapy

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The p53 protein activates the pro-apoptotic gene *FAS*, which encodes the death receptor for the FAS ligand (FASLG). Cancer cells are resistant to programmed cell death triggered by FASLG. We have discovered that two substances actinomycin D (ActD) and nutlin-3a (Nut3a) act synergistically in the activation of p53 and stimulation of a subset of p53-target genes. ActD stimulates kinases involved in the activation of p53, while Nut3a prevents

the interaction of p53 with its inhibitor, the MDM2 protein. ActD + Nut3a activates the expression of many genes connected with apoptosis, what unexpectedly does not result in extensive programmed cell death. We hypothesized that proposed drug combination (ActD + Nut3a) sensitizes cancer cells to the pro-apoptotic activity of FASLG. We exposed various cancer cell lines and normal human fibroblast to ActD + Nut3a for 45 h and next we treated cells with recombinant FASLG. We observed apoptosis by flow cytometry and activation status of caspase-3, -6, -8, -9, and -10 by immunoblotting (western blot). The cell viability was determined by the MTS assay and cell staining on culture plates. ActD and Nut3a strongly synergized in sensitizing cells to apoptosis triggered by FASLG. This combination killed almost all cells within 5 h. The cell death was accompanied by a strong activation of all examined caspases. In engineered p53-deficient cells this pro-apoptotic effect was completely lost. Hence, the observed cell sensitization is entirely dependent on p53. Therefore, the combination of ActD + Nut3a activates p53 in a way, which overcomes the resistance of cancer cells to apoptosis triggered by FASLG. The implementation of the proposed project opens the path to a completely new therapeutic approach in oncological treatment and provides new information on the effect of strong activation of the p53 protein. This research was funded by National Science Center (NCN), Poland, grant numbers: 2017/27/N/NZ5/01079 (B.Ł.-S.) and 2019/35/O/NZ5/02600 (M.R.)

P-35-007
Molecular characterization of pyrazinamide mode of action against *Mycobacterium tuberculosis*

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Tuberculosis (TB), caused by *Mycobacterium tuberculosis* (Mtb), remains one of the world's leading causes of death due to an infectious agent. Among the first-line drugs available, pyrazinamide (PZA) plays a crucial role in the treatment of TB. PZA is a prodrug, which needs to be converted by the bacterial enzyme PncA into pyrazinoic acid (POA), its active form, to exhibit antibacterial activity. Two main and conflicting modes of action are currently proposed in the literature. In the historical model, PZA/POA molecules are mainly active under acidic environmental pH conditions where they acidify intra-bacterial pH (pH_{IB}). However, recent studies have reported a new pH-independent model in which POA inhibits PanD, an aspartate decarboxylase involved in the coenzyme A (CoA) biosynthesis pathway. Thus, the molecular mode of action of this antibiotic remains poorly characterized. The aim of my PhD project is to decipher the contribution of acidic pH and the PanD protein in the molecular mode of action of PZA/POA molecules and their efficacy. To answer these questions, we carried out antimicrobial susceptibility testing in chemically defined media and further monitored the effect of PZA/POA molecules on Mtb pH_{IB} expressing a fluorescent reporter. Our results show that acidic pH potentiates the antimicrobial activity of PZA/POA, and disrupts Mtb pH_{IB} homeostasis in a dose-dependent manner. Finally, we show that exogenous pantothenate supplementation, a critical substrate involved in the CoA biosynthesis pathway downstream of PanD, strongly antagonized the antibacterial activity of PZA/POA regardless of the environmental pH, suggesting that this pathway may play a primary role in PZA/POA susceptibility. Such investigations aim at redefining PZA mode of action, and therefore providing new insights into the fundamentals of anti-TB therapy with important clinical implications.

P-35-008
HBV antigens produced in *Nicotiana benthamiana* with "humanized" N-glycosylation patterns show significantly improved immunogenicity and induce virus-neutralizing antibodies

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Hepatitis B Virus (HBV) infection affects over 290 million individuals globally, leading to 800,000 annual deaths. Vaccination remains the primary strategy to combat HBV spread and meet the WHO's objective to eliminate viral hepatitis by 2030. Enhancing the immunogenicity of HBV antigens is essential to overcome the drawbacks of existing vaccines, based on the small (S) envelope protein, such as non-responsiveness and inadequate defense against "vaccine-escape" mutants (VEM). We previously showed that inserting immunogenic epitopes from the large (L) envelope protein into the S antigenic domain significantly enhances the anti-HBV immunogenicity. However, protein production in mammalian cells is costly and restricts large-scale manufacturing. Plants are an appealing alternative for protein production due to their versatility and rapid scalability, and the recent generation of plants lacking β-1,2-xylosyltransferase and α-1,3-fucosyltransferase activities (FX-KO), via CRISPR/Cas9 editing, allows for the production of proteins with "humanized" N-glycosylation patterns. This study explores the influence of plant N-glycosylation on the immunogenic properties of the chimeric HBV S/preS1$^{16–42}$ vaccine candidate produced in both wild-type (WT) and FX-KO *Nicotiana benthamiana*. We showed that the absence of β-1,2-xylose and α-1,3-fucose in the FX-KO *N. Benthamiana*-produced HBV antigen significantly affects its antigenicity compared to the WT counterpart. Additionally, the "humanized" glycosylation pattern enhances the immune response in mice compared to the antigen produced in WT plants. Notably, antibodies triggered by the FX-KO-produced antigen more efficiently neutralized both WT HBV and a clinically relevant VEM. Thus, this study establishes glyco-engineered *N. benthamiana* as a markedly improved and cost-effective host for plant production of glycoprotein vaccines, emphasizing the impact of antigen glycosylation on the immunogenic properties of vaccine candidates.

P-35-009
G-quadruplex structures from the influenza A virus genome and their potential function during viral replication cycle

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Influenza A virus (IAV) causing pandemic outbreaks became an important research subject. Despite the high variability of its genome, viral RNA (vRNA) structure possesses features that remain constant between strains. Previous research demonstrates the significance of the vRNA secondary structure in the viral life cycle. Among the structures present within vRNA are G-quadruplexes (G4s) formed within G-rich sequences called PQSs. G4s have various functions during the replication cycle and thus are investigated as potential therapeutic targets. We searched the IAV genome for the presence of PQSs, studied their ability to fold into G-quadruplexes, and their potential role in the viral life cycle. Using bioinformatics tools, we identified twelve PQS motifs within the IAV vRNA. Then, by biophysical methods, we determined their propensity to form G4s. To this end, we used spectroscopic techniques and reverse transcription (RT) stop assay. The non-infectious IAV replication model, minireplicon, was then used to study the impact of G4-specific ligands, TMPyP4 and TMPyP2, on the virus replication in cell culture. Our results revealed that three of the selected PQSs from the IAV vRNA form stable RNA G4s. Moreover, these PQS motifs are present within segments encoding viral polymerase complex proteins. RT stop assay showed that G4-specific ligands can stabilize G4s restricting product synthesis. The biological studies demonstrated that TMPyP4 effectively inhibits IAV minireplicon replication and is a more potent inhibitor than TMPyP2. Taking into consideration the results, we concluded that G-quadruplexes are present within the IAV genome and can be targeted by specific ligands. What is more, via G4 stabilization, viral replication can be effectively inhibited. All our findings suggest that selected PQS motifs from the influenza A virus genome can serve as potential novel antiviral therapeutic targets. Previously published in Tomaszewska M et al. (2021) Int J Mol Sci 22, 6031.

P-35-010
Novel inhibitors of emerging coronaviruses entry

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The global health crisis triggered by the COVID-19 pandemic underscored the urgency for innovative therapeutic approaches. Our study explored three distinct antiviral strategies. First, we explored the specific interactions between the SARS-CoV-2 spike protein and the cellular receptor ACE2. We examined the potential of conjugates of cannabidiol (CBD) and non-steroidal anti-inflammatory drugs (NSAIDs) as novel antiviral dual-target agents against SARS-CoV-2/COVID-19. Results indicated that certain CBD–NSAID combinations exhibited superior antiviral activity against both SARS-CoV-1 and SARS-CoV-2, suggesting a potential therapeutic role in treating emerging coronavirus infections [1]. In the second approach, we investigated the viral entry based on the proteolytic activation of the SARS-CoV-2 spike protein facilitated by type II transmembrane serine proteases (TTSPs) like TMPRSS2. We defined the RNA secondary structure of TMPRSS2 coding sequence *in vitro*, and designed TMPRSS2-specific antisense oligonucleotides (ASOs) targeting specific structural motifs. Our findings demonstrated that TMPRSS2-specific ASOs effectively silenced the TMPRSS2 gene expression, leading to a reduction in the entry of emerging coronaviruses. For the third approach, we investigated inhibitors of surface protein interactions and proteolytic activation, screening on organometallic compounds to identify potent antivirals. Erlotinib and its organometallic conjugate compound 5 exhibited promising properties in inhibiting SARS-CoV-2. Collectively, our discoveries offer a promising and novel approach to address current and future viral emergencies. Reference: [1]. Pawełczyk, Anna, et al. Pathogens 12.7 (2023): 951.

P-35-011
A scaffold-free tissue engineering approach to create substitutes for cartilage repair by cell sheets

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Cartilage is a connective tissue with limited capacities of regeneration due to its low cell density and non-vascularization characteristics. To repair cartilage, different tissue engineering techniques were used to generate *ex vivo* tissue substitutes. Cell Sheet Technology (CST) is based on cell expansion *in vitro* and the recovery of the cell layer without enzymatic dissociation. This allows to preserve the extracellular matrix and different interactions, such as cell-cell and cell-matrix junctions, in order to mimic tissues physiology since cells are used as extracellular matrix sources. Our goal is to create auricular cartilage substitutes, using CST, by combining progenitor cells from the human

auricular perichondrium and the formation of cell sheets thanks to ascorbic acid in the medium. We sought to reconstitute cartilage substitutes by stacking several cell sheets, layer by layer, subjected or not to chondrogenic differentiation medium for several weeks. The constructs were analyzed by histological staining. Elastic cartilage markers (type II collagen, aggrecan, elastin) were evaluated at mRNA level (RT-qPCR) and localization by immunofluorescence within different cell sheet constructions. Dedifferentiation (type I collagen, alkaline phosphatase) and hypertrophic (type X collagen) markers were also evaluated. The use of chondrogenic medium combined with presence of ascorbic acid allowed an increase on the thickness of the constructions, with good adhesion between sheets. Analysis of the extracellular matrix by immunostaining shows the presence of cartilage-specific markers. Additionally, we performed proteomic analysis of the constructs and compared them to native cartilage. Our approach brings new perspectives and applications of the cell sheet technology in the field of tissue regeneration of cartilage engineering.

P-35-012
Trypsin digestion of protein in beef meat extract in the presence of microplastics

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Trypsin is the main protease in the intestine. Microplastics (MPs) have been previously shown to interact with and decrease the activity of some digestive enzymes, including pepsin and lipase. Red meat has been shown to be a source of allergy which has been linked to the galactose-alpha-1,3-galactose (alpha-Gal) post-translational modification of proteins. Our aim was to investigate the effect of two types of MPs commonly found in the environment – polypropylene (PP) and polyethylene terephthalate (PET) – on the digestion of protein in beef meat extract and preservation of protein harboring the alpha-Gal epitope. Digestion of beef meat extract has been performed with trypsin in simulated intestinal fluid (SIF) in the presence of MPs. After digestion was stopped with a specific inhibitor, bulk beef meat extract was separated through centrifugation from the MPs. Soft coronas were obtained by washing the MPs with SIF. The hard corona was obtained by addition of a reducing buffer for electrophoresis sample preparation to the MPs with a heating step at 95°C. All samples were analyzed with SDS-PAG electrophoresis. Selected samples were further analyzed with anti-alpha-Gal antibodies using western blot. There is an observable difference between the digestion patterns of meat extract after 1 and 2 h of digestion in the presence of MPs compared to the control. Evolution of digestion is similar for both types of MPs, without regard to plastic type. It has also been confirmed that preserved proteins possess the alpha-Gal modification. As MPs presence does not change trypsin specific activity, the change in digestion pattern is presumed to be due to steric effects and/or interplay of enzyme/protein in the corona. This study suggests that MPs presence influences trypsin digestibility of meat proteins, including alpha-Gal-bearing allergens. This project has received funding from the European Union's Horizon 2020 research and innovation programme under grant agreement No 965173.

P-35-013
The relationship of C-Triol and 7-KC Levels with macrophage activation indicators and carotid intima media thickness in children with familial hypercholesterolemia

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Hypercholesterolemia is an important risk factor for cardiovascular and cerebrovascular diseases and can induce atherosclerosis leading to coronary heart disease, stroke, and myocardial infarction. Oxysterols are oxidized derivatives formed from cholesterol enzymatically or non-enzymatically and play a role in the pathogenesis of atherosclerosis and coronary artery disease The aim of this study is to determine some important oxysterol levels in children with hypercholesterolemia and investigate the relationship of these levels with oxidative stress and the development of atherosclerosis. In this context, levels of Kolestane-3β,5α,6β-triol (C-Triol), and 7-Ketocholesterol (7-KC) were measured by LC MS MS methods. MDA levels were measured as an oxidant stress indicator, and SOD, catalase and paraoxanase levels were measured as an antioxidant capacity indicator by a colorimetric method. The study also evaluated chitotriosidase activity and YKL-40 levels as markers of macrophage activation, while intima media thickness, which indicates the development of atherosclerosis, was evaluated by ultrasonography. The study included 25 patients with familial hypercholesterolemia aged between 6 and 18 years and 22 healthy children. C-Triol and 7-KC levels were found to be significantly higher in the patient group. There was a positive high significant correlation between total cholesterol and C-Triol, while a weak positive correlation was found between 7-KC and total cholesterol. There was a significant positive correlation between LDL-cholesterol levels and C-Triol (high) and 7-KC (weak). C-Triol provides an excellent discrimination equivalent to CIMT between the two groups. As a result, this study is the first to determine and compare plasma oxysterol levels in healthy and hypercholesterolemic children. The high correlation of C-Triol levels with CIMT makes it a suitable candidate for the development of atherosclerosis.

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P-35-014
The identification and characterization of *E. coli*-specific phages from human urine samples

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Urinary tract infections (UTIs) are a type of bacterial infection that affect millions of people every year. These infections can be quite serious, with mortality rates ranging from 0.7% to 10% among hospitalized patients. The most common cause of UTIs is a type of bacteria called uropathogenic *E. coli*. Unfortunately, these bacteria are becoming increasingly resistant to antibiotics, making treatment more difficult. To combat this problem, researchers are exploring alternative treatments, such as phage therapy. The isolation of phages from human body fluids has not been extensively studied. Therefore, our project aims to isolate phages and bacteria from human urine and investigate their biological characteristics. Within the scopes of this study urine samples from 92 people were collected. We used bacteriological and biochemical methods such as the Api test, coagulase test, and oxidase test to isolate and identification E. coli from the same urine samples. We were able to isolate 32 urological strains of *E. coli*, and we determined the sensitivity of bacterial isolates to antibiotics using the Kirby-Bauer disc-diffusion method. We enriched the urinary samples to isolate 4 phages and studied their characteristics. We determined the host range using the spot test assay and the parallel streak methods, the morphology of the phage capsid using transmission electron microscopy (TEM), and the stability to environmental conditions such as temperature and pH conditions. Overall, we were able to isolate 32 new *E. coli* isolates and 5 urological phages. We studied the sensitivity of 21 antibiotics to the *E. coli* bacterial isolates and found that 2 out of 10 bacterial strains (20%) could be characterized as multidrug-resistant. Additionally, we found that 3 urological phages isolated from urine acted on one urological isolate. It's worth noting that we isolated the bacterium MEC3 and the phage NatsEC4 from the same source. These phages have a narrow spectrum of activity.

P-35-015
New challenges in embryo quality diagnostics from spent blastocyst medium in the *in vitro* fertilization (IVF) process

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The success of embryo transfer during an *in vitro* fertilization (IVF) cycle depends on many factors, the most important of which are a receptive endometrium and a quality embryo. During pre-implantation development, the embryo secretes substances into the culture medium, which could represent a suitable biomarker of embryo quality. Next-generation sequencing (NGS) analyses were used in a total of 63 SBM (53% successfully implanted embryos, 47% non-successfully implanted embryos), and 3 SBM were used for the qualitative and quantitative background. MicroRNA was isolated from SBM using the miRNeasy Micro Kit (Qiagen). Subsequently, a sequencing library using the Qiagen sequencing chemistry QIAseq miRNA Library Kit was prepared. NGS analysis using a NextSeq 500 on the Illumina Platform was performed. In our work among the number of detected molecules, 4 miRNA molecules with high specificity and sensitivity (AUC over 70%) were identified in predicting a high-quality embryo based on the sequencing and a combination of bioinformatics data analysis. A viable embryo for transfer is selected based on its morphology status. The non-invasiveness related to the analysis of the SBM represents a suitable tool for the study and characterization of the embryo, which could potentially increase the probability of successful embryo implantation. This work was supported by VEGA 1/0435/23, APVV-22-0357. *The authors marked with an asterisk equally contributed to the work.

P-35-016
Neurophage: molecular engineering of phage nanoparticles for non-invasive neuronal photostimulation

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The M13 phage has emerged as a versatile nanocarrier with a wide range of innovative nanobiotechnology applications. Its distinctive filamentous shape, coupled with the arrangement of different coat proteins along its structure, provides an exceptional cargo capacity for genetically fused or chemically conjugated molecules. In addition, the intriguing and unexplored characteristic of M13 phage to cross the blood–brain barrier (BBB), makes it a promising delivery agent for the treatment of different neurological diseases, overcoming challenges in the biomedical field. The mechanisms that enable M13 to cross the BBB were investigated *in vitro* on 2D and 3D BBB models. Furthermore, the high cargo capability and ease of genetic handling were exploited to enhance its crossing ability by displaying BBB interacting peptides in fusion with the phage's major coat protein (pVIII). Additionally, nanobodies were displayed on the phage's minor coat proteins (pIII), to enable the retargeting of the nanobiotechnological platform towards specific cell populations. As proof of concept, an anti-ALFA tag nanobody expressed in fusion with the pIII protein allowed the specific targeting of the phage to engineered neurons expressing a synthetic ALFA-transmembrane protein. After validating the BBB crossing ability and targeting specificity, further modifications are currently being introduced to this phage vector platform to target various central nervous system receptors implicated in pathological pathways. These modifications will involve genetic manipulation of mice and chemical conjugation of photovoltaic materials to the phage, with the overarching goal of demonstrating the non-invasive photostimulation of denervated neurons by the Neurophage nanobot.

P-35-017
Effects of the antiretroviral drugs Dolutegravir, Bictegravir and Raltegravir on 3T3-L1 mature adipocytes

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Integrase transfer strand inhibitors (INSTIs) are often prescribed as a first line treatment for patients with human immunodeficiency virus (HIV). Recently, weight gain has been observed in patients treated with INSTIs. In turn, weight gain might be correlated with serious comorbidities, including diabetes and cardiovascular diseases. A direct effect of INSTIs on adipocyte function and dysfunction may underlie this peculiar side effect. To investigate this aspect, three drugs, Dolutegravir (DTG), Bictegravir (BIC) and Raltegravir (RAL), were administered to 3T3-L1-derived mouse mature adipocytes, and the levels of markers of adipogenesis, fibrosis and inflammation were measured by western blot (WB) or qPCR. All three drugs increase in a dose-dependent manner the expression of adipogenesis markers including Pparγ, Cebp-α, Atgl, and adiponectin. INSTIs treatment increases the expression of fibrotic markers including fibronectin and vimentin, while α-SMA increased only following DTG treatment. Moreover, RAL and DTG strongly increase the inflammation marker CCL2. Cells were then stained with Red Oil O solution to assess eventual changes in the accumulation of fat in lipid droplets and treated adipocytes showed an increase of retrieved staining compared to control, this being correlated with an increasing of fat into lipid droplets. Together these results clearly demonstrated that INSTIs not only sustain adipogenesis but also promote adipocytes to acquire a fibrogenic/inflammatory/dysfunctional phenotype which may contribute to adipose tissue metabolic alterations. These results contribute to shed light on INSTIs' role on weight gain, but further studies are required to fully understand their role.

P-35-018
Selection of DNA aptamers for inhibition of the endopeptidase activity of the light chain of *Clostridium tetani* neurotoxin (TeNT)

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TeNT neurotoxin, which is produced by Gram-positive, anaerobic *Clostridium tetani* blocks the release of inhibitory neurotransmitter from synaptic vesicles and causes spastic paralysis. TeNT consists of two polypeptides, namely heavy (H) and light (L) chains, which are joined by a disulphide bond. L chain shows zinc endopeptidase activity and cleaves the integral membrane component of synaptic vesicle, VAMP (vesicle-associated membrane protein2/Synaptobrevin2/VAMP-2)[1]. In the literature, there are studies about developing aptamers for several pathogenic bacteria and their toxins. However, an aptamer specific for L or H chains of *C. tetani* TeNT neurotoxin has not been reported yet. The aim of this study was to develop specific DNA aptamers that can bind to L chain, which is critical for the action mechanism of TeNT toxin. Aptamers are short, specific, single-stranded DNA or RNA oligonucleotides, which can bind to their targets with high specificity. For this purpose, the L chain of *C. tetani* TeNT neurotoxin and VAMP-2 were recombinantly produced and purified in *E. coli* cells. Using the SELEX method, DNA aptamers binding to the purified L chain were selected and 10 candidate aptamers were identified. Further characterization studies were performed to identify the inhibitory potential of the aptamers on L chain and VAMP-2 interaction. The developed aptamer is expected to be potentially used in diagnosis/treatment of tetanus. Reference: 1. Yousefi M et al. (2013) Avicenna J Med Biotechnol. 5(4), 220–226. This work was supported by the Scientific and Technological Research Council of Türkiye (TUBITAK) (grant number 116Z024)

P-35-019
First steps towards the light-directed enzymatic *de novo* synthesis of DNA microarrays

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The recent efforts to develop approaches for the enzymatic *de novo* synthesis of DNA oligonucleotides have centered around the use of polymerases that show template-independent activity. To enzymatically generate a desired nucleotide sequence, the polymerization needs to be controlled to stall after each nucleotide addition. One strategy is to use nucleoside triphosphates with a transiently blocked 3′-OH: after incorporation, enzymatic strand extension cannot continue until the blocking group is removed. Having spatial control over this deblocking process would allow for large sets of sequence variants to be prepared in parallel on a single surface, a process that is known as microarray synthesis. One microarray fabrication technique is photolithography and uses patterned UV light to synthesize hundreds of thousands of unique oligonucleotides per surface in a process analogous to conventional solid-phase DNA synthesis. We now aim to transfer this principle to enzymatic DNA synthesis and generate DNA libraries at high throughput, with the added advantage of a more sustainable and less error-prone approach compared to chemical synthesis. In the first stage of the project, DNA triphosphates equipped with a photosensitive protecting group at the 3′-OH position were synthesized, with either a 2-(2-nitrophenyl)-propyloxycarbonyl (NPPOC) or a benzoylated derivative (BzNPPOC). After isolation and characterization, we tested these novel building blocks as substrates in untemplated primer extension reactions with the Terminal transferase (TdT) and Poly(U) polymerase (PUP). Surprisingly, while both polymerases accepted the modified triphosphates and quantitatively extended the primer under certain reaction conditions, analysis of the extended product suggests that the protecting group was prematurely removed by either enzyme.

P-35-020
Cold plasma (CP) induced secondary metabolites changes in *Stevia rebaudiana* Bertoni

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The most economically important source of natural low-calorie sweeteners, steviol glycosides (SGs) is *Stevia rebaudiana* Bertoni with stevioside (Stev) and rebaudioside A (RebA) being the most abundant. Pre-sowing seed treatment with cold plasma (CP) was shown to stimulate SGs biosythesis/accumulation up to several folds. In addition to SGs increase, the CP-induced increase of antioxidant activity was observed as well. This study aimed to evaluate the effect and it's universality of two types of CP on two different *Stevia rebaudiana* cultivars. The effect evaluted by comparing SGs, total flavonoid content (TFC), total phenolic content (TPC) and antioxidant activity (AA). Seeds of 2 stevia cultivars – SHUG A3-6 and SHUG HIGH A3 (HYBRID) – were treated for 2, 5 and 7 min. with two different CP equipment types – captively coupled (CC – CC2, CC5 and CC7 groups) and dieletric barrier discharge (DBD – DBD2, DBD5 and DBD7 groups) plasmas – before sowing. Both CC and DBD treatments stimulated SG production; however to different extents. In HYBRID CP5 induced the highest increase of 10% in RebA+ Stev concentration, in SHUG A3-6 the highest increase was observed in DBD5 group by 1.9 fold. The overall tendency to increase Stev and decrease RebA concentrations compared to control was observed in all groups. CP did not affect TFC and TPC except for SHUG A3-6 DBD5 group – where the increase in TPC concentration by 1.6 fold was observed. AA was stimulated in both cultivars by both plasmas. The highest AA increase was observed in DBD5 groups – by 1.7 fold in SHUG A3-6 and by 2.4 fold in HYBRID. In conclusion, seed treatment with two types of CP induced SGs concentration increase due to Stev biosynthesis stimulation which, in the case of DBD, correlated with the increase in antioxidant activity and TPC concentration rather than TFC in both cultivars. *The authors marked with an asterisk equally contributed to the work.

P-35-021
A tale of two models: Exploring defense priming in arabidopsis and tomato

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Plants are constantly confronted with different environmental conditions and must defend themselves against pests and pathogens. However, plants' inherent immunity often proves insufficient, leading to widespread crop infections and significant global socio-economic impacts. To address this problem, the concept of defense priming has been developed, which offers a more energy-efficient alternative by enhancing the plant's ability to respond without immediate activation of defense mechanisms. Understanding the molecular intricacies of defense priming is critical and underscores the importance of appropriate model plant selection. Our research focuses on defense priming in tomato induced by beta-aminobutyric acid (BABA), a potent compound. Surprisingly, our results suggest that the priming effect of BABA in tomato is mediated via the ethylene metabolic pathway, in contrast to previous findings in arabidopsis. This discrepancy underscores the importance of using multiple plant models to decipher the complex defense responses. With this in mind, we have extended our study to arabidopsis in addition to tomato. Using a detailed RNA-seq analysis, we investigated the transcriptomic changes in *Arabidopsis thaliana* Col-0 after BABA treatment and compared the results with those of tomato. In particular, the analysis revealed profound transcriptome alterations in the ibm1-4 mutant line of *A. thaliana* Col-0. The down-regulated genes showed enrichment in plant-pathogen interactions and photosynthetic processes, correlating with increased susceptibility to *P. syringae*. Remarkably, BABA treatment in the ibm1-4 mutant line upregulated previously repressed genes associated with plant-pathogen interactions, WRKY transcription factors, and biosynthesis of Phe, Tyr, and Trp. This was accompanied by increased SA/MeJA levels and significantly improved resistance. Our study highlights the need to use diverse plant models to unravel the mysteries of plant defense responses.

P-35-022
Perfluorooctane sulfonic acid increases VEGF expression in human granulosa cell line HGrC1

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The cause of female infertility is multifactorial including genetic and lifestyle factors and various health conditions. Recently, exposure to endocrine disruptors (EDs) has been highlighted as an important contributor to female infertility. Increased attention is placed on perfluorooctane sulfonic acid (PFOS), an ED and a "forever chemical" belonging to per- and polyfluoroalkyl substances. PFOS is widely distributed in the environment; hence, human exposure is inevitable. Women with higher levels of PFOS in serum and follicular fluid have 40% lower chance of becoming pregnant. However, little is known about the mechanism by which PFOS interferes with ovarian function. The main objective of this study was to identify the *in vitro* effects and the molecular mechanism of PFOS in human granulosa cell line HGrC1. HGrC1 cells were exposed to different concentrations of PFOS, and cell viability assay and RNA sequencing were conducted. PFOS at 5 μM and 10 μM were not cytotoxic to HGrC1 cells after 48 h of exposure. RNA sequencing analysis demonstrated that exposure to 10 μM PFOS affected 9 genes, including the gene encoding vascular endothelial growth factor (VEGF). We further exposed HGrC1 cells to 25 μM and 50 μM PFOS in different time points and conducted real-time quantitative PCR to reveal the time- and concentration-dependent effect of PFOS on VEGF mRNA. The highest increase in VEGF mRNA level was observed after 6-h-long exposure to 25 μM PFOS. Next, we analyzed the signaling pathways involved in the observed effect of PFOS on VEGF mRNA in HGrC1 cells. Western blot analysis revealed that 25 μM PFOS significantly increased phospho-AKT levels after 15 minutes of exposure. These preliminary results indicate that exposure to PFOS could interfere with VEGF through AKT signaling pathway. Additional research is needed to reveal a more detailed mechanism by which PFOS affects the expression of VEGF and ovarian function.

P-35-023
Pleiotrophin enhances mTORC1 activity and protein synthesis in endothelial cells through crosstalk of multiple receptors

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Pleiotrophin (PTN) is a secreted factor that enhances endothelial cell migration through its PTPRZ1 and $\alpha_v\beta_3$ integrin receptors. PTN also binds to vascular endothelial growth factor receptor 2 (VEGFR2), although the effect of this binding on endothelial cell activation is unclear. In the present work, we aim to elucidate the PTN signaling pathway in endothelial cells isolated from human umbilical veins (HUVEC) or the lungs (LMVEC) of *Ptprz1*$^{+/+}$ and *Ptprz1*$^{-/-}$ mice. PTN increases mTORC1 activity and protein synthesis, both eliminated by the mTORC1 inhibitor, rapamycin. PTPRZ1 is involved in this effect since *Ptprz1*$^{-/-}$ LMVEC have increased mTORC1 activity and protein synthesis, sensitive to rapamycin. Given the inhibitory effect of crizotinib on the enhanced angiogenic phenotype of *Ptprz1*$^{-/-}$ LMVEC, we tested the involvement of cMet in PTN activities. The cMet inhibitor crizotinib abolished the PTN-induced mTORC1 activation or the enhanced mTORC1 activity in *Ptprz1*$^{-/-}$ LMVEC, and PTN enhances cMet tyrosine phosphorylation through inhibition of the PTPRZ1. Considering the direct interaction between PTN and VEGFR2, we studied the role of VEGFR2 on the activation of mTORC1 by PTN. PTN enhances VEGFR2 tyrosine phosphorylation, while the selective VEGFR2 tyrosine kinase inhibitor nullified the PTN-induced mTORC1 activation and protein synthesis. Finally, the involvement of $\alpha_v\beta_3$ integrin was studied by using the selective anti-$\alpha_v\beta_3$ LM609 antibody or a PTN peptide that inhibits the PTN-$\alpha_v\beta_3$ interaction; both abolish the effect of PTN in endothelial cell migration. Surprisingly, LM609 and the PTN peptide induced cMet tyrosine phosphorylation and mTORC1 activation, providing a potential explanation for integrin inhibitors' failure in clinical trials. Our data suggest that PTN activates mTORC1 and protein synthesis in endothelial cells downstream of PTPRZ1, $\alpha_v\beta_3$, VEGFR2, and cMet, and give insights into the complex signaling regulating endothelial cell functions.

P-35-024
Characterizing 'sro': a singular repression system in arbitrium phages of the SPbeta family

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The arbitrium system, a novel quorum-sensing communication mechanism first identified in phages of the SPbeta family, governs the decision-making process between lysis and lysogeny[1]. These phages use peptides to communicate and coordinate the decision between lysis and lysogeny, but the mechanism by which these phages establish lysogeny remains unknown[2]. In our study of the SPbeta phage family, encompassing the species groups *Spbeta*, *eta*, *bimanducare*, *magnus*, and three other orphan phages[3], we identified a unique six-gene operon named the 'SPbeta phages repressor operon' (*sro*). Serving as an auxiliary repressor, SroD, in conjunction with the master repressors SroE and SroF, forms the repression module necessary for lysogeny establishment and maintenance. Additionally, we propose that the proteins SroABC within the operon constitute the transducer module, linking the arbitrium communication system to the activity of the repression module. Our *in silico* analyses also highlight variability within the transduction module among different species groups and open reading frames (ORFs). Significantly, recent laboratory findings, including the elucidation of the protein structure of the master repressor binding to DNA, enhance our comprehension of this intricate interplay. Overall, this research sheds light on the intricate and specialized repression system employed by arbitrium SPβ-like phages in making lysis-lysogeny decisions. References: 1. Erez Z et al. Nature. 2017;541 (7638):488-493. 2. Del Sol FG, Penades JR, Marina A. Mol Cell. 2019;74(1):59-72. 3. Kohm K et al. Environ Microbiol. 2022;24 (4):2098-2118. *The authors marked with an asterisk equally contributed to the work.

P-35-025
N4BP1 RNase binds to EDC4 and decapping complex in P-bodies

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N4BP1 (*Nedd4 Binding Protein 1*) is a ribonuclease consisting of 2 K-homology domains (*KH domain*), UBA (Ubiquitin Associated) domain, NYN (N4BP1, YacP-like Nuclease domain) domain and CoCUN (*Cousin of CUBAN*) domain. N4BP1 was proved to be an essential factor in the modulation of immunological response, signal transduction to NFκB (nuclear factor kappa-light-chain-enhancer of activated B cells) and degradation of viral mRNA. The aim of this research was to investigate the

interaction between N4BP1 and its bindings partner EDC4 by identification of the N4BP1 region responsible for this interaction. To establish which domain of N4BP1 is crucial for this interaction, we produced several genetic constructs coding for N4BP1-Fc, containing a deletion of a respective domain or fragment. We found that after deletion of the KH domain, the interaction with EDC4 was disrupted. EDC4 did not co-immunoprecipitate or colocalize with this deletion variant. KH domains contain a consensus GxxG motif responsible for the interaction with RNA. In N4BP1 these motifs are replaced by QSRG and GAES in KH1 and KH2 respectively. We introduced mutations into these motifs to abolish the interaction with RNA by the substitution of 2 internal amino acid residues to QDDG and GDDS in KH1 and KH2 respectively. Using immunoprecipitation followed by Western blot analysis we observed that the interaction between mutation variants of N4BP1-Fc (QDDG, GDDS, or both) and EDC4 was disrupted which suggests that this might be an RNA-mediated interaction. In conclusion, we were able to point out the amino acid residues crucial for the interaction with EDC4.

P-35-026
Cellular profiling of connexin isoform in retina with age

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Gap junctions, formed by structural elements known as connexins, are intercellular channels crucial for retinal homeostasis, facilitating the exchange of metabolites between neighbouring cells. These channels are therapeutic targets in different age-related diseases. However, to date, the majority of strategies have focused on Cx43, without considering the high diversity of connexins and their cell-dependent localization within the retina. A comprehensive understanding of the retinal connectome during aging remains aspirational, with no published systematic analysis. Here, retinas from C57BL/6J mice at 1 month, 4 months, 12 months, and 24 months were collected for western blot, qPCR and immunohistochemical analysis of various Cxs isoforms: Cx43 (expressed in RPE and ganglion cells), Cx50 (expressed in Müller cells), Cx36 (expressed in cones, rods, bipolar and amacrine cells), Cx45 (expressed in neural retina and ganglion cells), Cx57 (expressed in horizontal cells) and Cx30.2 (expressed in ganglion cells). Images were captured using the EVOS M7000 microscope, and 3D modelling was performed using the Celleste 3D Deconvolution module. Given the role of GJ channels in retinal inflammasome, we stained against GFAP, Galectin-3, and Iba1. As mice aged, differences in content or location were observed in an isoform-dependent manner. No major changes were observed until 12 months. Highest differences are found at 24 months with changes in expression and location of the different isoforms. Our findings provide new evidence regarding the relationship between Cxs-based communication and aging, uncovering a differential response of retinal Cxs isoforms with age. Funding: RYC 2018-024434-I, MINECO PID 2020-119466RB-I00, and FUSP-PPC-19-B53C4C64.

P-35-027
Oxygen sensing and oxygen-dependent signal transduction in oxygen sensor protein HemAT

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HemAT functions as an oxygen sensor in the chemotaxis control system of bacteria, consisting of a sensor domain and a signaling domain. The sensor domain in HemAT possesses a globin structure, with the heme group within the molecule serving as the core of the oxygen sensor. Binding of oxygen molecules to the heme in HemAT initiates chemotactic signal transduction. However, the specific mechanism by which HemAT recognizes oxygen and transmits the sensed information to the signaling domain is not well understood. To address this question, we aimed to elucidate the molecular structures of both the oxygen-bound (signal ON state) and reduced (signal OFF state) forms of HemAT and compare them. By X-ray crystallography, we determined structures of the sensor domains of HemAT in oxygen-bound and reduced forms at resolutions of 2.50Å and 2.36Å, respectively. In both cases, His119 coordinates with the heme as the axial ligand. In the proximal heme pocket of oxygen-bound HemAT, Tyr129 near the axial ligand His119 forms a hydrogen-bonding network with Glu168 though three water molecules. In contrast, this hydrogen-bonding network is absent in reduced HemAT. In oxygen-bound HemAT, the presence of this hydrogen-bonding network immobilizes the C-terminal helix of the sensor domain. In reduced HemAT, where the hydrogen-bonding network is absent, the C-terminal region is presumed to be flexible. The C-terminal helix of the sensor domain serves as a linker connecting the sensor domain and the signaling domain of HemAT. This suggests that binding of oxygen to the heme in HemAT induces a conformational change in the linker region, and this conformational change likely plays a crucial role in the oxygen-specific signal transduction by HemAT.

P-35-028
Expression profile of autophagy-related genes during aging of summer and winter worker bees (*Apis mellifera* L.)

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The honey bee (*Apis mellifera* L.) is a promising model for aging studies due to its phenotypic plasticity in relation to lifespan. Summer bees are characterized by intensive foraging behaviour and have a short lifespan of one month, while winter bees, which live up to six months, are less active and spend most of their time in the hive when conditions are unfavorable. Due to the seasonality of worker bee lifespan, it seems likely that the honey bee may become a key invertebrate model for understanding the molecular regulatory control of longevity. Autophagy is the major mechanism for recycling intracellular components (e.g. damaged proteins, cellular debris, whole organelles) in lysosomes. A decline in autophagy with age has been described extensively in a variety of systems. It is mediated by a set of evolutionarily conserved genes, the autophagy-related genes (*ATG*). The aim of

this study was to measure the expression of selected *ATG* genes in summer and winter honey bees. For aging studies, newly hatched bees were marked on the thorax and returned to the hives. Some of the bees were frozen and used as controls. The marked bees were collected weekly for 5 weeks (summer bees) and monthly for 4 months (winter bees). The relative gene expression of *ATG3*, *ATG5*, *ATG9* and *ATG13* was analyzed in the head and abdomen by qPCR. Gene expression of *ATG* genes was generally downregulated in winter bees both in the head and abdomen, probably due to their lower metabolism. In summer bees, *ATG* genes were upregulated in the abdomen at middle age, while upregulation in the head was more pronounced in older bees. The upregulation of *ATG* genes in summer bees could be the result of the transition from in-hive to foraging activity, leading to a higher metabolic rate and a greater need for autophagy to maintain homeostasis. Further research should aim to understand the mechanisms underlying the differences in *ATG* gene expression and incorporate other techniques to measure autophagy rate.

P-35-029
The lifespan extension of honey bees (*Apis mellifera*) living in low ambient temperatures is mediated by enhancing immunity

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Animals living in low ambient temperatures prolong their lifespans while living in high ambient temperatures shorten their lifespans. This system can be used to reveal the mechanisms of longevity and aging. Whether increased lifespan at low ambient temperatures and decreased lifespan at high ambient temperatures is related to immunity remains unclear. In this study, the 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 reared at 22°C, 30°C, or 38°C were collected at 25 days and their abdomen without digestive tract was used for RNA extraction. The immune genes, including abaecin, hymenoptaecin, defensin-2, glucose dehydrogenase, phenoloxidase, lysozyme, PGRP-LC710, and relish were assayed by qRT-PCR because these immune genes can be used to evaluate the immunity of honey bees. The results showed that the mRNA expression levels of abaecin, hymenoptaecin, defensin-2, and glucose dehydrogenase of bees reared at 22°C were higher than those of bees reared at 30°C or 38°C. The mRNA expression levels of phenoloxidase and lysozyme of bees reared at 22°C were lower than those of bees reared at 30°C or 38°C. The mRNA expression levels of PGRP-LC and relish were not significantly different at 22°C, 30°C, and 38°C. The high immunity of bees is manifested by high levels of abamycin, hymenomycin, defensin 2, and glucose dehydrogenase genes, and low levels of phenoloxidase and lysozyme genes. PGRP-LC and relish are highly expressed after pathogens and parasite infection. This study indicated that the immunity of bees reared at 22°C was higher than that of bees reared at 30°C or 38°C, and the high immunity was not related to pathogenic and parasitic infection. This study demonstrated that the lifespan extension of honey bees living at low ambient temperatures is mediated by promoting immunity.

P-35-030
Sigma-1 receptor antagonist BD-1063 attenuates Ca^{2+} responses induced by immunomodulators glutoxim and molixan in peritoneal macrophages

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Sigma-1 receptors are ubiquitous ligand-operated molecular chaperones in the endoplasmic reticulum membrane with a unique history, structure, and pharmacological profile. Acting as chaperones, sigma-1 receptors modulate a wide range of cellular processes in health and disease, including Ca^{2+} signaling processes. 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; leads to a more rapid regression of the disease severity to a milder form. Earlier, we have shown for the first time that glutoxim and molixan cause biphasic intracellular Ca^{2+} concentration, $[Ca^{2+}]_i$, increase due to Ca^{2+} mobilization from thapsigargin-sensitive Ca^{2+} stores and subsequent store-operated Ca^{2+} entry in rat peritoneal macrophages. To elucidate the involvement of sigma-1 receptors in the effect of glutoxim and molixan on $[Ca^{2+}]_i$ in rat peritoneal macrophages and in regulation of Ca^{2+} signaling processes in macrophages in general, we used sigma-1 receptor selective antagonist, compound BD-1063 (1-[2-(3,4-dichlorophenyl)ethyl]-4-methylpiperazine). Using Fura-2AM microfluorimetry we have shown for the first time that 20 mM BD-1063 significantly suppresses both Ca^{2+} mobilization (by $50.8 \pm 9.3\%$) from Ca^{2+} stores and subsequent store-operated Ca^{2+} entry (by $54.0 \pm 10.1\%$), induced by 100 mg/mL glutoxim or molixan in rat peritoneal macrophages. The data obtained indicate the involvement of sigma-1 receptors in the complex signaling cascade triggered by glutoxim or molixan and leading to $[Ca^{2+}]_i$ increase in macrophages. The results also suggest the involvement of sigma-1 receptors in the regulation of store-operated Ca^{2+} entry in macrophages. *The authors marked with an asterisk equally contributed to the work.

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P-35-031
Comparative analysis of pleural fluid antimicrobial peptides following open and closed thoracic surgeries

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Antimicrobial peptides (AMPs) play a crucial role in the innate immune system, showcasing a broad-spectrum antimicrobial activity[1,2]. This study aims to analyze the disparities in AMPs within pleural fluids (PF) derived from open and closed thoracic surgeries and to explore their synergistic interactions with antibiotics. 24 patients undergoing thoracic surgeries were enrolled (12 patients for each surgery type), and PF samples were collected at various intervals (2-4 h, 24 h, and 48 h). Specific ELISA kits were used to analyze AMPs, including Defensin 1β, Angiogenin, RNase7, and LL-37. Notably, PFs from open surgeries exhibited a distinctive AMP profile, emphasizing higher levels of DEF-1β, while closed surgeries demonstrated ANG predominance. Pooled PF-derived AMPs from open and closed surgeries were tested for their antimicrobial effect against two microorganisms (*S. aureus* and *E. coli*), where the open surgery PFs demonstrated a better effect against *E. coli*. In contrast, the closed surgery counterpart showed a higher effect against *S. aureus*. Furthermore, the interactions with the antibiotic cefazolin demonstrated a high synergy when combined with closed surgery PF against both microorganisms, while the open surgery PF only demonstrated this synergy when applied against *S. aureus*. The intricate interplay between AMPs following thoracic surgeries (open vs. closed) contribute to design of novel therapeutic strategies and management of post operative antibiotic therapy. *References*: (1) Duarte-Mata DI & Salinas-Carmona MC (2023) Front. Immunol., 14, 1119574. (2) Xuan J, et al. (2023) Drug Resist. Updat. 68, 100954.

P-35-032
Simulated microgravity induces changes in breast cancer cells

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Many astronauts have reported various side effects after long-term space missions in orbit such as cardiovascular changes, reduction of bone density and muscle atrophy. The effects of microgravity (μg) on cellular properties may be related to these health problems. Numerous studies have shown that μg has a major impact on cancer cells affecting proliferation, survival, migration and inducing breast cancer cells to adopt a less aggressive phenotype. Studies performed on MCF-7, a human breast cancer cell line ER-α positive, showed that in μg cells activate genes that are involved in the organization and regulation of the cell shape, cell tip formation, and membrane-to-membrane docking[1]. The purpose of this study was to evaluate the behavior of MCF-7 and SKBR-3 (human breast cancer cell line overexpressing HER-2) under simulated μg. 3D-μg simulator research cube provided by Litegrav was used in 3Dclinostat mode with random path distribution and μ-Slide 8 well for cell growth. Specifically, the evaluation of cancer cell behavior at different time points (1, 3 and 5 days) was performed by phase-contrast microscopy, cytoskeleton staining, viability assays and changes in gene and protein expression by real-time PCR with western blot confirmation. Morphological changes were observed in both cancer cell types under simulated μg, while cell viability was not affected. In particular, the difference in actin filament organization of cells in μg was confirmed by confocal laser scanning microscopy as well as differential gene expression. Data show how simulated μg induces changes in cell morphology and suggest the activation of specific gene programs, that may be involved in tumor development or the metastatic process. A deeper understanding of the mechanisms involved may lead to the development of new therapeutic strategies. Research conducted in simulated μg can provide a reliable tumor model to study different processes of cancer progression. Reference: [1] Kopp S, et al. (2016) Sci Rep. 6:26887.

P-35-033
2D and 3D *in vitro* model to assess the photothermal therapy of Anti-CD44 gold nanoparticles for endometriosis disease

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Because endometriosis and cancer share many pathophysiological features, some of the fundamental principles of cancer nanomedicine can be adapted to develop novel nanoparticle-based strategies. This study aims to evaluate the effectiveness of gold nanoparticles conjugated with anti-CD44 antibodies in targeting CD44 overexpressing endometriosis cells (Z12) after exposure to

photothermal therapy (PTT) in comparison to CD44 low expressing cells (Thesc) and fibroblast cell line (NIH-3T3). The study characterized pegylated gold nanospheres (PEG-GNPs) and gold nanostars (PEG-GNSs) conjugated with an anti-CD44 antibody using UV-visible spectroscopy (UV-Vis), dynamic light scattering (DLS) and Bicinchoninic Acid (BCA). The biocompatibility of both nanovectors were assessed using 2D and 3D cell models through MTT assay. Receptor recognition and internalization were confirmed using confocal microscopy (CLSM) and inductively coupled plasma-mass spectrometry (ICP). Finally, the lines treated with the conjugates, were exposed to an appropriate laser wavelength for PTT and cell viability was evaluated. The results from DLS, Zeta potential, UV-Vis analysis, dot blot, and BCA studies confirmed the antibody conjugation to both types of PEG-GNPs and PEG-GNSs. The viability assays demonstrated the biocompatibility of the conjugates with all three cell lines in 2D cell model. CLSM and ICP data revealed that the nanoconjugate recognized the receptor on Z12 cells better, resulting in higher internalization compared to the other cell lines. The nanosystem's PTT was observed *in vitro* only on Z12 cells, as compared to Thesc and NIH-3T3. Similar results were also obtained with the 3D model, in where both nanovectors were able to recognize the cells embedded into hydrogel and their PTT was observed only in Z12 cells compared to the other two cell lines. Further investigations are required to assess toxicity and the effect on endometriotic cells that overexpress CD44.

P-35-034
Effect of curcumin on platelet activation and ROS production induced by physiological and pathological agonists

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Platelets are key players in the processes of haemostasis and thrombosis. Recent evidence showed that platelets are also involved in Alzheimer's disease (AD) [1]. Fibrillar amyloid peptides, which accumulate in senile plaques in AD patients, are also present in cerebrovascular vessels and has been shown to induce platelet activation, aggregation, and ROS generation increasing the risk of vascular complications. Different natural compounds, such as curcumin, have been extensively used to treat human diseases for their anti-inflammatory, anti-oxidant, and anti-amyloidogenic effects [2]. The aim of this study is to investigate the possible effects of curcumin on platelet activation and oxidative stress induced by physiological agonists and by pathological amyloid peptides. Amyloid peptides Aβ40 and Aβ42, and corresponding scrambled peptides, were diluted in phosphate buffered saline at 37°C for 24 hours to promote amyloid fibril formation. Platelet aggregation was analysed by light transmission aggregometry, and protein phosphorylation was evaluated by immunoblotting with specific phospho-antibodies. Platelet intracellular ROS release was measured on washed platelets using H2DCFDA dye by flow cytometry. 25 μM curcumin reduced platelet aggregation induced by fibrillar amyloid peptides and standard agonists such as thrombin, GPVI agonist convulxin and TxA2 analogue U46619. Curcumin also diminished MAP kinases and phosphoinositide 3-kinase signalling pathways induced by fibrillar Aβ40 and Aβ42. Convulxin, thrombin and fibrillar amyloid peptides promoted significant intracellular ROS production in platelets, that is strongly reduced by curcumin. These results demonstrate the ability of curcumin to modulate platelet activation and ROS production induced by amyloid peptides and physiological agonists, paving the way to the use of curcumin in the treatment of cardiovascular disease and AD. *The authors marked with an asterisk equally contributed to the work. References: [1] Canobbio, I. et al. (2013) FEBS Lett 587(16):2606-11. doi: 10.1016/j.febslet.2013.06.041. [2] Bisceglia F. et al. (2019) ACS Chem Neurosci. 10(3):1420-1433. doi: 10.1021/acschemneuro.8b00463

P-35-035
Optimisation and characterisation of different strategies for siRNA loading into exosome

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The use of therapeutic RNAs has opened up a new avenue in drug discovery; however, their application is limited by the lack of appropriate systems ensuring efficient delivery and tissue specificity. Exosomes are extracellular vesicles produced by the majority of eukaryotic cells and appear to naturally host endogenous RNAs [1]. Given their biocompatibility, nanosized scale, low immunogenicity, and surface engineering feasibility, exosomes represent interesting biological carriers to deliver therapeutic RNAs. However, RNA loading into exosomes remains challenging, with poor loading efficiency and lack of technology standardization [2]. Here, electroporation, sonication, and transfection were evaluated as methods to improve loading efficiency of a siRNA against GFP transcript into HEK293T cells-derived exosomes, compared to passive loading. 10 μg of exosomes (~7.5×10^{10}) were mixed with 100 pmol of siRNA. Each loading method was carried out, followed by a clearing step to remove unloaded siRNA. All methods showed improvement of siRNA loading into exosomes compared to passive loading, with differences that are currently being investigated by gel electrophoresis and digital-PCR. None of the techniques induced physical alterations of exosomes, evaluated by tuneable resistive pulse sensing (TRPS) and TEM analysis, or in the amount of exosome markers, measured by western blot. Notably, ~50% of GFP silencing was observed after treatment of stably GFP-expressing HEK293T cells with selected loaded exosomes, confirming their ability to efficiently embed and deliver siRNA. Further studies on the effects of loading on the endogenous RNA content of exosomes by transcriptomic analysis are in progress. Overall, this study paves the way to the development and validation of a loading technology for using exosomes as RNA delivery systems. Previously published in: [1] O'Brien K et al. (2020) Nat Rev Mol Cell Biol. 21:585-606. [2] Zeng H et al. (2023) Cells, 12:1416.

P-35-036
ATM variants activation by endogenous ATM

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Ataxia telangiectasia (AT) is a rare neurodegenerative disease caused by biallelic mutations in the Ataxia telangiectasia mutated (*ATM*) gene, that codes for the protein of the same name ATM.

ATM is primarily involved in the repair of DNA double strand breaks (DSBs) but beyond this nuclear role, it is also present as an active dimer when triggered by reactive oxygen species (ROS) in the cytoplasm, working in an independent manner from DNA DSBs activation. Currently there is no cure available for these patients. However, the positive effects on neurological symptoms of AT patients treated with dexamethasone encapsulated within autologous erythrocytes (EryDex) during phase II and phase III clinical trials, led to a new hope for a possible treatment. The drug was able to induce an alternative splicing of the native ATM messenger producing shorter transcripts "ATM variants," which were detected *in vivo*, in the blood of treated AT patients. Some of the new ATM variants were characterized and examined in AT fibroblast cell lines and we were able to demonstrate their positive role in overcoming the absence of active ATM in AT cells. In addition, we have also investigated whether the activation of ATM variants depends on endogenous ATM, following oxidative stress. Preliminary investigations showed that a dimerization process occurred where a heterodimeric complex was formed between the ATM variant and the mutated endogenous ATM. Furthermore, this complex could restore ATM kinase activity, which is deficient or absent in AT cell lines, by phosphorylating the standard ATM targets. Further studies are required to better understand the potential interaction of endogenous ATM and the ATM variants but results to date provide an optimal proof of concept for the use of ATM variants in gene therapy to treat AT subjects.

P-35-037
Sulphonic compounds specifically interact with bacteriophages, acting as selective UV protectants

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The rapid spread of drug resistance among bacterial strains skyrocketed the costs of medical treatment. In the USA alone, the fight against drug-resistant microorganisms generates an annual cost of 55 billion USD. Therefore, there's a pressing need for novel antibacterial agents. Bacteriophages – viruses that infect bacteria – appeared as almost flawless antibacterials, due to their ability to evolve as fast as their bacterial hosts. During the research focused on developing efficient methods for bacteriophage stabilization against UV irradiation, we observed that all the UV protectants (Congo red, selected food dyes, phenol sulfonate derivatives) shared similar chemical characteristics – good solubility in water, hydrophilic side groups, and at least one aromatic ring. In further research, we found that for UV protection, the presence of at least one sulphonic group was essential. Using one of such molecules – Congo red dye – we verified the specificity of these interactions on several bacteria and bacteriophage species, including the unique enveloped phage Phi 6. We explained the mechanism behind these protective properties, the 'molecular sunscreen' mechanism, and we estimated the binding constant of the Congo red to T4 bacteriophage virions. Later, we confirmed that selected food colorants – including Brilliant blue FCF – can be used as the non-hazardous alternative for Congo red dye. We also examined the dynamics of the interactions between bacteriophages and sulphonic compounds, such as sodium dodecyl sulfate (SDS), MES, aromatic sulphons, and isatin sulfonic acid. The study allowed us to determine the simplest molecules capable of interacting with the proteins of bacteriophage capsid. Our study not only resulted in developing methods for the elimination of bacterial contaminations but also described the surface interactions of protein capsids with small molecular substances.

P-35-038
Expression of BAX and BCL-2 in bronchial epithelium in rat lungs in baclofen and baclofen ethanol poisoning

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Baclofen is a synthetic derivative of the inhibitory neurotransmitter, gamma-aminobutyric acid (GABA). This drug has a pronounced psychotropic effect and is often a subject to abuse especially among young people. One of the target organs in case of baclofen poisoning is the lung. The purpose of the study was to assess BAX and BCL–2 expression in the bronchial epithelium, alveolocytes type 1 and neutrophils 3 hours after baclofen and combined baclofen and ethanol administration. The experiment was carried out on 15 20-week-old male Wistar rats weighing 290-350 g, divided into 3 groups (5 rats each). The first group included intact rats. The second group included rats treated with baclofen at the dosage of 85 mg/kg. The third group included rats treated with baclofen at the same dosage and ethanol (7 mL/kg 40% vol.). During the immunohistochemical (IHC) examination of bronchial epithelium, alveolocytes type 1 and neutrophils of intact rats (group 1) the expression of BAX and BCL-2 was found to be weak. In group 2 (baclofen, 3 h.) rats BAX expression in the bronchial epithelium and alveolocytes type 1 was found to be strong, and BCL-2 expression was moderate. The expression of BAX and BCL-2 in neutrophils was moderate. In the bronchial epithelium and alveolocytes type 1 of group 3 animals (baclofen + ethanol, 3 h) the expression of BAX and BCL-2 was strong. In neutrophils, BAX expression was moderate, and BCL-2 expression was strong. The results obtained during the IHC study suggest the involvement of apoptosis in the development of bronchial epithelium and alveolocytes type 1 impairment. In addition, BCL-2 expression in the cells may play a certain role in the regeneration process.

P-35-039
ZFP57 is a regulator of postnatal growth and life-long health

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Early-life factors, including nutrition, shape long-term health outcomes. Despite the essential role of lactation in maternal nutritional support, the influence of epigenetic factors on lactation and postnatal growth remains poorly understood. Zinc-finger protein 57 (ZFP57), is an epigenetic regulator of genomic imprinting, a process that directs gene expression based on

parental origin, playing a vital role in mammalian prenatal growth. Our study employed diverse methods, including sorted mammary cell transcriptomes, molecular, biochemical, and histological techniques, *in-vivo* experiments, and cross-fostering experiments. These approaches enabled us to investigate mammary gland development, milk composition, maternal behaviour, and offspring growth and susceptibility to diseases. Here, we identify a novel function of ZFP57 in regulating the mammary gland, where it serves as a key modulator of postnatal resource control, independently of imprinted genes. ZFP57 influences multiple aspects of mammary gland function, including ductal branching and cellular homeostasis. Its absence leads to significant differential gene expression, related to alveologenesis, lactogenesis and milk synthesis, associated with delayed lactation and altered milk composition. This results in life-long impacts on offspring including the development of metabolic syndrome. Cross-fostering reveals intricate dynamics between mother and offspring during lactation. Pups raised by a dam of a different genotype than their birth mother exhibit exacerbated metabolic features in adulthood, providing additional insight into the programming of offspring long-term health by maternal context. This study deepens our understanding of the interplay between epigenetic factors, lactation, and postnatal resource control and identifies ZFP57 as a major regulator of both pre and postnatal resource control in mammals.

P-35-040
Temporal changes in the expression and activity of ENPP2/autotaxin, a primary lysophospatidic acid-generating enzyme, in reproductive tissues and circulating blood in pregnant rats

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ENPP2/Autotaxin plays a pivotal role in synthesizing lysophosphatidic acid (LPA), a bioactive lipid with diverse physiological and pathological implications in mammals, including reproductive and pathological functions. This study utilized quantitative RT-PCR analysis to investigate the gene expression of *Enpp2* in various gestational tissues (ovary, uterus, placenta, and fetal membrane) of normal pregnant rats. Our findings reveal that ovarian *Enpp2* expression starts at a low level and experiences a significant increase in the latter half of gestation. In the uterus, its mRNA levels exhibit a temporal rise, particularly on PRG15, while in the placenta, *Enpp2* expression increases proportionally with the aging of gestation. Fetal membranes show low *Enpp2* levels, escalating notably on PRG23, the day of parturition. These mRNA expression patterns were consistently reflected in the protein contents of the respective tissues, as demonstrated by Western blot analysis. Furthermore, the lysophospholipase D activity of ENPP2 in the tissues closely mirrors the observed protein levels. In the circulating blood plasma, both the content and enzymatic activity of ENPP2 increase temporally during late gestation, predominantly deriving from the placenta. These temporal regulatory patterns in mRNA expression, protein content, and enzymatic activity collectively suggest a finely tuned temporal regulation of ENPP2 in rat gestational tissues. Our comprehensive analysis underscores the notion that ENPP2-generated LPA plays multifaceted, tissue-specific, and systemic roles in gestation and parturition. The temporal regulation of ENPP2 expression in various gestational tissues highlights its dynamic involvement in processes crucial to successful pregnancy and childbirth. This study contributes valuable insights into the intricate regulatory mechanisms of ENPP2 and its downstream effects, paving the way for a deeper understanding of its implications in reproductive and pathological contexts.

P-35-041
Physiologically based pharmacokinetic (PBPK) modeling in the age of AI

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A physiologically based pharmacokinetic (PBPK) model is a set of quantitative hypotheses regarding descriptions of absorption, distribution, metabolism, and excretion (ADME) that are supported by scientific evidence and biochemical and physiological assumptions. PBPK models can provide a means for estimating internal dose metrics from applied doses of xenobiotics or exposures to environmental chemicals and are typically expressed as systems of ordinary differential equations (ODE). The traditional approach for building PBPK models is labor-intensive, time-consuming, and expensive. Each model must first undergo a tedious quality assurance review before use to ensure biological plausibility and correct implementation. This traditional approach for developing PBPK models cannot keep up with the increasing demand in chemical risk assessment and the needs of the pharmaceutical industry. Recent developments in machine learning (ML)/artificial intelligence (AI) have opened new avenues for integration in PBPK modeling to enhance efficiency and accuracy. Methods such as ODE training and neural ODEs can take advantage of substantial data sets accumulated for PBPK-related ADME. Preliminary neural ODEs also have the advantage of handling time-course observations at irregular intervals where classic neural network models would fail. Ultimately, these methods can potentially provide a new way to approach the parameterization of the complex models used in PBPK modeling. We can see several first steps in this direction that hold great promise for advancements in personalized medicine, data-driven parameterization, optimization of the clinical trial design, and real-time adaptive modeling. The future of PBPK modeling lies in the seamless integration of advanced modeling techniques and ML/AI technologies. As such, we will discuss some achievements and prospective directions of ML/AI implementations into ADME/PBPK research in the area of chemical risk assessment.

P-35-042
Effects of natural and synthetic boron-containing compounds in mice spermatogenesis and human sperm cultures

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In many mammals, sperm use fructose as their main energy source, and its transport inside the cell is carried out by the type 5 glucose transporter (GLUT5), which is specific for this saccharide. Some boron-containing compounds (BCCs) like tetrasodium

borate and boric acid have shown male reproductive impairment when administered at doses above 17 mg of boron/kg/day; however, the mechanisms for BCCs causing this effect in the male fertility have not been elucidated. On the other hand, a variety of carbohydrate-boron adducts derived from fructose and other saccharides have been reported, and their applications could be proved in multiple physiologic and pathologic processes. In this work, two synthetic fructose and arabinose adducts with boron were tested against some natural BCCs which have shown reproductive effects in different male species. Firstly, these BCCs were tested in a molecular docking on GLUT5 and adenylyl cyclase. At the beginning of the experimental phase the intraperitoneal administration of vehicle, boric acid, phenylboronic acid, and the two synthetic carbohydrate adducts in 5 groups (n = 8) of 6-week healthy male CD1-mice for 28 days and the extraction of the testis once finished the treatment to analyze the histological changes; the second part was the generation of human sperm cultures and their exposure to different concentrations of the BCCs to compare the sperm viability. The *in-silico* studio showed a better binding energy to both proteins docked for the carbohydrate adducts than their precursors. Some changes were observed in the histological sections right after the 28-day treatment, and in the human sperm cultures, the groups exposed to the boron-carbohydrate adducts showed impaired viability. The results presented *in-silico*, *in-vitro* and *in-vivo* could be potentially used in forward research of these adducts to be positioned as a new strategy for male anticonception.

P-35-043
Spermidine content and expression of genes involved in polyamine synthesis during aging in summer and winter honey bees

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The aging process is characterized by a progressive decline of several physiological and metabolic functions whose precise mechanism remains unclear. Event associated with aging is the decrease in polyamine content in the organism, particularly spermidine, one of dominant naturally occurring polyamines with a variety of biochemical functions that has been mostly analyzed in the context of aging. The honey bee (*Apis mellifera* L.) is an interesting organism for age-related studies due to its reproductive and temporal polyethism. The aim of this study was to monitor spermidine content and the expression of genes involved in polyamine metabolism during aging in the summer and winter generations of honey bee workers. Summer workers were sampled weekly for five weeks, while winter workers were sampled monthly for four months. For both summer and winter bees, the first sampling was considered the control group, consisting of newly hatched worker bees. The spermidine content was determined by HPLC analysis in bee's whole body, while the expression of ornithine decarboxylase (odc), spermidine synthase (sds), spermine synthase (sms), spermine oxidase (smox), and polyamine oxidase (paox) genes was measured by qPCR, in the abdomen of bees. The HPLC results showed that the spermidine content in the summer bees decreased with aging, while in the winter bees, it was lower during the first three months and increased in the oldest group. In summer bees, the expression of odc, sms, smox, and paox was up-regulated in older bees compared to newly hatched bees, while sds gene expression was down-regulated only in the oldest bees. In winter bees, the expression of sds increased in the second month, smox in the fourth, while the expression of sms decreased in the first month of life. The results suggest that there are differences in polyamine metabolism in different generations of honey bee workers, which may contribute to the understanding of polyamine role in the aging process.

P-35-044
Diversity of shape and size of Puumala virus in a cell

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Hemorrhagic fever with renal syndrome (HFRS) is a serious human disease, about 97% of cases in Europe are associated with Puumala orthohantavirus. There are no vaccines or antiviral drugs to treat HFRS. Despite extensive study, precise data on the morphology of Puumala hantavirus grown in cell cultures are lacking. The purpose of this work was to obtain data on the size and shape of Puumala virus particles infecting Vero cells, both inside and outside the cell. Puumala virus was isolated from culture medium obtained by growing infected Vero cells for 10–12 days and purified by ultracentrifugation in a 20–60% sucrose gradient. The presence of Puumala virus in the resulting sample was confirmed by western blot with antibodies obtained from convalescent sick people. Vero cells (3-day monolayer culture) were infected with Puumala virus incubated for 7–10 days and tested for the presence of viral antigen by the indirect fluorescence antibodies test. When 80% infection or more was achieved the cells were collected with a versene-trypsin mixture (to preserve the native form of Vero cells), fixed with 2.5% glutaraldehyde and 1% O_SO_4 and embedded in Epon 812. Thin sections were stained with uranyl acetate and examined using electron microscopy. The results revealed a variety of shapes and sizes in which Puumala virus was present inside the cell: round (62%), elliptical (35%) and irregular (3%). Moreover, outside the cell, virions were presented only in a round shape. The maximum size of Puumala virions observed inside the cell was 186 nm, the minimum was 105 nm. Average value 146.5 ± 41 nm. As for virions outside the cell, their size range was 112 ± 9 nm, which was probably determined by their regular round shape. From the literature, other types of hantaviruses also have a variety of shapes and sizes within the same species. Studying the diversity of the Puumala virus is of great importance for understanding the pathogenesis of HFRS and the development of vaccine preparations.

P-35-045
CBDA-mediated EIF2A activation alters protein homeostasis in glioblastoma cells

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Glioblastoma is an aggressive, fast-growing and brain tumor refractory to conventional chemotherapies and radiotherapies. Since tumor aggressiveness is due to the wide variety of genetic and epigenetic variations, a combination of targeted therapies is strongly required. In this context, the study of the molecular targets of action of bioactive compounds allows the identification of new potentially druggable proteins. Our previous studies led to the identification of eukaryotic initiation factor 2A (EIF2A) as the protein target of the bioactive native phytocannabinoid cannabidiolic acid (CBDA) in glioblastoma cells. EIF2A was reported to regulate protein expression under cell stress conditions. Interestingly, although CBDA was not a cell stress inducer, we observed that its binding to EIF2A in glioblastoma cells increased the affinity of the protein for eukaryotic translation machinery. Based on these results, we investigated the effect of the interaction CBDA-EIF2A on the nascent proteome. To this end, we carried out pulsed stable isotope labeling by cell culture amino acids assay, followed by an enrichment of newly synthesized proteins via Click chemistry. This approach showed that CBDA induced proteome remodeling in a short-time treatment (4 h), in terms of significant increase of the synthesis of molecular chaperones involved in the stress-related unfolded protein response, intracellular vesicle formation/trafficking process and the activation of the protein ubiquitination process. Remarkably, WB analysis of profile expression confirmed a significant increase in the amount of ubiquitinated proteins upon CBDA treatment. Moreover, p62 and LC3-II, specific markers of autophagosome formation, were found to be significantly modulated. In conclusion, these findings suggested that CBDA-mediated EIF2A activation could affect protein homeostasis machinery suggesting that EIF2A plays a pivotal role in regulating protein turnover in cancer cells.

P-35-046
Perfluorinated compounds impact bone cell homeostasis in a zebrafish model for dominant osteogenesis imperfecta

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Perfluorinated compounds (PFASs) are widely used in commercial and industrial products due to their chemical inertness. Nevertheless, they are poorly biodegradable and accumulate in the environment. Excessive exposure to PFASs is known to be toxic for healthy individuals and it could even have worst effect on more fragile people. We aim to investigate the impact of PFASs on individuals affected by osteogenesis imperfecta (OI), a rare heritable brittle bone disease modelling juvenile osteoporosis. It is characterized by defects in bone cell differentiation and activity associated to poor mineralization, resulting in fragile and misshaped bones. To investigate the impact of PFASs on OI skeleton we exploited the zebrafish Chihuahua (*Chi*/+), a well validated model for dominant OI. WT and Chi/+ crossed with the transgenic line *Tg(OlSp7:nlsGFP)*, expressing GFP under the early osteoblast marker Osterix, were used to follow *in vivo* bone cell differentiation. Sp7: GFP+ WT and *Chi*/+ were treated with perfluorooctanoic acid (PFOA) and perfluorooctanesulfonic acid (PFOS) from 1 to 6 days post fertilization, then osteoblast differentiation and bone mineralization, apoptosis, osteoblast and adipocyte differentiation markers were investigated. PFOA reduced mineral deposition in mutants, while both pollutants impaired pre-osteoblast differentiation in *Chi*/+ larvae. Apoptosis was activated following PFOA exposure in both WT and mutants, while only in *Chi*/+PFOS stimulated the expression of *pparα*, a marker of lipid metabolism. Oil red O staining confirmed the lipidic disregulation that resulted in an increased lipid droplets in the liver. Our data represent a first warning sign, suggesting a negative impact of PFASs exposure on skeleton even worse in presence of bone fragility.

P-35-047
Development of injectable keratin-fibrinogen hydrogel for hemorrhage control

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Hair-derived keratin exhibits significant potential in biomedical applications, particularly in hemostasis and wound healing. This potential arises from its inherent ability for integrin-mediated platelet activation and adhesion. This study delves into the potential of a keratin-based fibrin hydrogel with biodegradability and cell compatibility as a promising alternative to traditional fibrin dressings. Moreover, our objective was to develop an injectable and biocompatible keratin-based hemostatic agent by chemically combining fibrinogen and keratin, leading to the rapid formation of hemostatic biomaterials incorporating thrombin. Scanning electron microscopy revealed increased pore size and fiber porosity upon cross-linking fibrinogen and keratin, facilitating platelet aggregation and erythrocyte coagulation in the thrombus. The resulting hydrogel demonstrated heightened water-binding capacity, confirmed injectability through rheological analysis, and adaptability to tissue injury site morphology. Additionally, the study introduces keratin-fibrinogen conjugated hydrogel (KNFH), an injectable synthetic polymer combining human hair keratin protein with fibrin monomers. The hydrogel exhibited mechanical stability, optimized thrombin (Thr) concentration for injectable hemostasis, and non-cytotoxicity characteristics. Leveraging the fibrin network's structural advantages, KNFH effectively addressed irregular wound sites, promoting rapid bleeding control, coagulation, and neovessel formation. Hemostatic efficacy was assessed in a rat model of whole blood and liver hemorrhage, revealing reduced clotting time, bleeding volume, and increased neovascularization. Consequently, this research examines the multifunctional roles of keratin, contributing to a comprehensive understanding of the developed hemostatic materials for acute bleeding management in irregular or

severe wounds. *The authors marked with an asterisk equally contributed to the work.

P-35-048
Platelet activation induced by CpG-rich oligodeoxynucleotides: the involvement of the C-type lectin receptor CD93

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CpG-oligodeoxynucleotides (CpG-ODNs) are short single-stranded synthetic DNA molecules containing unmethylated CpG motifs, which mimic bacterial DNA and act as pathogen associated molecular patterns (PAMPs). As other PAMPs, CpG-ODNs induce platelet activation, but the molecular mechanism of this process is poorly characterized. Platelets are emerging players in innate immunity and express several pathogen recognition receptors (PRRs) for pathogen sensing and immune response. The C-type lectin (CTL) receptor CD93 is a type I transmembrane glycoprotein involved in inflammation, angiogenesis, and cancer. In nucleated cells, the CTL domain of CD93 binds CpG-ODNs, suggesting a possible novel function for CD93 as a previously unidentified PRR. In this study, we have investigated the role of CD93 in platelet activation induced by CpG-ODNs. Type C CpG-ODNs (ODN2395) were selected for this study and their effect on the activation of control wild type (WT) and CD93-knockout (KO) murine platelets was investigated. CpG-ODNs induced aggregation of WT platelets, accompanied by integrin αIIbβ3 activation and α-granule release. Platelets lacking CD93 exhibit a significant impairment in α-granule secretion, whereas integrin activation and platelet aggregation were only marginally affected. Immunoblotting analysis revealed that the CpG-ODNs stimulated the tyrosine phosphorylation of different signalling proteins in WT platelets, including PLCγ2 and Pyk2, and these responses were significantly defective in the absence of CD93. Moreover, activation of the AKT/GSK3 axis and of the MAP kinases cascade by CpG-ODNs was markedly dependent on CD93 expression. In conclusion, CD93 is involved in platelet stimulation induced by CpG-ODNs by regulating the activation of protein kinase-dependent pathways required for platelet secretion. Further studies are required to clarify the relevance of CD93 in functional platelet responses to CpG-ODNs in thrombosis and immunity.

P-35-049
Charge distribution contributes to *in vitro* protein desiccation protection of a group of highly charged anhydrobiotic intrinsically disordered proteins

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All life forms on the planet need water to survive. There are organisms that can tolerate losing more than 90% of their water content (desiccation), an ability called anhydrobiosis. Examples of anhydrobiotic organisms are some species of tardigrades, arthropods, nematodes, resurrection plants and most plant seeds. To tolerate desiccation, anhydrobiotic organisms share the accumulation of intrinsically disordered proteins (IDPs), proteins that lack a stable tridimensional structure. *In vitro* experiments have shown that IDPs act as non-classical chaperones that protect proteins from denaturation/aggregation during desiccation. However, there's little information about the molecular features that drive IDPs protein protection during desiccation. In this study, we found that for a group of five highly charged IDPs from the tardigrade *Hypsibius exemplaris* and the insect *Polypedilum vanderplanki*, the degree of charge distribution correlates nonlinearly with the *in vitro* protein protection efficiency. Using *in vitro* lactate dehydrogenase (LDH) activity protection assays, we found that highly charged IDPs with segregated charged residues with opposite charge protect LDH more efficiently from desiccation. Charge distribution is known to alter the global dimensions of IDPs. Global dimension analysis using ALBATROSS revealed that proteins with low asphericity protect LDH in a more efficient way. Our results suggest that charge distribution together with asphericity contribute to protein protection of highly charged IDPs during desiccation in vitro. These results point out that a general characteristic such as the global dimensions may conduct the protection efficiency of IDPs.

P-35-050
Evolution of enzyme regulation and the mechanistic drift hypothesis: The case of the activation by AMP in archaeal ADP-dependent sugar kinases

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Allosteric regulation of archaeal carbohydrate metabolism is a scarcely studied phenomenon. It has recently been demonstrated that AMP activates the bifunctional ADP-dependent phosphofructokinase/glucokinase from the methanogenic archaea *Methanococcus maripaludis* (MmPFK/GK) in both activities [Previously published in: Vallejos-Baccelliere G et al. (2022) FEBS J. 289 (23):7519-7536]. This enzyme is a member of the ADP-dependent sugar kinase family, which includes bifunctional ADP-GK/PFK, specific ADP-GK, and specific ADP-PFK enzymes. In this work, we perform a comprehensive evolutionary study using ancestral protein reconstruction to trace the trajectory of the appearance and disappearance of this regulatory trait. We found that AMP regulation is an ancestral trait conserved only in bifunctional

ADP-PFK/GK lineages, which exclusively includes enzymes belonging to methanogenic organisms. Although activation by AMP is a conserved trait, the underlying kinetic mechanism of activation presents important divergences through evolution. Mechanisms ranging from an increase in either sugar or MgADP affinity to an increase in catalysis or a combination of them, are randomly distributed across the evolutionary trajectory of AMP activation. Moreover, important differences are found between the activation mechanisms of both activities in a single enzyme. Based on these results, we propose the mechanistic drift concept, which can be extrapolated to a wide range of phenomena in macromolecular evolution and has interesting theoretical and philosophical consequences to be explored. Funding: FONDECYT 1231263. *The authors marked with an asterisk equally contributed to the work.

P-35-051
The cytotoxicity and antibacterial activity of *Moringa oleifera*-mediated silver nanoparticles

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Bacterial resistance to antibiotics is one of the major health threats of the XXI century. Current preclinical pipeline includes various nanoparticles (NPs) as a possible alternative to antibiotics. To use NPs in biomedicine, it is important to study their cytotoxicity and antibacterial activity. In the current work the effects of silver nanoparticles (AgNPs) biosynthesized using *Moringa oleifera* plant extract on red blood cells, as well as on HeLa cells (CCL-2), are investigated to determine their cytotoxicity. Moreover, the NPs effects on proton-translocating F_oF_1-ATPase activity of *Escherichia coli* BW25113, *Enterococcus hirae* ATCC9790, and *Staphylococcus aureus* MDC5233 has been studied. *Moringa*-mediated AgNPs showed concentration-dependent hemolytic activity against healthy humans' erythrocytes. Significant inhibition of HeLa cells growth was observed at the lowest concentration tested (6.25 μg/mL) after exposure to nanoparticles for 24 and 72 h. The IC50 values for these exposure times were 0.305 and 0.37 μg/mL, respectively. This indicates that 24 h exposure time is sufficient for AgNPs to effectively demonstrate their growth inhibitory effects. The bacterial F_oF_1-ATPase activity was investigated to find out the possible target of AgNPs. The ATPase activity of bacterial membrane vesicles was suppressed by the inhibitor N,N'-dicyclohexylcarbodiimide (DCCD) by ~1.5 fold in all tested bacteria. AgNPs (10 μg/mL) inhibited DCCD-sensitive ATPase activity in *E. coli*, *E. hirae*, and *S. aureus* by ~3.3-, 2.5-, and 1.7-fold, respectively. The results indicate that AgNPs are involved in intracellular mechanisms, inducing changes in the structure and permeability of cell membranes. The low concentrations of biosynthesized AgNPs are not toxic to healthy cells, which makes their application in biomedicine possible. The work was supported by the Basic Support and Higher Education and Science Committee of RA, in the frames of the research project № 21T-1F179.

P-35-052
Identification and comparisons of SNPs hybrid camels in Kazakhstan

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The domestication of camels (genus Camelus) is an important resource not only for the production of meat, milk, and wool but also for tourism, sports racing, and logistics. Of the three extant species, two are domesticated (*Camelus dromedarius* and *Camelus bactrianus*), and one remains wild (*Camelus ferus*). In scientific work, one-humped and two-humped camels have been studied quite a bit. However, the offspring (hybrids) obtained by crossing these animals, which are superior to the parent species in many important traits, have not yet been studied. Hybrid camels are currently widely used in two countries: Turkey and Kazakhstan. Therefore, we present whole genome sequencing (WGS) data from five hybrid camels bred in the Almaty region (Kazakhstan). We compared these data with WGS data on one-humped, two-humped, and wild camels obtained from the database. A total of 43,552,164 single nucleotide polymorphisms (SNPs) were detected in the studied groups. Further comparison of these SNPs revealed the following numbers of private SNPs among the populations: hybrid camels (3,271,083), wild camels (2,515,591), Bactrians (1,244,694), and Dromedaries (531,224). The genetic structure of the studied animals is described, and a phylogenetic tree is constructed to assess their genetic distance. It was established that the studied hybrids are genetically closer to Dromedary camels, since they were on a close branch of the phylogenetic tree. The results of the study will contribute to further study of the genetic resources of this mammal, as well as the conservation of these resources.

P-35-053
Redistribution of membrane receptors for chemoattractants during electrotaxis of mouse 3T3 fibroblasts

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Electrotaxis, the directed migration of cells in an electric field (EF), is vital for wound healing and embryonic development. Although the exact mechanism of EF detection is not fully understood, it is believed that the movement of membrane proteins, including chemoattractant receptors, under the influence of the EF, is a significant factor in guiding cell polarisation and migration directionality. The study investigated the dynamics of receptor redistribution, focusing on how quickly chemoattractant receptors, notably EGFR, PDGFRA, and TGFBR1, relocated during the electrotaxis of mouse 3T3 fibroblasts. We tracked the locations of these receptors tagged with GFP before and after EF application. It was shown that EGFR gathered on the cathodal side of the cells, aligning with their cathodal migration. The redistribution was more efficient under alkaline conditions and depended on the intensity of the EF. The dynamics of this redistribution were particularly notable near the cell base, as observed

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through TIRF microscopy, but decreased when the glass was coated with poly-L-lysine. Such observations indicate that electroosmosis plays a dominant role in this redistribution process. While EGFR redistribution aligned well with the primary response of the cells to the application of EF, it did not correlate with their rapid reaction to the reversal of EF, which occurred within 1-2 min, when the orientation of the receptor was not yet reversed. A similar pattern of cathodal redistribution was observed for PDGFRA, whereas TGFBR1 did not exhibit this behaviour. In conclusion, our study suggests that the mechanism of EF detection with EGFR or PDGFRA redistribution (potentially through electroosmosis) may contribute to the electrotactic behaviour of 3T3 cells. However, this mechanism does not explain the cells' swift response to changes in EF direction. This work was supported by a grant from the National Science Centre 2018/31/B/NZ3/01750, Poland. *The authors marked with an asterisk equally contributed to the work.

P-35-054
Alterations in alternative splicing and polymorphism of nucleotide variants of genes related to metabolism of antihelminths in L3 invasive larvae of *Anisakis simplex* s.s.

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Significant changes in natural and social factors, changes in lifestyle and diet affect the spread of parasitic diseases and bring new challenges for the prevention, control, clinical diagnosis and treatment of these threats. Nematodes of the genus *Anisakis* parasitize marine mammals, and in humans the larvae cause a disease called anisakiasis. The main source of infection is food containing raw fish or shellfish. Due to recently observed treatment resistance, there is a great need for new classes of antiparasitic agents with novel mechanisms of action. The aim of this study is to identify transcriptomic variations in L3 larvae of *A. simplex*, with particular emphasis on alternative splicing and the modulation of expression-specific variants under the influence of the drugs albendazole (ALB), ivermectin (IVM) and pyrantel (PYR). Multivariate differential transcriptomic splicing analysis (rMATS v.3.2.5) was used to find potential alternative splice (AS) sites, and single nucleotide variant (SNV) variability was estimated from allele frequency differences between drug-treated and non-drug-treated larvae using bcftools tools and R environment libraries. A study on the effects of three drugs (ALB, PYR and IVM) on the transcriptome of *A. simplex* showed variability in alternative gene splicing. Sixty-eight protein-coding genes were potential candidates that showed allelic variations after treatment of *A. simplex* larvae 6 with the drug ALB. Two other drugs showed allelic specificity at 83 (PYR) and 95 (IVM) protein-coding genes. The gene splicing events in moesin/ezrin/radixin and trehalose metabolism found in each of the comparisons in this study suggest the theory that genetic markers of the digestive system may be targeted by the three drugs in *A. simplex* larvae. This work was supported by National Science Centre of Poland, grant no.2020/37/N/NZ9/03312.

P-35-055
Influence of margarine diet alone and on the background of feeding every other day on the antioxidant system of mouse liver and heart

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Consumption of diets with margarine containing trans-unsaturated fatty acids causes oxidative stress (OS) in animals and humans. This work aimed to check if in mice fed with margarine every other day feeding (EODF) may decrease OS intensity. Three C57Bl/6J mouse groups for four months the control group consumed laboratory chow, the margarine group was given 70% fat margarine in addition to their normal diet, and EODF group was given chow with margarine every other day. The level of lipid peroxides (LOOH) in the liver of males in the margarine group was 55% lower than that in the control group, whereas in females it was 67% higher than in the control animals. Females of EODF group had 56% and 74% lower hepatic LOOH content than the control and margarine groups, respectively. The activity of glutathione-S-transferase (GST) in the liver of females on EODF group was 88% and 44% higher than that in the control and margarine groups. Hepatic glutathione peroxidase (GPx) activity was 91% higher in females on the margarine diet and 86% higher in the EODF diet compared to the control. LOOH levels in the heart of males on the EODF diet was four times higher than in control animals and three times higher than in mice that ate margarine. Female hearts demonstrated 66% higher GST activity on the margarine diet compared to the control group. EODF group had by 26% lower GST activity compared to the margarine group. The activity of GPx in the hearts of males and females which consumed margarine was 3 times and 61% higher than the control values, respectively. Eating food every other day resulted in 2.5 times higher GPx activity in males and 51% in females compared to the control. Thus, our data show that EODF may enhance efficiency of antioxidant defense system in mice which provides an avenue for future studies to develop cheap and effective strategies to prevent negative effects of foods containing trans-unsaturated fatty acids. *The authors marked with an asterisk equally contributed to the work.

P-35-056
Regulatory functions of APE1 on non-canonical secondary structures in DNA and RNA

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The apurinic/apyrimidinic endodeoxyribonuclease 1 (APE1) is a protein mostly known for its role as a DNA repair enzyme, as it is involved in the repair of abasic (AP) sites in DNA. AP sites, which affect both DNA and RNA, can be generated spontaneously or by the action of glycosylases. Recently, new roles of

APE1 were discovered, mainly regarding DNA G-quadruplex (G4) biology and RNA metabolism. Indeed, some regions of DNA, as well as some RNA molecules, can form alternative secondary structures, influencing biological processes involving telomere maintenance and miRNA biogenesis. Between those structures, the most recognized are G4, in guanosine-rich strands, and i-motif (iM), in the complementary cytosine-rich strands. Data from our laboratory have demonstrated that APE1 can repair AP sites embedded in DNA G4, but it is unknown if this function is conserved also towards other secondary structures, as iM or G4 in RNA (rG4). Here, we focused on the role of APE1 on the processing of non-canonical structures in DNA and RNA. Regarding iM, we chose as our model the sequence of the telomeric cytosine-rich strand. Interestingly, the position of AP site affected iM stability and APE1 cleavage efficiency, as AP sites in the core were processed better than the ones in the loop. Currently, we are investigating if any APE1 partner protein might increase its cleavage activity on damaged iM. Regarding rG4, we chose as our model the precursor form of miR-92b, which contains a rG4 in a dynamic equilibrium with the canonical hairpin, influencing its biogenesis. *In vitro* and cellular assays showed that APE1 can bind this rG4, impacting on the maturation process of miR-92b. We plan to further study how modifications like oxidation or AP site damages influence the rG4 folding and the role of APE1 towards damaged rG4 structures. In summary, we highlighted two undescribed roles of APE1 in the context of non-canonical nucleic acids secondary structures.

P-35-057
The analysis of secondary metabolism gene clusters of the thermophilic strain *Streptomyces* sp., isolated from the endemic cold-water sponge of Lake Baikal

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Microorganisms isolated from extreme ecological niches have a variety of adaptations to unfavorable conditions and a low degree of exploration. This makes them highly efficient factories for the production of new natural compounds. Thus, the aim of this study was to assess the composition of gene clusters related to secondary metabolism in the thermophilic strain *Streptomyces* sp. isolated from Baikal cold water sponge. The genome was sequenced using the Illumina MiSeq platform. The genome was annotated using the Prodigal service (v. 2.6.3), and functional annotation was performed using eggNOG-mapper (v. 2.1.9). The potential for secondary metabolite synthesis was analyzed using the antiSMASH tool (v.7). During the study, a cluster of type III polyketide synthase was identified, responsible for the synthesis of compounds such as flaviolin and 1,3,6,8-tetrahydroxynaphthalene. Flaviolin is a product of the spontaneous oxidation of 1,3,6,8-tetrahydroxynaphthalene, which is synthesized by bacteria. Additionally, a cluster of type II polyketide synthase responsible for the synthesis of kuramycin was also detected. Kuramycin is an antibiotic with a polyketide backbone consisting of modified orsellinic acid. Furthermore, a cluster responsible for the synthesis of geosmin was identified in the genome of the thermophilic strain. Geosmin is a metabolite responsible for the earthy smell. Moreover, a cluster responsible for desferrioxamine E synthesis was identified. Desferrioxamine E acts as an antioxidant, antibiotic, and siderophore. Finally, a cluster related to ectoine synthesis was also discovered. Ectoine protects cell membranes from oxidative damage and was previously described in extremophilic microorganisms. Thus, extremophilic strains isolated from Lake Baikal represent large sources of natural compounds. The study was carried out with the financial support of Ministry of Science and Higher Education of the Russian Federation projects FZZE-2024-0003, FZZE-2024-0013.

P-35-058
Extensive oligomerization of human RNase 1 through 3D domain swapping: investigations on the antitumor activity of its oligomers

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Human pancreatic ribonuclease (RNase 1) recently showed to form enzymatically active oligomers, upon acid lyophilization, through the 3D domain swapping (3D-DS) of both its N- and C-termini [Previously published in: Noro I et al (2023) Int J Biol Macromol 249, 126110], i.e., similarly to, but more abundantly than bovine RNase A [Previously published in: Libonati M & Gotte G (2004) Biochem. J. 380, 311–327]. Notably, RNase 1 is four-residues longer than RNase A, and we found that a RNase 1 mutant displaying a shortened C-terminus that resulted as long as RNase A, can self-associate at a higher extent than wt-RNase 1. This result suggested a specific influence of the C-terminus in the oligomerization of each RNase, as it was confirmed by mutants of onconase (ONC), an amphibian RNase forming only a N-swapped dimer [Previously published in: Gotte G et al. (2021) Int J Biol Macromol 191, 560-571]. Since the oligomers of both RNase A and ONC are active against tumor cells, principally because they can escape the cellular RNase inhibitor [Previously published in: Gotte G et al. (2021) Int J Biol Macromol 191, 560-571], we are now measuring the antitumor activity of the oligomers of both wt- and C-term-shortened RNase 1 variants. Preliminary results show a low activity against A375 human melanoma cells of monomers and dimers of both RNase 1 variants. However, ongoing tests are comparing dimers displaying the "start" Met-1 with the ones in which this residue is specifically cleaved. In this way, we investigate the role of Met-1 affecting the N-terminus in both oligomerization tendency and antitumor activity of RNase 1. Moreover, we are also accumulating RNase 1 trimers to test their activity, that might be hopefully higher than dimers, like it is with RNase A [Previously published in: Libonati M & Gotte G (2004) Biochem. J. 380, 311–327]. Hence, oligomeric derivatives of a RNase of human origin might open new therapeutic possibilities against scarcely curable cancers.

P-35-059
A eumelanin-based bio-dynamic molecular scaffold to design a new potential galectin inhibitor class

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Melanin represents one of the most spread and heterogeneous family of polymeric pigments. Among the five classes of melanin (eumelanin, neuromelanin, pheomelanin, pyomelanin and allomelanin), eumelanin is mostly associated with dark pigmentation and its role in photoprotection against photodamage was investigated in Zamudio Dìaz DF at al. (2024) Sci Rep 14, 3488. The main building block of eumelanin molecules is represented by the 5,6-dihydroxyindole (DHI), a molecule with spontaneous polymerization capacity that is gaining attention as a powerful scaffold to build complex compounds of biomedical interest. With this in mind, the DHI monomer was derivatized with a lactose unit to create a new soluble molecule, LactoDHI, proposed as a lead compound for a new potential galectin inhibitors class. Indeed, the self-assembly ability of LactoDHI monomers could pave the way for an innovative target-scavenging method to sequester the overabundance of secreted galectins that is often a trademark in tumor development and metastasis formation, as described in Guo Y et al. (2020) Oncol Rep, 5 (44), S. 1799–1809. The existing literature on galectin inhibitors revolves mainly around two categories of compounds: small synthetic disaccharides and natural-derived polysaccharides, as studied also in Pirone L et al. (2022) Int J Mol Sci 23, 8273. In our preliminary experiments, the reaction of polymerization of LactoDHI in solution was followed by UV-Vis Spectroscopy (UV-Vis) and Dynamic Light Scattering (DLS) techniques. Subsequently, the interaction between the two forms of LactoDHI (monomeric and polymeric) and recombinant human galectin-3, a galectin commonly overexpressed in tumors, was investigated using two techniques: Isothermal Titration Calorimetry (ITC) and BioLayer Interferometry (BLI). All together, these pivotal data suggest that the distinctive intrinsic dynamicity of LactoDHI compound could open new horizons in galectin inhibitors rational design field.

P-35-060
Primary evaluation of the effect of biologically active compounds synthesized by Baikal oxyphilic *Janthinobacterium* sp. on human spermatozoa

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Oxidative stress is one of the main causes of reproductive dysfunction. Despite the significant development of assisted reproductive technologies, infertility treatment is often ineffective. In this regard, there is a need to search for natural compounds that can act as prototypes of active substances in the development of new pharmaceuticals. According to our previous studies, Baikal microorganisms can synthesize antioxidants for protection against oxidative stress. Thus, the aim of this study was to analyze the effect of Baikal oxyphilic *Janthinobacterium* sp. 2021M8 extract, which has antioxidant activity, on spermatozoa *in vitro*. The effect of the extract on spermatozoa was assessed using a PLS-MY-B041A-3 microscope, Semen and Sperm Quality Analysis System software (V1.12, China), and a 96-well plate. Dried methanol and semen were applied as a negative control. For experiment, a concentrated methanol extract of the strain was used. Also, the concentrated extract was diluted with methanol 10 times Additionally, the extract underwent fractionation on a chromatographic column filled with Sephadex LH-20 to evaluate the impact of fractions on the physiological parameters. Parameters were measured after 1, 3, and 6 hours of incubation at 37°C. The analysis was conducted through 3 analytical repetitions. According to the results, the concentrated extract caused the death of spermatozoa after 1 hour of exposure. However, spermatozoa retained their viability after 6 hours of exposure to the diluted extract. In contrast, under control conditions, sperm death was observed after 6 hours. Fractionation of the extract revealed that at least half of the fractions had spermicidal effect. At the same time, some fractions had the effect of increasing the number of motile forms and sperm velocity. The study was carried out with the financial support of Ministry of Science and Higher Education of the Russian Federation project FZZE-2024-0003.

P-35-061
Genomic stability meets cytoskeletal maintenance: unraveling genetic interactions in yeast

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Cytoskeletal integrity and genomic stability are vital for cellular function. Microtubules, composed of α- and β-tubulin heterodimers, are essential for processes such as transport and cell division. Specific cofactors, including Alf1 in *Saccharomyces cerevisiae*, facilitate proper folding of tubulin subunits. Meanwhile, Rad51 plays a central role in homologous recombination, crucial for DNA repair. In this study, we explore the genetic interactions between the DNA double-strand break repair pathway and microtubule metabolism in *S. cerevisiae*. The *rad51 alf1* double mutant exhibits slowed growth and heightened sensitivity to caffeine and DSB-inducing drugs, indicating synergistic effects. Furthermore, nuclear Rad52 foci, indicative of DSB damages, accumulate more in the *rad51 alf1* mutant compared to single mutants. Interestingly, the absence of Alf1 ameliorates the highly-mutated phenotype of *rad51* mutants. Additionally, overexpression of Alf1 rescues the impaired growth observed in *alf1* mutants. These findings suggest specific genetic interactions between genomic stability and cytoskeletal maintenance, highlighting the intricate relationship between cellular pathways involved in maintaining cellular integrity.

P-35-062
The effect of royal jelly on biomass accumulation and antioxidant enzyme activity of the yeast *C. guilliermondii* NP-4

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In recent years, natural antioxidant supplements have gained popularity due to their ability to reduce oxidative stress without side effects, unlike pharmaceutical drugs. Royal jelly (RJ), a nutrient-rich bee product, has been widely used in traditional and modern medicine. It can be used to contribute to high yeast biomass production, which is valuable as a source of vitamins and complete protein. The aim of our study was to examine the impact of RJ on yeast biomass accumulation, lipid peroxidation processes, and antioxidant system activity. RJ added to the growth medium of yeasts at concentrations ranging from 25 to 150 mg/mL, leads to an increase in yeast biomass accumulation during the stationary phase. The most effective concentration of RJ was found to be 50 mg mL^{-1}, resulting in an 18% increase in yeast biomass. The growth rate (μ) of yeast in the presence of RJ increased by 1.5 times during the exponential growth phase, with the difference in growth between yeast grown with and without RJ diminishing during the logarithmic and stationary phases. Additionally, the presence of RJ in yeast cells led to a 48% decrease in malondialdehyde levels, indicating a reduction in lipid peroxidation processes. Furthermore, catalase activity was stimulated by 21.3% and superoxide dismutase (SOD) activity by 32% in yeast cells grown with RJ. We can suggest that certain bioactive components in RJ, particularly peptides and hydroxy-fatty acids, prevent further oxidation of membrane unsaturated fatty acids by being oxidized themselves. So, RJ creates favorable conditions for reducing oxidative stress through the neutralization of reactive oxygen species (ROS). Overall, the results of this study contribute to our understanding of the mechanisms involved in neutralizing oxidative stress in eukaryotic cells and highlight the potential agricultural importance of RJ in enhancing yeast biomass production for fodder enrichment with complete protein.

P-35-063
Targeting the oligomerisation interfaces of *Plasmodium* oocyst rupture proteins for anti-malarial inhibitor discovery

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The dimerisation of two oocyst rupture proteins (ORP1 and ORP2) from *Plasmodium berghei* is essential for sporozoite egress and malarial infection of the human host via a mosquito bite. Immediately prior to oocyst rupture, ORP2 relocates from the cytoplasm to the oocyst capsule, where it meets ORP1, forming a heterodimer via their Histone-fold domains (HFD). The HFD is common to histones from eukaryotes and archaea, and proteins involved in transcriptional regulation, thus it is curious why this domain should be present in non-nuclear proteins. We present the crystal structure of the ORP1/2 heterodimer and show that ORP1 and ORP2 are structurally similar to the HFD subunits NF-YB and NF-YC, respectively, of the pioneer transcription factor NF-Y. The NF-Y complex forms a heterotrimer with a third subunit, NF-YA that attributes specificity in the DNA-binding mechanism. Based on the structural homology of ORP1/ORP2 with the NFYB/C dimer, and preliminary data that show that ORP1/2 can bind DNA in complex with human NFY-A, we seek to confirm an analogous interaction between ORP1/2 and a third ORP (ORP3), since oligomerisation interfaces represent possible druggable targets. In this context, a GAL4-based Yeast Two-Hybrid-based combinatorial library of cyclic peptides was used to identify cyclic peptides that interact with ORP1, revealing five top candidates for further investigation and testing, *in vitro* and *in vivo*.

P-35-064
Effects of Treatment with montelukast and levocetirizine in patients with allergic rhinitis

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Montelukast and levocetirizine are used in the treatment of allergic rhinitis (AR) Conflicting opinions have been put forward about the effectiveness and safety of these drug. However, these studies lacked measurement methods that allowed accurate assessment of montelukast and levocetirizine levels. With this study, we aimed to measure montelukast and levocetirizine levels in blood samples taken from AR patients with a robust, simple and validated high-performance liquid chromatographic (LC-MS/MS) method and to investigate the relationship between these levels and some parameters. Thirty-three adults with AR admitted to the hospital were randomly assigned to receive montelukast 10 mg and levocetirizine 5 mg at admission and every evening for 4 weeks thereafter. The validated LC-MS/MS method was applied to measure blood montelukast and levocetirizine levels of patients with AR taken after 4 weeks. Serum montelukast levels of patients with AR using 10 mg of montelukast daily were 273.0 (12.6-4330.0) ng/mL and serum levocetirizine levels of patients with AR using 5 mg of levocetirizine daily were 362.0 (11.8-2152.0) ng/mL. There was a negative correlation between WBC, MCV, MCH, NEU and LYM, and montelukast levels (<0.05), while a negative correlation was between WBC (<0.05), NEU (<0.05) and LYM (<0.005) levels with levocetirizine levels (<0.05). Montelukast had the positive correlation with disease duration (<0.005) and levocetirizine levels (<0.001). Also, levocetirizine had the positive correlation with disease duration (<0.01). Montelukast and levocetirizine are clinically effective and have the capacity to improve the quality of life associated with rhinitis-asthma. The developed validated tandem mass spectrometric method has been successfully applied for the measurement of serum montelukast and levocetirizine levels in patients with AR, and these levels were found to be compatible with the reported levels. *The authors marked with an asterisk equally contributed to the work.

P-35-065
Effects of prenatal Δ9-tetrahydrocannabinol exposure on cannabinoid type 1 and dopamine D2 receptors gene expression in pre-adolescent and adolescent rats

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Despite a common misconception that *Cannabis sativa* is a harmless substance, its use may represent a major potential risk for psychosis throughout life. In this context, epigenomic changes could be the molecular mechanisms underlying the transcriptional and behavioural effects of cannabis. In line with the neurodevelopmental hypothesis of several psychopathologies and considering the well-documented dysregulation of the endocannabinoid and dopaminergic systems in mental illness, here we investigated the gene expression of these two systems by qRT-PCR in the prefrontal cortex of rats prenatally exposed to the main psychoactive component of cannabis the Δ9-tetrahydrocannabinol (THC), at two different time-points: pre-adolescence and adolescence. Moreover, miRNAs expression targeting genes of our interest was preliminary assessed by qRT-PCR. According with our previous results [1,2], we highlighted a significant increase in the expression of the Cnr1 gene, coding for cannabinoid type 1 receptor, as well an increased expression of the Drd2 gene, coding for the dopamine D2 receptor, both in pre-adolescent and in adolescent rats. Preliminary data showed an inverse correlation between Drd2 and its relative miRNa, miR-9a-5p, suggesting a possible epigenetic regulation of the gene. Interestingly, prenatal THC exposed rats showed behavioural impairments in the social interaction test and in the novel object recognition test as index of social withdrawal and cognitive deficit, respectively. Thus, our results confirm the key role of the Cnr1 and Drd2 genes regulation on the effects evoked by THC exposure during early and sensitive neurodevelopmental stages. References: [1] D'Addario, C., et al. (2017). Schizophr Res 188, 132-140. [2] Di Bartolomeo, M., et al. (2021). Pharmacol Research 164:105357.

P-35-066
Phage-related sequences and antiphage defense systems in *Sinorhizobium meliloti*

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The abundance of phage-related sequences and antiphage defense systems in the genomes of nitrogen-fixing economically valuable rhizobia strains forming symbiosis with legume forage grasses was assessed. The object of the study was *Sinorhizobium meliloti* strains which are symbionts of plants of the genus *Medicago* spp. growing in the territory of the Primary Center of Origin of Cultivated Plants in the NW Caucasus region. The whole-genome sequences of the strains were determined by NGS and NNGS methods. Phage-related sequences had different completeness (intact, incomplete, questionable) and were also represented by genomic islands; all sequences listed were site-specific integrated. Intact prophages were predominantly (frequency of 0.35) localized on the chromosomes of these strains. The size of the studied sequences, with some exceptions, ranged from 10 to 80 kb, and their number reached 30 sequences per genome. Analysis of phage-related sequences showed that they mainly contain late genes responsible for the synthesis of viral particle proteins, which may lead to the development of specific homoimmune defenses in bacterial cells, whereas early and silent genes necessary for phage DNA replication were lost with an average frequency of 0.83. Thus, phage-related sequences actively lose their potential mobility and as a result are "anchored" in bacterial genomes. This structural degradation of phage-related sequences occurs under impact of bacterial antiphage defense systems. We have detected a number of distinct abortive infection antiphage defense systems and at least three different restriction-modification systems in *S. meliloti* strains. The elements of the adaptive antiphage system were predicted to attribute to CRISPR/Cas type 1E. Thus, we proposed to characterize promising rhizobia strains for practice based on the creation of barcode immune systems, which certainly has a demand in biotechnology. The work was supported by the RSF 24-26-00274. *The authors marked with an asterisk equally contributed to the work.

P-35-067
Tissue-specific effects of mesenchymal stromal cell secretome on the regeneration of spermatogonial stem cell niche

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The functioning of stem cells is maintained by a stem cell niche. After damage a key role in niche regeneration belongs to mesenchymal stromal cells (MSCs). It was previously shown local injection of MSC secretome contributed to the restoration of the spermatogonial stem cell (SSC) niche. However, the ability of resident testicular MSCs to restore the SSC niche remains poorly understood. The murine model of spermatogenesis damage by doxorubicin was performed. Testes were analyzed using immunohistochemical staining with antibodies to one of the main MSC markers CD90. Leydig cells and MSCs were isolated from intact

or damaged testes. Secretome of MSCs from murine testis and adipose tissue, human dental or adipose tissue was added to Leydig cells following by the measure of testosterone concentration in medium by enzyme-linked immunosorbent assay. After damage the number of CD90+ cells in the testicular interstitium increased, and injection of human MSC secretome further enhanced this process. *In vitro* testosterone production was higher after adding of MSC secretome from murine intact testes on intact Leydig cells compared to secretome of MSCs from damages testis or adipose-derived MSCs. Ability of Leydig cells from damaged testis to secrete testosterone also decreased. Secretome of human dental and adipose MSCs were comparable in the stimulation of testosterone secretion. So, the paracrine effects of MSCs on SSC niche turned out to be tissue-specific. After damage the number of testicular MSCs increases. However, their regenerative potential mediated by secretome seems insufficient to support the regeneration of SSC niche. Exogenous MSC secretome may be promising for the restoration of SSC niche and spermatogenesis. Our data are valuable for the further development of novel medicines based on MSC secretome for the treatment of spermatogenesis disorders. The research was supported by the Russian Science Foundation, grant 19-75-30007, https://rscf.ru/project/19-75-30007/.

P-35-068
Investigating bevirimat derivatives: an examination of HIV-1 maturation and resistance

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HIV-1 is a retrovirus that causes AIDS. Although current antiretroviral therapy is highly effective, the error-prone nature of reverse transcriptase has led to the emergence of drug-resistant strains and there is a need for novel inhibitors that target different stages of the HIV life cycle. Maturation inhibitors, such as bevirimat (BVM), represent one such class, even though its testing was halted in phase II due to reduced viral sensitivity caused by a common polymorphism at the drug's binding site. We screened second-generation BVM derivatives for efficacy against HIV-1 infectivity and maturation. We preselected the three most potent BVM derivatives against BVM-resistant HIV variants commonly found in patients undergoing BVM treatment. We used a combination of *ex vivo* cell-based lentiviral systems and *in vitro* assembly and maturation assays to assess the impact of inhibitors on the assembly of mature and immature HIV-1 particles. Furthermore, we examined the effects of these BVM derivatives on the maturation of HIV-1 Gag-derived polypeptides. In addition, we investigated the potential effect of combining BVM derivatives with small polyanions, such as IP6, which naturally bind and stabilize the HIV-1 hexameric lattice in both immature and mature forms. Both second-generation BVM derivatives and IP6 have been shown to affect particle folding and maturation. Of the three most potent inhibitors tested, we have identified one that is effective against a BVM-resistant mutant. This study was supported by the project National Institute of Virology and Bacteriology (Programme EXCELES, ID Project No. LX22NPO5103) – Funded by the European Union – Next Generation EU.

P-35-069
Antioxidant activity and phenolic compounds in strawberries after preharvest treatment with plant extract

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Strawberry fruits have a pleasant taste, beneficial nutritional value, and are consumed worldwide. Rich in antioxidant and phenolic compounds, strawberries are a valuable berry for human health. Alternative natural substances are investigated as plants growth or immunity enhancers when applied directly on plants. In parallel, the quality of the fruit takes on great importance after using such measures and should be investigated equally. During this research, we investigated antioxidant capacity and total phenolic compounds in strawberries (cv. 'Malwina'), treated with *Syzygium aromaticum* L. extract preharvest. Non-treated strawberries were used as control. 4 repetitions were performed for each treatment. Experimental field contained plots in randomized block design. Preharvest strawberry plants spraying with 0.75% (v/v) extract was performed 4 times every 7-10 days from flowering. Samples of healthy, similar in appearance fruits were collected from experimental plots, homogenized, and stored in freezer until analysis. Total phenolic compounds (TPC) were analyzed using Folin-Ciocalteu method. Additionally, DPPH and ABTS antioxidant activities were determined. Results of TPC were 186.6 mg GAE/100 g FW in preharvest treated strawberries and 211.3 mg GAE/100 g FW in untreated strawberry fruits. The tendency in antioxidant capacity was similar: ABTS and DPPH antioxidant activities were 31.5 and 11.6 μmol TE/g FW in preharvest treated strawberries, and respectively 33.8 and 12.4 μmol TE/g FW in non-treated strawberries. Results suggest that *S. aromaticum* extract slightly decreases TPC and antioxidant activity in strawberry fruits, when applied preharvest. This could be related to the effect of *S. aromaticum* extract on strawberry antioxidant system when applied directly on plants. Further research could include evaluation of specific phenolic compounds and other biochemical strawberry constituents to expand the effect of plant extract treatment on strawberry fruit quality.

P-35-070
Cold plasma-aeroponics technology coupling for improvement of *Stevia rebaudiana* Bertoni morpho-biochemical traits

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Seed treatment with cold plasma (CP) is considered a green technology to stimulate seed germination, plant morphometric parameters, biomass production, and disease resistance by inducing changes in plant biochemical phenotype. *Stevia rebaudiana* Bertoni is an economically valuable plant due to its secondary metabolites steviol glycosides (SGs) which are widely used as health-beneficial sweeteners. Our research group recently reported for the first time that pre-sowing seed treatment with CP can be used as a powerful technique for the stimulation of SGs biosynthesis and/or accumulation when stevia is grown in soil. This study aimed to investigate the effect of coupled technology of

pre-sowing seed treatment with two types of CP and plant cultivation in aeroponics on some economically valuable morpho-biochemical traits [dry leaf weight, SGs concentration, antioxidant activity (AA), total phenolics and flavonoids content (TPC, TFC)] of stevia. We have demonstrated that a short (2–7 min) seed treatment with two types of CP (dielectric barrier discharge (DBD) and capacitively coupled (CC) CP) have different effects. CC substantially decreased dry leaf weight (by 30%), didn't change the total concentration of most abundant SGs rebaudioside A (RebA) and stevioside (Stev) but increased RebA/Stev ratio (an indicator of taste quality) from 0.43 (control) up to 0.71. CC also increased TPC (1.6–2.4-fold), TFC (40–55%) and AA (17–52%). DBD, contrary to CC, increased dry leaf weight by 30–83% but decreased SG concentration by 34–51%. However, SGs amount per plant or area unit after 5 min treatment was 21% higher compared to control. DBD decreased AA with no change to TPC and 20% higher TFC. We have demonstrated that CP has the potential to substantially enrich plant material with valuable secondary metabolites when stevia is grown in aeroponics. This work was supported by the Research Council of Lithuania (S-MIP-23-8).

P-35-071
PTEN reactivation by a small molecule-based approach

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Phosphatase and tensin homolog (PTEN) is a pivotal tumor suppressor and its inactivation results in cellular proliferation and various diseases. Recent investigations have underscored the significant role played by HECT ligases in the inactivation of PTEN. Notably, it has been shown that some NEDD-4 like HECT ligases (WWP1, WWP2 NEDD-4) induce ubiquitination of PTEN, resulting in its degradation. There is potential for therapeutic strategies targeting this process to address crucial oncological conditions, including prostate cancer, colorectal cancer, and acute myeloid leukemia. Our group, utilizing computational screening and DEL technology, identified a set of promising compounds capable of modulating the functions of some HECT ligases and, consequently, the growth of prostate cancer cells. To date, we are employing a combination of biochemical, biophysical, and molecular biology approaches to enhance our understanding of the compounds' mechanisms and to guide the optimization of the molecules.

P-35-072
Investigation of the biological and mechanical properties of a ZnO contents incorporated dental resin-reinforced glass ionomer cement materials

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This study is aimed to develop zinc oxide incorporated resin reinforced glass ionomer cement dental restorative material for helping biological and mechanical properties. After the setting time and mechanical properties of RMGIC incorporating varying amounts of ZnO (0, 1, 2, and 4 wt% in powder) were characterized, the surface morphology and composition of the resulting ZnO-RMGIC materials were investigated. To elucidate the surface properties, scanning electron microscopy, and energy-dispersive spectroscopy were used and setting time, solubility, sorption, micro vickers hardness and were compressive strength analyzed to evaluate the physical properties. Chemical properties were investigated by pH change and ion release measurements. The antibacterial effects of the bioactive set ZnO-RMGIC were tested with *Staphylococcus aureus* (*S.aureus*, ATCC 6538, USA) and the viability of human exfoliated human dental pulp stem cells (hDPSC) with this biomaterial was examined. There was no significant difference of 0, 1, 2, and 4% ZnO-RMGIC in compressive strength, but the higher the ZnO-RMGIC 2% content. An hDPSC cell viability of less than 70% was observed with 25% diluted extract in ZnO-RMGIC. The content of ZnO nanoparticles has increased and antibacterial effects have increased. All ZnO-RMGIC showed moderate-to-severe cytotoxic response. When ZnO nanoparticles are mixed, the compressive strength is maintained, and the hardness is increased. ZnO were found to reduce *S. aureus* growth and inhibit biofilm formation remarkably. This work was supported by the National Research Foundation of Korea (NRF) grant funded by the Korea government (MSIT) (No. 2021R1C1C1010005), by the National Research Foundation of Korea (NRF) grant funded by the Korea government (MSIT) (No. 2022R1F1A1074892).

P-35-073
Cholesterol crystal solubilization by reconstituted high-density lipoproteins: a biophysical exploration and evaluation of their efficacy in removing extracellular cholesterol deposits *in vitro*

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Cholesterol crystal (CC) accumulation within atherosclerotic plaques is closely linked to inflammation, plaque instability, and adverse cardiovascular outcomes. Existing management strategies often encounter limitations in effectively addressing CC-induced

inflammation and CC-solubilization. In this context, the exploration of the potential effects of reconstituted high-density lipoproteins (rHDL) in solubilizing CCs represents an innovative therapeutic approach. This strategy not only targets the dissolution of CCs but also has the potential to contribute to plaque stabilization. In this study, we have used biophysical techniques to investigate the capability of rHDLs in cholesterol crystal solubilization and their impact on inflammation induced by the presence of cholesterol crystals in macrophages. rHDL composed of apolipoprotein A1 (ApoA1) and 1,2-dipalmitoyl-sn-glycero-3-phosphocholine (DPPC) demonstrated a notable capacity for cholesterol crystal solubilization. Moreover, the inflammatory response of THP-1 cells treated with DPPC-rHDL in the presence of CCs was significantly lower than that observed in cells not exposed to DPPC-rHDL. Our findings indicate that DPPC-rHDL effectively solubilizes cholesterol crystals. Furthermore, *in vitro* experiments demonstrate a significant reduction in THP-1 cell inflammatory responses when treated with DPPC-rHDL in the presence of cholesterol crystals. This highlights the use of DPPC-rHDL as a promising strategy for innovative atherosclerosis interventions. *The authors marked with an asterisk equally contributed to the work.

P-35-074
Optimizing the production of recombinant human Galectin-1: a general protocol for maintaining stability and lectin activity in the long run

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Galectins are a family of lectins characterized by their binding affinity for β-galactosides and a shared amino acid sequence motif in their carbohydrate recognition domain. Galectins are involved in the modulation of inflammatory responses and mediation of immune suppressive mechanisms in tumour progression and evasion, making them attractive targets for research. Galectin-1 is comprised of a β-sandwich stabilized by a hydrophobic core and interactions at the dimer interface. Galectin-1 is highly susceptible to oxidative inactivation due to the presence of six cysteines in its sequence and their participation in the formation of intramolecular disulfide bonds that destabilize the folding of the protein, thus leading to the loss of its lectin activity [1]. Activity of Galectin-1 is dependent on the (i) correct folding of the carbohydrate-recognition domain, (ii) maintenance of the reduced state of its cysteines, and (iii) preservation of the spatial arrangement of the residues involved in ligand binding even in the carbohydrate-ligand-free form of the protein. With this in mind, we designed four constructs to be purified either by affinity chromatography on a lactosyl-sepharose column or by IMAC, and we characterized the protein variants in a time-dependent manner by SDS-PAGE, DLS, nanoDSF, SEC, CD, UV-Vis spectroscopy, and FP assays with high-affinity ligands, to establish a general protocol for the preparation of recombinant Galectin-1 for studies where long-term stability and maintenance of lectin activity are a requirement. Our findings show a clear improvement in the stability of the tag-less cysteine-to-serine mutant form of Galectin-1 over the remaining variants. The observations provided valuable insight into the factors relevant to maintaining the stability and lectin activity of Galectins. Reference: [1] López-Lucendo MF et al. (2004) J. Mol. Biol. 343, 957-970. This work was supported by CSF (23-08490L), MEYS CR (LM2023042), and Charles University (GAUK 358322).

P-35-075
Plasma proteome profiling using multi-nanoparticle protein corona for early detection of diseases

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Understanding the interactions of nanoparticles (NPs) with biological mileau is crucial for developing effective nanotechnologies. An important aspect of nano-bio interactions is the formation of a protein corona (PC) when NPs they encounter biological fluids. The composition of the PC depends on factors such as the size, shape, and surface properties of the NPs, as well as the specific biological milieu (e.g. diseased blood or healthy blood) (1). Recent research demonstrated the utility of the PC in proteomic profiling to facilitate the identification of disease-specific biomarkers (2). Considering that the PC's composition is affected by NP surface properties, NPs with different surface properties adsorb different proteins from biofluids, thus increasing the potentiality of the PC for biomarkers discovery. In this context, using blood samples from patients, our group has employed a multi-NPs array to create PC profiles, where the obtained information was used to train a machine-learning algorithm able to distinguish Alzheimer's patients from healthy individuals (3). The same method was successfully applied to the diagnosis of coronary artery disease (4). More recently, we used a PC-based approach for detection of superficial plaque erosion, a cause of residual thrombotic complications in atherosclerosis, in an *in vivo* model recapitulating features of the pathophysiological condition. Being able to detect the plaque erosion could lead to targeted therapies, thus greatly affecting clinical management. We demonstrated that metal of frame NPs act as plasma protein concentrators allowing the identification of a higher number of proteins, compared to unfractionated plasma. Nonetheless, several plasma proteins were identified exclusively in the superficial erosion group and might thus serve to discriminate the condition. References: 1. Corbo et al. (2017) Biomaterials Sci 5(3):378-387. 2. Kamaly et al. Nanoscale 2022. 3. Corbo et al. Adv Health Mat 2021. 4. Lee et al. Small 2023

P-35-076
Chromatin remodeling, alternative RNA processing and m6A RNA modification in Arabidopsis

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The genomic DNA is arranged within a nucleoprotein complex known as chromatin. Epigenetic mechanisms, including DNA methylation, histone modifications, and chromatin remodeling, regulate the accessibility of genomic DNA, thereby influencing gene expression and governing a complex interplay among various checkpoints involved in transcript biosynthesis, editing, and processing. In Arabidopsis, 40-60% of expressed genes possess alternative transcript isoforms, enhancing genome coding capacity. During plant development or in response to stress, a single gene may exhibit differential expression and editing in a tissue-specific manner through alternative RNA processing or m6A mRNA methylation. Our investigation revealed that subunits of the SWI/SNF chromatin remodeling complexes (CRCs) play a role in determining alternative transcription start site (TSS) choice in an organ-specific manner, impacting both the level of gene expression and the formation of alternative transcripts. Additionally, we observed a direct interaction between the CDC5 subunit of the spliceosome activating complex (NTC) and the SWI3C subunit of the SWI/SNF CRC. These proteins may cooperate in regulating alternative exon usage by directly modulating chromatin structure and histone modification during transcription. Notably, core subunits of both complexes were found to interact with MTA, a protein involved in mRNA methylation in Arabidopsis. Subsequent analysis revealed that mutations in *SWI3C* and *CDC5* genes decreased m6A levels in mRNA from *swi3c* and *cdc5* Arabidopsis mutants. In summary, our results and literature data support the hypothesis that transcription and alternative RNA processing in Arabidopsis are subject to regulation through cross-talk between SWI/SNF chromatin remodeling machinery, m6A mRNA methylation, and spliceosome activating processes in an organ-specific manner. Funding: National Science Centre: 2015/16/S/NZ2/00042; 2018/29/B/NZ1/01935 and 2021/43/D/NZ2/02461 given to SK.

P-35-077
Optimized RNA extraction strategy from whole human saliva

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Saliva has emerged as a promising source of biomarkers for predicting, diagnosing, and monitoring various diseases, particularly those within the oral cavity. Utilizing saliva for biomarker detection and quantification offers numerous advantages over blood collection due to its noninvasive and painless sampling method, making it suitable even for children. However, saliva is a complex biofluid containing DNA, proteins, RNA, metabolites, and other components, presenting experimental challenges, especially in isolating nucleic acids, particularly RNA. In this study, we investigated different protocols for RNA extraction from human saliva, with and without a stabilization solution. We evaluated the efficacy of these methods for downstream gene expression studies using quantitative real-time RT-PCR (RT-qPCR), considering parameters such as RNA quality, integrity, and yield. Among the methods tested, an "in-house" protocol demonstrated superior robustness, reproducibility, and cost-effectiveness in extracting high-quality RNA from human saliva. RT-qPCR analysis of a panel of genes, including a housekeeping gene, consistently yielded reliable Cq values across all samples. In summary, our proposed "in-house" method offers a reliable means of extracting RNA from human saliva without the use of toxic chemicals like phenol and chloroform. This strategy has the potential to quantify salivary genetic biomarkers in clinical settings, thereby advancing the development of personalized medicine for both oral and systemic diseases. 1. Previously published in: Gandhi, V et al. (2020) Biomarker Insights, 15, 1–9.

P-35-078
The advantage of speeding up bacteriological urine analysis using coherent fluctuation nephelometry (CFN)

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The rapid development of technologies in laboratory medicine along with significant increase in the productivity of instruments give opportunity to conduct a large number of studies within one laboratory. At the same time, screening methods come to the forefront, allowing to quickly separate samples that require further analysis. It helps to reduce the financial burden. Thus, according to our data about 50% of the urine samples are negative. The goal was to study was to compare a new screening method for indicating the growth of bacteria in urine – coherent-fluctuation nephelometry (CFN, Nefelyzer, Medtechnopark LLC, Russia) – with the well-known method of laser nephelometry (LN, Alifax HB&L, Italy). The reference method was the inoculation of 10 µl of urine using InoqulA Kiestra (BD, USA) on solid culture media (Biomedia LLC, Russia): Colombian lamb blood agar, chromogenic agar for uropathogenic bacteria and Sabouraud agar. A total of 2288 urine samples were analyzed. Identification of microorganisms was carried out by the MALDI-TOF method on a Microflex LT/SH device (BrukerDaltonics, Germany). It was found, that CFN showed growth detection in the first 60 minutes 21.7% more often than LN. The diagnostic specificity of the CFN method (n = 2288) was 92%. About 50% of urine samples contained residual antimicrobial activity. 2% discrepancy was found with growth of *Enterobacteriaceae* family on plates and negative instrument screening test. It should be noted that it was observed in samples with a negative test for residual antibiotics. We believe that this can be caused by the low growth rate of individual strains, and it requires further study to improve the growth qualities of liquid screening media.

P-35-079
From HMP-P to pyridoxal: evolutionary trajectories and specificity transitions in the vitamin kinase enzyme family

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The biosynthesis of vitamins B1 and B6 involves the phosphorylation catalyzed by ATP-dependent vitamin kinases family belonging to the Ribokinase superfamily. Within these enzymes, two homologous can perform the phosphorylation of hydroxymethyl-pyrimidine (HMP), an intermediate in the synthesis of vitamin B1: 1) ThiD2-PLK/HMPK, a bifunctional enzyme that phosphorylates HMP to produce hydroxymethyl-pyrimidine-phosphate (HMP-P), besides the phosphorylation of pyridoxal (vitamin B6) to pyridoxal-5-phosphate. 2) ThiD-HMPPK, A hydroxymethyl-pyrimidine phosphate kinase that phosphorylates HMP to HMP-P, and also phosphorylates HMP-P to produce hydroxymethyl-pyrimidine-diphosphate (HMP-PP). This second phosphorylation reaction is unique in the Ribokinase superfamily, as it is performed on a phosphate group and not on a primary alcohol. Interestingly, phylogenetic analyses indicate that ThiD2-PLK/HMPK enzymes diverged from ThiD-HMPPKs, suggesting that the HMP-P phosphorylation would have been lost during evolution, while the pyridoxal phosphorylation appeared as an evolutionary novelty in the ThiD2-PLK/HMPK group. In this work, we have determined the crystal structure of an ancestral ThiD-HMPPK from the order Enterobacteriales (AncEnHMPPK) in ternary complex with either HMP or HMP-P with non-hydrolyzable analogs of ATP and identified crucial residues for HMP-P binding and catalysis. To determine how HMP-P phosphorylation evolved towards pyridoxal phosphorylation, we reconstructed ancestral sequences of the lineage, analyzed key residues conservation, and traced them through evolution, elucidating the point mutations that would have occurred during the divergence of ThiD2-PLK/HMPK. These analyses indicate that mutations A110C, H179A, and T211A would have been the main changes in ThiD-HMPPK during the evolution to ThiD2-PLK/HMPPK, showing that the appearance of pyridoxal phosphorylation was concomitant with the disappearance of the HMP-P phosphorylation. FONDECYT 1221667.

P-35-080
Glycogen phosphorylase from the methanogenic archaea *Methanococcus maripaludis*: Unique regulatory properties of a PLP independent phosphorylase

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Glycogen phosphorylase (GP) catalyzes the rate-limiting step in glycogenolysis to release glucose-1-phosphate from glycogen. While all phosphorylases require the cofactor pyridoxal-5'-phosphate (PLP) for catalysis, their regulation varies. Phosphorylases from vertebrates exhibit complex allosteric regulation, while the evidence for enzymes from archaea and bacteria is scarce and points to a minor regulatory role. To analyze phosphorylases from different phylogenetic groups, we inferred the phylogenetic tree for the family. Sequences from the Methanococcales group of archaea revealed a substitution (Lys678Thr) that might hinder PLP binding. Biochemical characterization of the recombinant GP from *Methanococcus maripaludis* (MmGP) was performed. MmGP exhibited a strong preference for large, branched substrates like glycogen, with a Km of 0.3 mM for phosphate and a half saturation constant of 0.3 mg/mL for glycogen, values similar to those reported for bacteria and eukaryotic enzymes. However, PLP analysis by fluorescence spectroscopy and mass spectrometry indicated the absence of the cofactor, challenging the established requirement of PLP for phosphorylase activity. Additionally, MmGP displayed complex allosteric regulation, unlike previously reported archaeal GPs. The enzyme was inhibited by various metabolites, including NaPPi, F6P, PEP, ADP, ADP-glucose, and UDP-glucose, and activated by fructose-1,6-bisphosphate (FBP). These findings suggest a more intricate metabolic control of glycogen metabolism in archaea than previously thought. Overall, the results demonstrate that phosphorylase activity is possible in the absence of PLP and suggest that glycogen metabolism in methanogenic archaea might be regulated by multiple effectors. Further studies, like site directed mutagenesis and structure determination, are in progress to identify residues involved in catalysis. Funding Fondecyt 1231263.

P-35-081
The contribution of *Staphylococcus* genus to pathogenicity and virulence in the hospital environment

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Nosocomial infections pose a significant threat to patient health and safety worldwide, with *Staphylococcus* sp. emerging as a leading cause of morbidity and mortality in healthcare settings. Understanding the role of virulence factors in the pathogenesis

of *Staphylococcus* sp. is essential for developing effective nosocomial infection treatments, especially given the variety of mechanisms involved, including adherence factors, toxins, immune evasion strategies, and biofilm formation. Due to high patient density, invasive medical procedures, and prolonged antimicrobial exposure, *Staphylococcus* sp. and antibiotic resistance thrive in hospitals, causing outbreaks and nosocomial infections. In this context, we examined the diversity and phenotypic profiles of pathogenicity and virulence factors in *Staphylococcus* sp. strains isolated from inert surfaces and healthcare workers' skin from 207 samples collected from 25 healthcare facilities. The strains, characterized by MALDI-TOF MS, exhibited high diversity, with 31 different bacterial species identified, predominantly belonging to the *Staphylococcus* genus. Most staphylococcal strains were isolated from the skin of healthcare personnel, indicating the contribution of resident skin microbiota to nosocomial transmission and healthcare-associated infections, likely due to non-compliance with hygiene, sterilization, and disinfection protocols. Phenotypic analysis performed on 52 staphylococcal strains showed high production rates for most analyzed virulence factors, including coagulase and hemolysins, amylase, lecithinase, lipase, DNase, and esculinase. Among the species analyzed, *S. aureus* displayed the highest pathogenicity and virulence index, followed by *S. haemolyticus* and *S. warneri*. Thus, identifying and understanding nosocomial pathogen virulence factors is crucial for implementing effective infection control measures and developing specific therapeutic strategies to mitigate the impact of these infections. *The authors marked with an asterisk equally contributed to the work.

P-35-082
YD-repeats as a building block for tailored protein delivery systems

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Bacterial YD-repeat-containing proteins are examples of "polymorphic toxins" that share a common architecture, consisting of an N-terminal domain involved in protein secretion, multiple YD-repeating motifs and a hypervariable C-terminal toxin. This protein family has been implicated as virulence effectors associated with various toxin delivery systems, including Type V and VI Secretion Systems and the tripartite ABC or Tc toxin systems. Our previous pioneering structural studies on YD-repeat proteins focussed on the TcB-TcC subcomplex of bacterial ABC toxin complexes, revealing that multiple YD-repeat sequences form an extended spiralling β-sheet, which makes a shell-like structure that encapsulates the toxin domain prior to its delivery. The hypervariability of the toxin domain suggests the system can be engineered to deliver non-native cargo proteins instead of the native toxic payload. To investigate the extent to which this general mechanism of toxin packaging, assembly and release is conserved in YD-repeat-containing proteins found in other functional contexts, here we report several structures of YD-repeat-containing proteins found in different biological contexts. Our results demonstrate that the YD-repeat is a simple structural motif that, in the context of tandemly arranged repeats, acts as the core building block for hollow, proteinaceous cages of varied sizes and capacities.

P-35-083
Bacterial ferroptosis-like death induced by pleurocidin in *Vibrio vulnificus*

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Pleurocidin is a 25-mer peptide with a net positive charge and amphipathic α-helical structure, derived from *Pleuronectes americanus*. Recent study reported the novel form of programmed cell death in *Vibrio vulnificus*, emphasizing the need for further investigation of bacterial cell death mechanisms. In this study, pleurocidin triggers the accumulation of reactive oxygen species and cells undergo iron-dependent fenton reaction which exerts potent influence in bacterial metabolism. The reaction catalyzes poly unsaturated fatty acid (PUFA) oxidation, exerting oxidative damage in cells. Lipid peroxidation, a major hallmark of ferroptosis-like death, was observed after treatment of pleurocidin. Glutathione depletion and lethal oxidative damage cause disruption of homeostasis, leading to DNA damage in bacterial cells but without fragmentation. These results cast the possible occurrence of ferroptosis-like death in pathogenic bacteria, enhancing the understanding of microbial cell death.

P-35-084
Regulation of the fructose operon via DeoR family transcriptional regulator FruR in *Faecalibacterium prausnitzii* A2-165

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Faecalibacterium prausnitzii, a dominant member of healthy human gut microbiota, relies on the phosphoenolpyuruvate (PEP):carbohydrate phosphotransferase system (PTS) to import fructose, simultaneously phosphorylating it to fructose 1-phosphate (F1P). In a recent study, we identified that *F. prausnitzii* has two distinct HPrs (HPr1 and HPr2) as histidine-containing phosphocarrier proteins. While HPr1 has one phosphorylation site (histidine15) and is majorly involved in the PTS sugar transport, HPr2 has two phosphorylation sites (histidine15 and serine46) and may participate in numerous physiological regulations. Here, we show that FruR, a DeoR family transcriptional regulator, interacts with both serine46-phosphorylated (Ser46~P) HPr2 and F1P for its sophisticated transcriptional regulation of the fructose (*fru*) operon. FruR can facilitate the RNA polymerase binding only in the presence of both HPr2(Ser46~P) and F1P. The formation of FruR-HPr2(Ser46~P)-F1P complex has distinct binding motif with Apo-FruR in the *fru* promoter. The binding sequences located in between the -35 and -10 element, and downstream of transcriptional starting site (TSS) is crucial for appropriate binding and transcriptional initiation of the FruR complex. DeoR family proteins consist of an N-terminal DNA binding domain, the C-terminal domain, where sugar derivatives such as F1P can bind, and a linker domain that connects the two domains. F1P not only increases the binding affinity of HPr2 to the C-terminal domain of FruR, but also induces a structural modification in the linker domain. This gives a flexibility to the N-terminal DNA binding domain and decreases the DNA binding strength of FruR, but rather allows the RNA polymerase binding to the *fru* promoter. Based on the molecular biological and structural analysis of FruR, we suggest how its DNA recognition is influenced by each interacting molecule.

P-35-085
Mechanisms behind the role of mannose PTS regulator ManR on cell–cell aggregation in *Vibrio cholerae*

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The phosphoenolpyruvate (PEP):carbohydrate phosphotransferase system (PTS) is known for its role in carbon catabolite repression in addition to the regulation of various physiological processes in bacteria. Owing to these functions, the transcription of PTS-encoding genes undergoes meticulous regulation by transcriptional regulators that respond to substrate availability. In our previous study, we reported that deletion of the *Vibrio cholerae* mannose-specific PTS (PTS^{Man}) regulator, *manR*, led to an increase in biofilm formation, while colonization in the host decreased. The distribution of PTS^{Man} is relatively constrained among bacterial species, primarily found in those linked to animal hosts, hinting at the potential significance of the mannose transporter in hostbacteria interactions. ColabFold and ligand-fishing experiments were utilized to identify interacting partners of ManR. Additional biochemical and molecular experiments were conducted to elucidate the underlying mechanisms of how ManR regulates autoaggregation in the host environment.

P-35-086
Optimization of recombinant viral protein VPg expression in heterological *Escherichia coli* system and its purification

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The *Potyviridae* family includes about a third of known plant viruses that cause large crop losses. The genome this family is represented by +ssRNA to the 5-end of which the viral protein VPg is covalently attached mimicking the mRNA cap structure which binds to the plant translation initiation complex. As a result, the translational machinery of a host plant is switched to produce viral proteins. Interestingly, the resistance of plants to virus strains largely depends on the composition of isoforms of the host translation initiation complex with which VPg interacts. Thus, studying the VPg interactome by proteomics methods (i.e. by IP-MS) will make it possible to determine the core of the protein complex necessary for the progression of the infection. In our study, we focused on creating a simple and effective system for inducing the expression and purification of the VPg protein of potato virus Y (PVY) – one of the most agriculturally significant plants viruses – to obtain pure VPg protein in large quantities for the purpose of antibody production. In this work, we created a genetic construct for the expression of recombinant VPg in *E. coli* cells, containing the VPg gene fused with 6xHis-SUMO tag for purification of the recombinant protein. We carried out induction of protein synthesis in various strains of *E. coli* (BL21(DE3), Rosetta (DE3), Lemmo21 (DE3)), in two media (LB with the addition of IPTG and auto-inducible ZYP-5052) and with varying temperatures (37°C, 30°C, 20°C). The highest yield of recombinant protein in soluble form was observed when the Rosetta strain (DE3) in LB medium (0.5 mM IPTG) at 20°C was used. We purified the protein to electrophoretic homogeneity using IMAC (Ni^{2+}), then performed specific proteolysis of the 6xHis-SUMO tag and separated the tag-free protein using subtractive IMAC. The yield of purified VPg was 1.5 mg per 1 g of *E. coli* cells. This work is supported by the Russian Science Foundation Grant №21-76-10050.

P-35-087
Heparin and heparan sulfates interaction with bone morphogenetic protein 6 and hemojuvelin

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Heparin and heparan sulfates (HS) are highly sulfated glycosaminoglycans that can bind a plethora of proteins, modulating a wide range of biological pathways. In this context, it has been reported that heparin interferes with the hepcidin pathway, that regulates iron homeostasis, binding and sequestering some actors of the bone morphogenetic protein (BMP) complex. Using different approaches, such as computational analysis, recombinant proteins, heparin-sepharose column, competition binding in immobilized heparin studies, we investigated the capacity of heparin to bind different components of the BMP6 complex. We have already demonstrated that BMP6 showed a high heparin binding properties mainly involving regions at C-terminal (including K126, K127 and R129) and N-terminal (R5, R6 and R7). Here we show that ALK2 and ALK3, the BMP type I receptors respectively mediating BMP6 and BMP2 signalling, and BMPR2, representing the promiscuously utilized BMP type II receptors did not interact with heparin or HS. Interestingly, the co-receptor Hemojuvelin (HJV) is enriched with basic residues and mainly the ones of its N-terminal fragment, generating an exposed positive patch, showed a high and specific affinity for heparin/HS binding. To identify the heparin binding site, we generated mutants HJV for each of two putative domains (HBD1, HBD2) or both (HBD1 + 2), that further will be analyzed for their heparin binding affinity and the effect on the hepcidin pathway. Our biochemical studies provide structural basis for the understanding the mechanism of hepcidin inhibition by heparin and a novel regulation mechanism of hepcidin expression, in which hepatic HS sustain the signalling activation, attracting the BMP ligands toward the membrane, and facilitating the assembly of active BMPR complexes, contacting HJV.

P-35-088
How a single non-conventional amino acid can modify the effectiveness of amphibian antimicrobial peptides

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Antimicrobial peptides (AMPs) are considered as alternative new weapons to fight antibiotic-resistance. The AMP Esculentin-1a (Esc 1-21) (peptide 1) is known for its ability to inhibit the growth of Gram-negative bacteria, while it is weakly affecting Gram-positive bacteria [1,2]. Here, three analogues of peptide 1 were designed by the replacement of a single amino acid with a non-coded residue (peptide 2), or with a Proline (peptide 3) or D-Proline (peptide 4) and investigated for their structure-activity relationship and mechanism of action on living and model systems. The results indicated that peptide 2 displayed enhanced activity against Gram-positive bacterial strains, without being cytotoxic to mammalian cells. In addition, fluorescence assays in bacteria or lipid vesicles mimicking the microbial cell membranes demonstrated that peptide 2 possesses a membrane-perturbing activity. Nuclear magnetic resonance and circular dichroism spectroscopy were used to analyse the structure of the most active peptide 2. Our results demonstrated that the biological activity of peptide 2 is the result of a combination of different features, including increased biostability, increased alpha helical content as well as the adoption of a deformed helix bent and the capacity to decrease the membrane fluidity. Remarkably, this study has shown how a single amino acid substitution can modify the spectrum of activity of the parent peptide, thus assisting the optimization of AMPs for the development of new broad-spectrum anti-infective agents. References: 1. Luca V et al. (2013) Cell Mol Life Sci 70, 27732. 2. Di Grazia V et al. (2015) Amino Acids 47 2505. This research was supported by EU funding within the NextGenerationEU-MUR PNRR Extended Partnership initiative on Emerging Infectious Diseases (Project no. PE00000007, INF-ACT) to M.L.M and Fondazione Italiana per la Ricerca Cistica (Project FFC#4/2022) Delegazione FFC Ricerca di Roma e della Franciacorta e Val Camonica.

P-35-089
The effects of radiation therapy fractionation on the sparing of healthy tissue in FLASH radiotherapy

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Ultra-high-dose rate FLASH radiotherapy (FLASH-RT) for lung cancer shows reduced toxicity to surrounding healthy tissues, offering an advantage over conventional RT (CONV-RT) that causes adverse effects such as pulmonary fibrosis. This sparing phenomenon is known as the "FLASH effect".[1] The FLASH effect, at present, has only been observed in studies that administer the radiation dose in a single fraction and by using a single field; however, in a clinical perspective, this modality allows to treat only superficial tumors with low-energy (<12 MeV) electrons. To treat also deep tumors in FLASH mode, it is necessary to use high energy electron beams (VHEE, E >100 Mev) and use multiple fields at different angles to adequately conform the dose distribution, introducing a delay time between the irradiations at each field angles[2]. Herein, *in vitro* study on 16HBE human bronchial epithelial cells was conducted explored the FLASH effects (with clonogenic survival and redox state), in comparison to CONV-RT, by delivering a total dose of 4 Gy, both in a single irradiation (train of consecutive pulses at a fixed frequency) and in two irradiations separated by varying delay times (from 0.5 seconds to 5 minutes). Preliminary results indicate significant lesser toxic effects on cells with a delay time of 10 seconds, compared to both other assessed times and dose delivered with a single irradiation. These data suggest that the Flash effect depends on the amount of time between two irradiations, rather than on the total delivered dose. Further analysis on the impact of oxidative stress is ongoing, indicating the need for more research to optimize FLASH-RT treatment protocols in a clinical context. References: 1. Bourhis J et al (2019) Radiotherapy and Oncology 139, 11–17 2. Armstrong JG et al (1993) Int. J. Radiation Oncology Biol. Phys. 26, 685-689. Thanks to Fondazione Pisa and PNRR Tuscany Health Ecosystem, Spoke 1 "Advanced Radiotherapies and Diagnostics in Oncology" CUP I53C22000780001.

P-35-090
Exploring the potential of PLGA nanoparticles for enhanced barrier integrity and cellular uptake: an *in vitro* study with skin and lung co-culture models

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The *in vitro* co-culture models offer a promising approach to understanding the cell barriers' complexities, facilitating the study of drug permeation and toxicity in a physiologically relevant context. Poly(lactic-co-glycolic acid) (PLGA) nanoparticles (NPs) stand out as versatile tools, leveraging their unique properties to enhance drug stability, bioavailability, and targeting efficiency. This study focuses on the characterization of fluorescent PLGA NPs interaction with dermal and pulmonary barriers for refining drug delivery strategies. The lung barrier model was obtained by co-culturing A549 epithelial cells and MRC-5 fibroblasts (1:1 ratio), and the skin barrier model was a co-culture of HaCaT keratinocytes and CCD-1070Sk fibroblasts (1:1). The PLGA NPs were added in the apical compartment at a non-toxic concentration (25 μg/mL), and were removed after 24 h, and cells were monitored 3 days more. Firstly, the inflammation status was checked by measuring the levels of interleukins IL-6 and IL-8, which were maintained almost similar to control up to 96 h. Secondly, the effect of PLGA NPs on barriers' permeability was evaluated by transepithelial electric resistance (TEER) measurement. There was an increase of TEER values over time for both control and PLGA NPs-treated cells until 48 h, with no differences between the groups. Further, for both types of co-culture models we observed higher TEER values after 96 h compared to control, suggesting a strengthening of barriers and decreased permeability. Based on the fluorescence intensity quantification, we noticed that more than 90% of NPs were internalized during the first 24 h, without being transported into the basal compartment. Taken together, these findings suggest a higher probability of PLGA NPs-based drug delivery systems being retained within the targeted tissue or cell types, minimizing systemic toxicity. Acknowledgements: this study was funded by UEFISCDI, grant no. PN-III-P1-1_1-TE-2021-1375 (81TE/2022). *The authors marked with an asterisk equally contributed to the work.

P-35-091
Analysis of very long chain fatty acids in tail skin in a mouse model of IKSHD disease using GC-MS

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IKSHD (ichthyotic keratoderma, spasticity, mild hypomyelination and dysmorphic features) is a genetic disease caused by p.S165F mutation in a fatty acid elongate 1 (*ELOVL1*) gene [1]. The major IKSHD symptoms appear in the central nervous system and the skin. The mutation causes changes in very long chain FA (VLCFA) content with significant impact on lipid metabolism. VLCFA plays a crucial role in the skin barrier maintenance and the myelin integrity [2]. The tail skin tissues obtained from Elovl1$^{p.S165F/p.S165F}$, Elovl1$^{p.S165F/wt}$ and wild type (C57BL/6J; WT) mice at the age of 6 months. The lipids were extracted from abovementioned samples using the Folch method [3]. The lipid extracts were derivatized into FA methyl esters and analyzed by gas chromatography mass spectrometry (GC-MS). To examine statistical significance of differences in FA levels between mice groups, the one-way ANOVA was used. The differences in FA content between genders were determined using the non-parametric T test. Significant differences in VLCFA profiles were observed between Elovl1$^{p.S165F/p.S165F}$ and WT mice. In Elovl1$^{p.S165F/p.S165F}$ female were observed accumulation of C20:0-C22:0 and deficiency of C24:0 compared to WT females. Level of C24:0 was lower in Elovl1$^{p.S165F/p.S165F}$ compared to WT males. The levels of C19:1 and C21:0 were higher in Elovl1$^{p.S165F/p.S165F}$ compared to Elovl1$^{p.S165F/wt}$ male. Higher content of C20:0–C26:0 was noticed in males compared to females. The results indicate the mutation in question changes the VLCFA content in all examined mice: Elovl1$^{p.S165F/p.S165F}$, Elovl1$^{p.S165F/wt}$ and WT, as well as depending on individual sex. These disorders may result in disorganization of the skin barrier and progressive abnormalities in its functioning. Funding: National Science Center, grant no. 2020/37/B/NZ4/00821. References: [1] A. Kutkowska-Kaźmierczak et al. J. Med. Genet. 55:408-414 (2018), [2] A. Kihara. J. Biochem. 152 (2012) 387–395, [3] J. Folch et al. J. Biol. Chem. 226 (1957) 497–509.

P-35-092
Utilization of near-infrared fluorescence bile acid derivatives for non-invasive assessment of hepatobiliary secretory function

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Impairment of biliary secretory machinery is common in various liver diseases. Conventionally used serum markers do not always accurately reflect this dysfunction. To develop a non-invasive method for assessing hepatobiliary secretory function using extracorporeal monitoring of cholephilic fluorescent bile acid (BA) derivatives. These compounds (NIRBADs) were synthesized, using "click reactions," by conjugating chemically modified cholic, glycocholic, and taurocholic acids to fluorochromes emitting near-infrared (NIR) fluorescence. Flow cytometry with CHO cells stably expressing liver transporters (NTCP, OATP1B1, and OATP1B3) was used to evaluate NIRBAD uptake *in vitro*. In anesthetized rats, the NIR fluorescence emitted by the liver after intravenous NIRBAD administration was extracorporeally recorded using high-resolution imaging equipment. NIRBADs were primarily taken up by OATP1B3 and NTCP, and their uptake was inhibited by rifampicin (OATP1B3) and taurocholic acid (NTCP). Docking analysis supported their interaction with BA transporters. After intravenous administration, NIRBADs were rapidly cleared from the blood and secreted into bile. The time course of extracorporeal monitoring of hepatic NIR fluorescence closely correlated with that directly measured on the liver surface in rats with mid-ventral laparotomy. No changes in serum markers of hepatic and renal toxicity were found after NIRBADs administration. In rats with phalloidin-induced liver damage, which resulted in 50% reduction of bile flow, a significant decrease in serum clearance and secretion into bile of NIRBADs was determined. This was associated with prolonged hepatic retention and extended NIR fluorescence half-life extracorporeally recorded. Novel BA derivatives with NIR fluorescence enable non-invasive assessment of secretory hepatobiliary function, offering promising prospects for clinical applications.

P-35-093
Carbosilane dendrimers conjugated with caffeic acid and PEG interact with human serum albumin

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Dendrimers are monodispersed synthetic macromolecules that have potential applications for drug and RNA delivery platforms. One of the most important systems which should be considered for drug carrier evaluation are the serum proteins that bind various exogenous and endogenous substances and determine their blood levels [1]. This study examined the interaction of newly synthesized polyphenolic dendrimers with human serum albumin at a constant protein concentration and various ratios of dendrimer. Considered polyphenolic dendrimers functionalized with polyethyleneglycol and caffeic acid residues could be a useful tool in biomedical applications due to their significant antioxidant and antiradical properties [2]. Zeta size, zeta potential, circular dichroism and transmission electron microscopy techniques were applied to analyze the binding mode, morphology and conformation of human serum albumin / dendrimer complexes. Obtained results show that polyphenolic dendrimers are able to interact with human serum albumin, change its structure and electrical properties. References: [1]. Kubczak, M. et al. Colloids Surf B Biointerfaces 227, 113359 (2023). [2]. Grodzicka, M. et al. Sustain. Mater. Technol. 33, e00497 (2022) Disclosure of Funding: This work was supported by grant 2018/30/Z/NZ1/00911 of the Project "NanoTendo" and by grant 2023/07/X/NZ7/00026 of the Project "MINIATURA7" and innovation programme under grant agreement no 685451, co-financed by grant of the National Science Centre of Poland under "Beethoven Life 1" program, no: 2018/31/F/NZ5/03454, and grant PID2020-112924RBI00 (MINECO).

P-35-094
Computational prediction of (alpha-1-antitrypsin)-elastase Michaelis complex reveals an exosite contributing to high-affinity interaction

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Neutrophil elastase (NE) is a protease released by activated neutrophils during an inflammatory response and has an anti-microbial function, but when unregulated exerts proteolytic activity on elastin and other extracellular matrix components. Alpha-1-antitrypsin (AAT), an abundant plasma serpin, acts as an important regulator by irreversibly inhibiting NE. The protective activity of AAT is highlighted by the development of early onset emphysema in patients with alpha-1-antitrypsin deficiency. Here the structure of the Michaelis complex formed by AAT and NE was studied by computational methods. Standard molecular dynamics and essential dynamics techniques were used to simulate the docking process using, as a model, the crystal structure of the Pittsburgh variant of AAT in complex with trypsin. A good candidate for the AAT-NE structure was found and was demonstrated to satisfy the geometric conditions required for enzymatic activity, and to be stable over time in the range of hundreds of nanoseconds. Analysis of the interaction interface reveals key contacts including a strong double hydrogen bond involving the P3 residue of AAT and several hydrophobic contacts in the central part of the reactive center loop of AAT. A region located upstream strand S4C of AAT, comprising three acidic residues (D202, E199, E204), was found to strongly interact with Arg 147 of NE. Recombinant AAT variants in which all three acidic residues were mutated to either alanine or serine as well as the single D202R mutant showed reduced association rate to NE and confirmed the predicted acidic region of AAT as an exosite contributing to high affinity interaction to NE.

P-35-095
Silk spheres targeting VEGF receptors for oligotherapeutics delivery – a new approach for cancer treatment

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The selective and efficient delivery of siRNA remains a challenge to its successful therapeutic application. Bioengineered silk is a material with great potential in drug delivery. It can be functionalized with a recognition peptide or nucleotide-binding sequence, enabling the transport of nucleic acids to specific target cells. The tumor microenvironment (TME) consists not only of cancer cells but also a multiplicity of cell types, including endothelial cells of the vascular system. Thus, targeting the VEGF receptors overexpressed in cancer and endothelial cells of TME with oligonucleotide-loaded spheres is a promising cancer treatment strategy. Herein, two bioengineered silk proteins able to target VEGFR-positive cells were constructed. The functionalized silks were based on the MaSp1 spidroin sequence of *N. clavipes* fused to VEGFR1 and VEGFR2 receptor-binding peptides. MS2KN silk, an MS2 variant based on the MaSp2 silk protein functionalized with nucleic acid binding domain KN, was used to prepare blends with VEGFR-targeting silks. Agarose gel electrophoresis was performed to determine oligonucleotides binding to the silk proteins. Next, obtained proteins were processed into spheres and characterized in terms of morphology, size, zeta-potential, cytotoxicity, and siRNA-loading into particles. The uptake of constructs by VEGFR-positive endothelial and lung cancer cells was assessed using flow cytometry and confocal microscopy. The silencing effect of siRNA on target mRNA (*HIF1A*) was measured with real-time quantitative PCR. Blending VEGFR-functionalized silks with MS2KN improved the siRNA-binding compared with plain complexes. VEGFR-targeting spheres loaded with siRNA bound with high efficiency and were actively internalized by the VEGFR-positive cells. The silencing effect of *HIF1A* mRNA was observed for HIF1α-siRNA confined in silk spheres. The results indicated VEGFR-targeting silk spheres as a promising tool for effective siRNA delivery into TME.

P-35-096
Microfluidic diazoxide diazoxide-loaded solid lipid nanoparticles for the mitigation of oxidative stress in Friedreich ataxia

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Friedreich ataxia (FRDA) is a hereditary autosomal recessive disorder characterized by frataxin deficiency, impacting mitochondrial function and causing oxidative damage leading to a progressive neurodegeneration. Diazoxide (DZX), a potent vasodilator used in the treatment of systemic hypertension, has shown promise in preclinical models but faces challenges in crossing the blood–brain barrier and potential toxicity at higher doses. Solid lipid nanoparticles (SLNs) are a novel approach to improve blood–brain barrier (BBB) penetration and reduce side effects. In this study, we utilized a microfluidic technique to load SLNs with DZX (SLN-DZX). We observed improved permeability across the BBB compared to plain DZX, using an *in vitro* cell model. Additionally, FRDA fibroblasts treated with SLN-DZX exhibited internalization of SLNs and a significant reduction in ROS levels. These findings suggest that the SLN-DZX formulation holds promise as a potential therapeutic strategy for FRDA. *The authors marked with an asterisk equally contributed to the work.

P-35-097
Development of screening methods for inhibitors of cap-binding process in influenza A virus

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Influenza A virus encodes RNA-dependent RNA polymerase, which is composed of three subunits – PA with endonuclease activity, PB1 with polymerase activity and PB2 with binding site for host five-prime cap. Influenza virus can replicate only with host five prime cap, so cap-snatching process is crucial step in the influenza life cycle and the cooperation of these three subunits is essential for stealing the host five-prime cap and for subsequent viral replication. Pimodivir (also called VX-787) is a known inhibitor of interaction between PB2 cap-binding domain and host five-prime cap. Clinical trial with pimodivir was recently discontinued. For this reason, there is an effort to find new inhibitors of the cap-snatching process in influenza. In this study, we developed several methods (for example based on AlphaScreen and DSF) for high-throughput screening of potential cap-binding inhibitors. We found that these methods can be used for inhibitor screening. We also synthesized new derivatives of pimodivir and measured the strength of interaction between these novel compounds and PB2 cap-binding domain. We found a compound whose affinity for the PB2 cap-binding site is similar to pimodivir. These results will be used for further synthesis and screening of potential inhibitors.

P-35-098
Toxicity assessment of conjugated nanocomposites with quercetin

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The mechanism of wound healing is an important physiological process that plays a key role in maintaining haemostatic balance. Wound contamination, infection or an extensive area of skin damage led to abnormal functioning of the haemostatic system. Pathologies that interfere with wound healing can prolong a patient's recovery, affecting his or her quality of life and leading to high financial costs. Bimetallic silver and gold nanoparticles combined with polyphenols exhibit a number of properties desirable from a medical point of view, including anti-inflammatory, antimicrobial and antioxidant effects. For this reason, we propose silver and gold nanocomposites with quercetin as potential factors supporting the process of wound healing. However, it is very important to test their effect *in vitro* on the cells with which they will have direct contact, i.e. skin and blood cells. The aim of this study was to assess the degree of toxicity of quercetin-conjugated nanocomposites to human erythrocytes and human skin fibroblasts *in vitro*. The biological material included human erythrocytes and a human skin fibroblast (BJ) cell line. The haemotoxicity of the nanocomposites was assessed based on the degree of haemolysis and the degree of haemoglobin oxidation in erythrocytes. On the other hand, cytotoxicity to skin cells was determined using the MTT assay. Determinations were made using spectrophotometric methods. Based on the results, it was concluded that nanocomposites combined with quercetin in the concentration range (0.5-25 μg/mL) did not show significant toxicity towards erythrocytes. No toxicity of the tested nanoparticles to skin cells was observed. The obtained results suggest that the tested nanocomposites are biocompatible with the tested biological material and can be used for further research assessing their properties useful in the wound healing process.

P-35-099
Searching for the epigenetic background underlying the regulation of fish fertilization capacity

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In vitro fertilization in fish reproduction is an essential practice in nowadays methods of breeding in fish aquaculture. Only recently, reproductive success has been correlated with the uneven quality of spermatozoa population produced by a fish individual. The sperm quality includes important biochemical properties, vitality and motility which affect their fertilizing ability and ultimately influence the embryo development and quality of the progeny. It is common sense that a large number of high-quality spermatozoa increases reproductive success. Thus, the investigation of spermatozoa subpopulations of varied quality is vital for improvement of the overall fish reproductive success. Identification and description of signalling mechanisms influencing the quality of sperm physiological properties during external fertilization are key in our understanding of the relevance of the spermatozoa subpopulations in their reproductive success. This knowledge will be highly instrumental in better managing *in vitro* fertilization procedures, including sperm storage and cryopreservation. This study focuses on identifying spermatozoa subpopulations based on the differences in their physiological properties directly linked to their fertilizing ability and their potential correlation with DNA methylation of spermatozoa. Using the WGBS method followed by the differential methylation analysis of the control group with spermatozoa subpopulations separated based on the different quality of their physiological properties may unveil new factors influencing spermatozoa physiological properties regulated via epigenetic mechanisms. Analogical analysis of DNA methylation in produced progeny may further decipher the complexity and importance of epigenetic regulation in fish fertilization.

P-35-100
Phylogenetic insights and molecular dynamics simulations of CsqR, a transcription factor in *Escherichia coli*

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CsqR is a local transcription factor from *Escherichia coli* that controls expression of the *yih* genes involved in the utilization of sulfoquinovose (SQ), a derivative of glucose. SQ and its derivatives sulforhamnose (SR), and sulfoquinovosyl glycerol (SQG) might be effectors of CsqR. Preliminary results from our laboratory indicate that CsqR may exist in an alternative truncated form. In this work, we aimed to investigate the evolutionary patterns of CsqR and predict its binding properties with candidate effectors SQ, SR, and SQG. According to phylogenetic analysis, CsqR homologs were found in both the Actinobacteria and Proteobacteria phyla. The structure of the phylogenetic tree indicated that *csqR* was duplicated at some point. Among Enterobacteriales species, CsqR homologs had a highly conserved Met25 residue. We hypothesized that CsqR may exist in two alternative variants: the full-length long one (CsqR-l) and the short one devoid of 24 N-terminal residues (CsqR-s). Molecular dynamic simulations unveiled two potential arrangements of the CsqR-s structure, where the interdomain linker appeared either as a disordered loop or an α-helix. This helix facilitated the hinge-like rotation of the N-terminal domain, enabling CsqR-s to transition between two conformations termed "open" and "compact." Molecular docking demonstrated that CsqR-s might better differentiate between putative ligands and other compounds compared to CsqR-l. It is quite rare for bacteria to have multiple forms of a single protein, with only a few instances described to date.

P-35-101
Investigation on biological effects of the emergent environmental pollutant 4-octylphenol in human cell lines

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The emergent pollutant 4-octylphenol (4-OP) originates from the environmental degradation of alkyl phenol ethoxylates, compounds largely used in several industrial applications. Once released into the environment, it accumulates in waters, sediments and air. For its structure, 4-OP acts as an endocrine-disrupting chemical, causing damaging effects on reproduction and development of aquatic organisms. Increasing evidence indicates that 4-OP also represents a potential health risk for animals and humans which can be exposed to it through contaminated food and water and through inhalation and dermal absorption. In the present work, we characterized the cytotoxic ability of 4-OP towards several human cell lines, representing the potential main targets in the human body (i.e. hepatic, intestinal, pulmonary, renal and dermal), also comparing its effect with that of 4-nonylphenol (4-NP) and of a mixture of both 4-OP and 4-NP in a range of concentration between 1 and 100 mM. Viability assays demonstrated that each cell type had a peculiar sensitivity to 4-OP and that, in some cases, a combination of the two alkylphenols displayed a higher cytotoxic activity with the respect to the single compound. Then, we focused the attention on a liver cell line (HepG2) in which we observed that 4-OP reduced cell cycle progression and increased apoptosis. Moreover, 4-OP showed an interference with protective physiological cell processes such as the unfolded protein response, autophagy and the antioxidant response. On the whole, our data highlight a possible contribution of this pollutant to deregulate the normal homeostasis in human cells. *The authors marked with an asterisk equally contributed to the work.

P-35-102
Toothpastes with different detergent contents affect morphogenesis and redox system parameters in zebrafish embryos

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The process of embryogenesis is critical and affects future life. This study aims to assess the impact of three types of toothpastes with different detergent components on the embryonic development of zebrafish. Zebrafish embryos were exposed to sodium lauryl sulfate (SLS) and cocamidopropyl betaine (CAPB)-containing toothpastes, while the TP3 group was exposed to toothpaste containing no detergent until 72 hours post fertilization (hpf). The control group consisted of healthy embryos in the embryo medium (E3). Morphological analyses were performed every 24 hours under a stereomicroscope. At the end of 72 hpf, redox system parameters including lipid peroxidation (LPO), nitric oxide (NO), and glutathione S-transferase (GST) were evaluated in the embryo homogenates using spectrophotometric methods. Pericardial edema and a lack of pigmentation were observed in the SLS and CAPB-containing toothpastes. Depending on the detergent content of the toothpastes, significant variations were found in the LPO, NO levels, and GST activities, while the toothpaste group with no detergent did not induce a significant change. Our studies continue to elucidate the molecular mechanisms of the effects of toothpastes and their detergent contents on embryogenesis.

P-35-103
Development of a novel assay to measure alpha-1-antitrypsin inhibitory activity toward neutrophil elastase

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Alpha-1-antitrypsin deficiency (AATD) is a genetic disorder that mainly manifests in adults with lung emphysema and liver cirrhosis. Pathogenesis of the liver disease arises from misfolding of alpha-1-antitrypsin mutants such as Z-AAT (E342K) that results in accumulation of Z-AAT polymers in the endoplasmic reticulum of hepatocytes. On the other hand, emphysema derives from AAT deficiency in the bloodstream leading to reduced inhibition of AAT target proteinases such as neutrophil elastase (NE) in the lungs. In general, clinical assays implicitly equate circulating AAT levels to anti-protease activity. However, the activity of circulating Z-AAT variant is further reduced by conformational modifications that impair the inhibitory mechanism and by the presence of inactive polymers released by hepatocytes. Standard assays developed in the past to measure AAT inhibitory capacity in plasma require an experimental set-up that may limit their routine implementation in most clinical laboratories. To overcome this, we developed a new sandwich ELISA to determine NE inhibition by plasma AAT. The inhibitory activity determined by this assay on both purified AAT or control plasma samples were consistent with those determined by classical methods and was not affected by alpha-2-macroglobulin, another abundant inhibitor of serine proteinases in plasma. Application of the new method to plasma samples from patients with the ZZ genotype showed that 20 to 50% of circulating Z-AAT is inactive toward NE. This is partially due to the intrinsic dysfunction of the Z-AAT molecule as well as to the presence of inactive circulating polymers, which we previously showed to range from 8 to 33% of total plasma AAT content in a cohort of ZZ patients. Measuring the anti-elastase protective activity provides an additional level of detail to explain the different severity of lung manifestations observed among patients, and could thereby improve the current diagnostic pipeline of AATD.

P-35-104
New insights into sperm storage, mating behavior and sperm competition in Portunidae based on the invasive swimming crab *Charybdis hellerii* (Crustacea: Decapoda)

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The Portunidae crabs are known as sperm plug producers that copulate with a soft exoskeleton, avoiding sperm competition. *Charybdis hellerii* is a worldwide invasive species originally from the Indo-Pacific Ocean. We studied mating behavior and the chemical composition of sperm storage in the seminal receptacle (SR) as the egg paternity in this invasive species. The SR were analyzed under histochemistry and transmission electron microscopy. We amplified four microsatellite loci of *Charybdis feriata* and PCR products were screened after electrophoresis. The sperm plug is absent in *C. hellerii* and the ventral-type SR maintains a constant volume throughout the ovarian cycle. Spermatophores and spermatozoa are organized in sperm packets or strata in the RS after three consecutive laboratory copulas and the female copulates in hard condition (intermolt). Each copula produced three spermatozoa masses separated by secretion forming three strata. The strata secretion among the spermatozoa masses consists of neutral and acidic polysaccharides, poor in proteins. The dorsal region of the SR produces glycoprotein secretions in a stratified epithelium that are released primarily via holocrine mechanism. The paternity test using Cfe01 microsatellite loci effectively demonstrates polyandry in 20% of the *C. hellerii* eggs from ovigerous females collected in the field. Thus, the polysaccharide-rich strata are efficient in producing sperm competition where the last male to copulate has priority to fertilize the oocytes. In conclusion, the absence of sperm plugs and the presence of sperm packets in the SR, which maintains its morphology during ovarian development conciliated to more than one male mating with hard-shelled female *C. hellerii*, differs from all Portunidae recorded. More than one copula is effective in producing sperm competition by forming strata and only in a few cases occur polyandria, probably due to a mix of spermatozoa from different copula during the fertilization.

P-35-105
Vimentin-targeted doxycycline-loaded chitosan nanoparticles inhibit MMP activity in a colorectal cancer epithelial–mesenchymal transition (EMT) model

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Colorectal cancer (CRC) is the third most common cancer worldwide. Epithelial–mesenchymal transition (EMT) is vital in cancer progression and metastasis. EMT has gained prominence in understanding the aggressive behavior of CRC and in therapeutic resistance. Matrix metalloproteinases (MMPs) have emerged as key players in facilitating the EMT by orchestrating extracellular matrix remodeling. The dysregulation of MMP expression has been associated with the invasive potential of CRC, making it an attractive target for therapeutic intervention. In this study, it was aimed to investigate the consequential alterations in the expression of MMP subtypes in an *in vitro* EMT model of Caco-2 cells and to target an inhibitor for the MMP subtypes upregulated in EMT which is loaded in a nanocarrier system to enhance the treatment effectiveness by adding this new formulation to the adjuvant therapy protocol. For this purpose, self-transition to a mesenchymal phenotype in Caco-2 cells through prolonged incubation (30-90 days) was induced and transition was validated. Gene expression and protein levels of MMP-2 and MMP-9 were determined in epithelial (E) and mesenchymal (M) phenotypes. Both phenotypes were treated with doxycycline (Dox), which inhibits MMP-2 and MMP-9 activities. Dox-loaded chitosan nanoparticles (CS-NPs) were prepared by ionic gelation method,

labelled with vimentin antibody (Vim) and characterised for their physicochemical properties. MMP-2 expression levels increased 3.2-fold in the M phenotype validated by an increase in vimentin (1.5-fold) and N-cadherin (1.7-fold) expression levels. CS-NPs with 23% loading capacity (240-280 nm) released 74% doxycycline at 48 hours. Vimentin conjugation efficiency was 57%. 3.2 μg/mL CS-NPs inhibited MMP-2 activity by 65%, while 11.4 μg/mL free doxycycline inhibited it. In the light of these preliminary data, further studies will be conducted to evaluate the ability of targeted CS-NPs to improve clinically used adjuvant therapy. *The authors marked with an asterisk equally contributed to the work.

P-35-106
Rosmarinic acid methyl ester induces apoptosis and inhibits migration in triple-negative breast cancer

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Breast cancer is the most common diagnosed cancer with the leading cause of cancer-related deaths in women worldwide. Almost 15% of all breast cancer is diagnosed as triple negative breast cancer (TNBC). Treatment of TNBC presents challenges due to limited targeted therapies, inefficacy of chemotherapy, and severe side effects. Thus, new strategies which overcome toxicity and drug resistance in TNBC are gaining attention. Rosmarinic acid (RA) is an abundant phenolic ester showing anti-inflammatory, anti-diabetes, anti-cancer and anti-metastatic effect. Epithelial–mesenchymal transition (EMT) is a key link to regulate tumor invasion and metastasis and studies show that RA can regulate this process. The current study investigates the anti-cancer effects of RA and its derivatives in cell line MDA-MB-231. Cytotoxicity of RA and its derivatives was observed in a dose and time dependent manner in TNBC cell line MDA-MB-231 and normal mammary epithelial cell line MCF-10A. However, the therapeutic index of rosmarinic acid methyl ester (RAME) was higher than other RA derivatives. Further growth inhibition studies revealed that RAME significantly inhibited clonogenic survival of MDA-MB-231 cell line. Cell cycle arrest at SubG0 phase shows improved anti-cancer characteristics and qPCR results showed significant increase in the apoptosis related gene expression level. RAME inhibited the cell migration and invasion ability by modulating EMT in MDA-MB-231 cell line. Furthermore, RAME regulated EMT through the downregulation of the mesenchymal markers, N-cadherin and upregulation of epithelial markers in MDA-MB-231 cell line. Taken together, our findings demonstrate the therapeutic potential of RAME in TNBC. *The authors marked with an asterisk equally contributed to the work.

P-35-107
Distribution of membrane-bound sialidase NEU3: the effect of lipid raft patching

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The plasma membrane-associated sialidase NEU3 removes sialic acid from oligosaccharides, glycosphingolipids and glycoproteins directly on the cell surface and shows high substrate specificity toward gangliosides that are abundant in lipid rafts. Lipid rafts are small, dynamic domains enriched in cholesterol, sphingomyelin, gangliosides and proteins involved in biological processes with pivotal physio-pathological implications. In this study the distribution of sialidase NEU3 inside and outside lipid rafts, its influence on the ganglioside composition, and its behaviour upon lipid raft patching has been described in COS7 and HeLa cells transiently transfected with the murine NEU3 cDNA. Upon NEU3 expression, the enzyme reaches a maximum activity that is 10/13-fold higher compared to non-transfected cells and equally distributes between the lipid raft and the non-raft membranes. The enzyme modifies the ganglioside content of the lipid bilayer with a decrease in GD1a and an increase in GM1. In NEU3 transfected cells, lipid raft patching induced by cholera toxin subunit-B treatment results in the recruitment of the enzyme inside lipid rafts. Confocal microscopy analysis of HeLa cells confirms NEU3 differential distribution between non-raft membranes and lipid rafts of the enzyme and its recruitment in lipid rafts upon raft patching. Aggregation of lipid rafts has been demonstrated in several biological events such as the regulation of haematopoiesis, the response to death receptor agonists in endothelial cells, the vesicular transport in polarized cells and, finally, in the series of events leading to malignancy. In this perspective, the recruitment of NEU3 from non-raft membranes into clustered lipid rafts may be relevant to explain the functional implications of the enzyme in raft biology. Moreover, NEU3 could be a possible novel target to modulate the behaviour of lipid rafts in cell biology. *The authors marked with an asterisk equally contributed to the work.

P-35-108
Unveiling the role of membrane-anchored calpains as regulators of growth and development across organisms

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Calpains, a class of modulatory proteases, hold pivotal roles in various cellular processes across eukaryotes. While animal cytosolic calpains have been extensively studied, membrane-anchored calpains remain enigmatic. In our study, attention is directed towards exploring the structural and functional characteristics of

membrane-anchored calpains, alongside their phylogenetic distribution. The analysis identifies four types of membrane-anchored calpains, with Types 1 and 2 demonstrating widespread distribution among unicellular protists and streptophytes, indicating their ancient evolutionary origins. Conversely, Types 3 and 4 diversified early and are found in brown algae and oomycetes. Of particular interest is the plant DEK1 protein, the sole representative of membrane-anchored calpains subjected to functional scrutiny. The study presents a comprehensive overview of DEK1, covering its structural features, putative regulation, posttranslational modifications, and biological roles. Furthermore, the work delves into potential model organisms and available tools for studying membrane-anchored calpains with unexplored biological roles. Understanding the mechanisms underlying membrane-anchored calpains holds promise for shedding light on fundamental cellular processes such as cell polarization, cell fate determination, and morphogenesis, extending beyond the realm of plants. This study serves as a valuable resource for researchers seeking to unravel the intricacies of membrane-anchored calpains and their broader implications in cellular biology. This work was supported by the Slovak Research and Development Agency grant APVV-21-0227

P-35-109
Transformation of sequence-structure properties of protein tandem repeats induced by shifted reading frames

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A minor variation at the nucleotide level can cause a shift in reading frame, which often results in significant changes in protein sequences. Alterations in repetitive regions of proteins, which are prevalent and play a pivotal role in biological functions, are especially significant. These regions are often characterized by a biased amino acid composition that leads to a high concentration of specific physicochemical properties. These properties may be completely altered due to frameshifting. Moreover, certain tandem repeats at the nucleotide level are known as "hotspots" for frameshifting events. In our research, we generated frameshifted sequences from reference proteins across 50 proteomes from both eukaryotes and prokaryotes. We then performed a detailed, large-scale comparative study of the reference and frameshifted sequences within tandem repeats, uncovering several captivating correlations. For instance, we found that frameshifted sequences tend to have a shorter repeat unit, which correlates with an increase in hydrophobicity, a decrease in the number of intrinsically disordered regions, and a higher tendency to aggregate compared to the reference sequences. Additionally, frameshifted tandem repeat sequences showed a significantly higher percentage of arginine. Our findings shed light on the dual consequences of frameshifts, namely the disruption of protein functionality and the facilitation of genetic variability, which is essential for evolutionary selection.

P-35-110
PDZ2-functionalized-nanoparticles for targeted inhibition of SARS-CoV-2: a promising therapeutic approach

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The COVID-19 pandemic, triggered by SARS-CoV-2, has underscored the pressing need for innovative antiviral strategies in the battle against viral infections. Although the spike protein has received a lot of attention as a viable target for global vaccination approach, other proteins have also been carefully considered. Among them, the once-underappreciated SARS-CoV-2 E-protein is now recognized as a key component in viral pathogenesis, coordinating the budding, assembly, and spread of the virus. A PDZ-binding motif (PBM), essential to SARS-CoV-2 pathogenicity, is present in the C-terminus of the E-protein. This motif is identified and bound by the PDZ2 domain of the human tight junction protein ZO-1. Many studies have been conducted on the complex interaction between the C-terminal portion of SARS-CoV-2 E-protein and the PDZ2 domain of ZO-1. As result, we developed an innovative therapeutic approach using PLGA-based nanoparticles with PDZ2-ZO1 external functionalization as intracellular baits, targeting the E-protein in infected cells. Unlike traditional methods, our approach disrupts virus assembly, replication, and spread by leveraging the E protein-PDZ2 interaction. Additionally, ZO1's PDZ2 domain exhibits versatility by interacting not only with SARS-CoV-2 but also with other coronaviruses like MERS-CoV and SARS-CoV through their Envelope protein. This interaction, acting like a potent "sponge," holds promise for novel antiviral treatments. In vitro studies on cellular models confirmed the significant reduction of SARS-CoV-2 virulence, emphasizing the therapeutic potential of targeting viral-host interactions. *The authors marked with an asterisk equally contributed to the work.

P-35-111
Is chemical composition a reliable index of the nutritional value of foods?

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To raise consumer awareness of the importance of limiting unhealthy foods, the nutrition label has become mandatory and other evaluations such as the nutri-score are underway. The nutrition label and the nutri-score are based on the chemical composition of the food and do not consider bioaccessibility, i.e., the percentage of the nutrient that is released from the food matrix during digestion and becomes available for absorption. The food matrix significantly influences bioaccessibility – and consequently bioavailability of nutrients. Therefore, the chemical composition of foods could be similar but their nutritional value different. To evaluate how the food matrix modulates the bioaccessibility of lipids and starch, some high-fat foods with different degrees of processing and some starchy foods with different dietary fiber contents were examined. The foods were digested in vitro according to the INFOGEST protocol. To evaluate starch digestion, the protocol was modified to include amyloglucosidase, a brush border enzyme. Digestion gave rise to a soluble fraction (SF) containing bioaccessible nutrients. Lipids were extracted from SF, weighed, methyl-esterified, and the resulting fatty acid methyl esters quantified by gas chromatography. Free glucose was quantified spectrophotometrically. The bioaccessibility of total lipids, fatty acids and glucose was extremely different in the examined foods. Furthermore, the fatty acid profile in SF did not fully reflect that of undigested food. The amount of lipids/fatty acids/glucose released during digestion depends on the food matrix and processing. This highlights that the nutrition label does not reflect the content of nutrients which are actually available for absorption and can therefore exert any effect within the body. This must be carefully considered not only in calculating the energy provided by a food but also in evaluating its possible effects on health. Acknowledgment: Project code PE00000003 – ONFOODS. *The authors marked with an asterisk equally contributed to the work.

P-35-112
Seeding of primary fibroblasts and microvascular fragments into PLLA scaffolds for the treatment of chronic wounds

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In the wound-healing process, internal or external factors can interfere with one or more stages, leading to the formation of a chronic wound, commonly known as an ulcer. Chronic wounds are a severe health problem, and current medical treatments are often inefficient. The work aims to develop a regenerative medicine approach by using PLLA (poly-L-lactic acid) scaffolds supplemented with species-specific dermal fibroblasts and microvascular fragments in order to promote and improve the healing rate. Primary fibroblasts were isolated from Wistar rat dermis and characterized through immunostaining. These cells were then seeded onto PLLA scaffolds treated with type I collagen from rat tail. The proliferation rate was assessed through viability assays, and cell morphology was observed via scanning electron microscopy (SEM), revealing cell adhesion and proliferation along the scaffold's surface. Simultaneously, an extraction protocol was optimized to isolate microvascular fragments from adipose tissue (ad-MVFs); the resulting ad-MVFs were analyzed via SEM and cultured in a 3D collagen-gel system to maintain their vessel structure. Ongoing studies are focused on co-culturing fibroblasts and microvascular fragments onto the scaffolds. In this context, fibroblasts would promote the healing rate by releasing growth factors and extracellular matrix components, while ad-MVFs, interconnecting with the circulatory system of the host, will ensure a supply of oxygen and nutrients. This construct will be tested *in vivo* for treating skin and diabetic ulcers, introducing a new approach in wound care.

P-35-113
Vanillin flavor in e-liquids: is it dangerous for endothelial cells? The Replica Project

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The e-cigarette seems to be a safer alternative to tobacco combustible cigarette and a valuable tool to reduce the tobacco harm caused by smoke. It is a battery-powered device that simulates smoking by heating an e-liquid to produce an aerosol that the user inhales. E-liquid typically contains a combination of propylene glycol (PG), vegetable glycerin (VG), nicotine, and chemical flavors. Flavors are generally recognized as safe (GRAS) for food applications, but little is known about their application in vaping. In this study the international Replica group investigated the effects of vanillin, one of the most widespread flavors in vaping, on the endothelium when vaporized from an e-cigarette. We vaped e-liquids containing PG, VG, and vanillin in two settings,

regular (1 Ohm) and sub-ohm (0.3 Ohm), to verify how vanillin behaves towards aortic endothelial cells, to replicate a study conducted by Fetterman and colleagues in 2018 which shown endothelial dysfunction induced by vanillin in e-liquids. We evaluated cytotoxicity, oxidative stress, and nitric oxide bioavailability, by covering some gaps reported in the original study by Fetterman. We observed a certain harmful effect mostly attributable to ethanol, mistakenly used to dilute vanillin in the original work by Fetterman and colleagues, but no harmful effect either on the viability of the cells or on their ability to produce nitric oxide. Even a certain protective effect against oxidative stress for vanillin seems to be observed. Our results confirm the endothelial cell dysfunction observed in the original paper but clarify that these observations are attributable to the ethanol and not to vanillin, which instead, in a more appropriate and realistic model of exposure, seems to exert a protective effect, particularly in a regular setting of the e-cigarette, compared to the most extreme setting (sub-ohm).

P-35-114
Potential kidney aging delay effect of COD in old mice

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Although the aging process itself does not cause kidney disease, aging kidneys undergo complex changes that predispose them to kidney pathology. Cornus officinalis DW Extract (COD), a traditional medicine in Korea, is used for the treatment of lowering blood glucose, anti-inflammatory, antioxidant, anti-apoptotic properties, and kidney conditions, including renal fibrosis and diabetic nephropathy. The study aimed to explore the potential anti-aging effects of COD on kidneys. It divided an aged mouse model into five groups: young (3 months), old (24 months), senolytic drugs (PC, dasatinib 0.5 mg/kg/day + quercetin 5 mg/kg/day), COD 100 (100 mg/kg/day), and COD 300 (300 mg/kg/day). Aging processes were investigated in C57BL/6J mice aged 18-24 months, equivalent to 56-69 human years, administered saline (Old group), PC, and COD orally for 6 months. In this study, COD clearly increased the decline in activity that occurs with aging. However, grip strength increased in COD compared to the old group, but it was not significant. Furthermore, we have demonstrated that COD increases urine volume, osmolarity, and creatinine clearance. However, it appears that COD does not have a significant impact on electrolyte concentrations. Additionally, the concentration of Klotho, known as an anti-aging factor, significantly increased at COD 300, leading us to supposed that COD may be associated with the inhibition of aging. We identified 100 genes that significantly changed due to aging in the kidneys of aged mice, and among them, we discovered 51 target genes that were altered by COD. We assessed the expression of senescence target genes (Ndufb2, Cycs, Dld, Pdss1, CoQ10, and Atp5e) to evaluate the effect of COD on the anti-renal aging function in aged mice, and found that it was significantly suppressed. The above results indicate that COD may play a protective role in kidney aging. *The authors marked with an asterisk equally contributed to the work.

P-35-115
Secondary structure of subgenomic RNA N as target for inhibition of SARS-CoV-2 replication

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RNA viruses are dangerous threats to human and animal health. Several of them are significantly hazardous because of their high epidemic or pandemic potential, e.g., influenza, SARS (including SARS-CoV-2), ebola, zika, and MERS viruses. Constant changes occurring within the genomes of RNA viruses, including the SARS virus, lead to the emergence of new virus variants and strains. Hence, a better understanding of the SARS-CoV-2 virus biology could help develop more effective drugs against COVID-19. The presented research aims to better understand the role of RNA structure in the replication process of SARS-CoV-2 and to use this knowledge to design potential inhibitory tools. In our study, we utilized selective 2'-hydroxyl acylation analyzed by a primer extension (SHAPE) method and chemical mapping to generate a secondary structure model of SARS-CoV-2 subgenomic RNA (sgRNA) N *in vitro*. Based on the achieved RNA structure, we designed and synthesized a group of modified antisense oligonucleotides and small interfering RNAs targeting the sequences within the coding region of SARS-CoV-2 sgRNA N. To test their inhibitory effect, we generated an N gene plasmid system containing green fluorescent protein (eGFP) that allowed us to validate the oligonucleotide antiviral effect in the HEK293T cell line. The level of plasmid system inhibition upon the addition of oligonucleotides was measured by flow cytometry. Our research demonstrated that the most prominent inhibitory effect was obtained for four of ten tested oligonucleotides. Moreover, one of them showed a very high, 90%, reduction of fluorescence level indicating the inhibition of virus replication. Overall, we suggest that presented here new, modified oligonucleotides designed based on the revealed secondary structure of the sgRNA N can be potential therapeutic tools against SARS-CoV-2 virus. *The authors marked with an asterisk equally contributed to the work.

Molecular basis of diseases – Part B

P-36-001
Conformational investigations on a recombinant lipocalin allergen: the route toward molecular-based allergy diagnosis

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Mus m 1 lipocalin is the cause of allergic disease in sensitized individuals. Its structure is a β-barrel and includes a disulfide bond and a free Cys placed in the α-helix that runs alongside the barrel. Diagnosis of Mus m 1 allergy is based on serum IgE testing and relies on a mouse epithelial extract as antigen source.

Given the heterogeneous and variable composition of the extract, we investigated the potential of recombinant Mus m 1 (rMus m 1) as a single allergen component in serum IgE testing. A preliminary prediction of conformational epitopes of rMus m 1 3D structure suggested that rMus m 1 is endowed with specific antigenicity, a prerequisite for allergenicity and specific IgE-binding ability. We developed an indirect ELISA assay based on the rMus m 1 component as antigen. The diagnostic assay performed with reasonable diagnostic accuracy (AUC = 0.87) compared to a routine diagnostic test that exploits the native extract. These findings revealed that rMus m 1 is a valuable tool for studying the fine epitope mapping of specific IgE reactivity. However, the modest resistance of rMus m 1 to aggregation makes it unsuitable for biomedical preparations since its free Cys is recognized as responsible for thiol/disulphide exchange, an event that favors protein misfold/aggregation. Molecular dynamics simulations, focusing on the hydrophobic interface between the β-barrel and the α-helix motifs, confirmed that the free Cys substitution with Ala produces a stabilizing effect by reducing the α-helix movement. HINT (Hydropathic INTeractions) program was used to evaluate the conformational variation of this mutant, unveiling that Ala is involved in a hydrophobic cluster generated by interface residues. Since the α-helix conformational movement could be responsible for rMus m 1 propensity to aggregate, the mutant might be an attractive lead molecule for the development of a diagnostic kit.

P-36-002
Functional analysis of a PTPMT1 splice-site mutation in patient-derived cells: A novel mitochondrial disease gene

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Mitochondrial disorders represent a considerable portion of all rare neurogenetic disorders. Recently, our team identified a novel splice site variant [(NM_175732.3): c.255G>C (p.Gln85His)] in the PTPMT1 (Protein Tyrosine Phosphatase Mitochondrial 1) gene through trio whole exome sequencing (WES) analysis in a pediatric patient with neurological symptoms. PTPMT1 is a mitochondrial lipid and protein phosphatase localized in the mitochondrial inner membrane and has crucial roles in cardiolipin synthesis and ATP production. Herein, for the functional characterization of the novel disease gene, mitochondrial energy metabolism, organization of the oxidative phosphorylation (OXPHOS) complexes within mitochondria, and mitochondrial network morphology were analyzed in the patient dermal fibroblasts. Functional studies showed reduced mitochondrial respiration and disrupted mitochondrial network morphology in patient dermal fibroblasts compared to healthy controls. To further characterize the molecular mechanisms underlying the PTPMT1-related disease and to develop a therapeutic approach, we performed omics analysis (lipidomics, proteomics, and transcriptomics) and identified repurposable drug candidates. Our team tested a drug candidate identified based on the lipidomics data for the potential rescue of defective mitochondrial energy metabolism and network morphology in patient cells and showed promising results for its efficacy. Although each mitochondrial disease is rare, it is important to provide molecular diagnosis and better understand the disease mechanisms to develop targeted therapies in similar groups with converging pathways. This work was supported by the Newton-Katip Çelebi Fund (#216S771) and the Scientific and Technological Research Council of Türkiye (TUBITAK) (#220S734).

P-36-003
Putative RNA methyltransferase NSUN7 orchestrates assembly of sperm tail accessory structures in mice

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NSUN7 belongs to the NOP2/Sun domain protein family, where all members catalyze the methylation of the 5th position of cytosine. *NSUN7* is exclusively expressed in spermatogenic cells and mutations in it lead to male sterility in humans and mice [previously published in: Harris T et al. (2007) Biol of Rep 77, 376–382)]. We created a *Nsun7* knockout mouse line ($Nsun7^{-/-}$) and a mutant line with NSUN7 bearing single amino acid substitution of one of the catalytic cysteines with alanine ($Nsun7^{C\rightarrow A}$). We observed a significant decrease of motility and disturbances in the trajectory of $Nsun7^{-/-}$ sperm, while $Nsun7^{C\rightarrow A}$ mice exhibit a normal phenotype. On the ultrastructure of $Nsun7^{-/-}$ spermatozoa we found that the attachment pattern of fibrous sheath longitudinal columns (LC) to the axonemal microtubule doublets had changed. Normally, the presence of LC attached to the doublets 3 and 8 should restrict flagella bending in a plane passing through the central pair of microtubules. The normal phenotype of $Nsun7^{C\rightarrow A}$ mouse may suggest either the change in the mechanism of methylation reaction or the fact that methyltransferase activity is dispensable for spermiogenesis. In order to get an understanding of molecular pathways associated with the function of NSUN7, we performed a comparative proteomic analysis of testis from wild-type and knockout $Nsun7^{-/-}$ mice. We identified 39 deferential proteins, most of which are associated with cilia development and endoplasmic reticulum. The most interesting observation was the downregulation of the ubiquitin ligase RNF151 in $Nsun7^{-/-}$ mice. The loss of function mutations in a gene of the other ubiquitin ligase *Ube2b* results in the similar phenotype as we observe for $Nsun7^{-/-}$ mice [previously published in: Escalier D (2003) Biol of Rep 69, 373–378]. Taken together, these and previous findings suggest the existence of a mechanism that ensures the correct positioning of the longitudinal columns of spermatozoa.

P-36-004
Dysregulations of microtubule plus end proteins in an *in vitro* spinal muscular atrophy model

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Spinal muscular atrophy (SMA) is a rare neurodegenerative disease, which is caused by deficiency of survival motor neuron (SMN) protein. Absence of SMN causes alterations in cytoskeletal elements, including microtubules. Microtubules are the most dynamic filaments of the cytoskeleton and microtubule-associated proteins (MAPs) regulate its structure and functions. We previously demonstrated reduced microtubule stability together with the altered levels of several MAPs [1]. Therefore, in this study, we created an interaction network of SMN with MAPs using bioinformatic tools. Protein interaction network was constructed by retrieving the list of all MAPs, and both direct and indirect interactions with SMN were collected. Among all, $p150^{Glued}$ was visualized as a hub protein in the network, while APC was shown to connect SMN complex with MAPs. Since both proteins bind to microtubule plus ends, they were selected for *in vitro* analysis, and protein levels were analyzed in SMN knock-down motor neuron-like NSC34 cells. Western blot studies demonstrated a significant upregulation in $p150^{Glued}$ protein level, and APC was found to be downregulated in SMN deficient cells compared to controls. Consistently, immunofluorescence stainings and quantitative image analysis showed that comet-like dots formed by $p150^{Glued}$ and APC were significantly altered in the proximal part of the neurites in the absence of SMN. $p150^{Glued}$ is a subunit of dynactin complex, whereas APC is involved in *beta-catenin* destruction, therefore our results indicate that their dysregulations could have an impact on both microtubule dynamics, retrograde transport, and Wnt signaling pathway. Studies are ongoing to understand molecular mechanisms of microtubule dysregulations in SMA pathomechanisms. This study is supported by The Scientific and Technological Research Council of Turkey (TÜBİTAK, Project number;121S884). Previously published in: [1] Zobaroğlu Özer P et al. (2022) Mol Cell Neurosci 120, 103725.

P-36-005
Type 2 diabetes risk alleles in peptidyl-glycine alpha-amidating monooxygenase influence GLP-1 levels and response to GLP-1 receptor agonists

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Patients with type 2 diabetes vary in their response to currently available therapeutic agents (including GLP-1 receptor agonists) leading to suboptimal glycemic control and increased risk of complications. We show that human carriers of hypomorphic T2D-risk alleles in the gene encoding peptidyl-glycine alpha-amidating monooxygenase (PAM), as well as Pam-knockout mice, display increased resistance to GLP-1 *in vivo*. Pam inactivation in mice leads to impaired gastric emptying: this persists during GLP-1R agonist treatment and is rescued when GLP-1R activity is antagonized, indicating resistance to GLP-1's gastric slowing properties. Meta-analysis of human data from studies examining GLP-1R agonist response (including RCTs) reveals a relative loss of 44% and 20% of glucose lowering (measured by glycated hemoglobin) in individuals with hypomorphic PAM alleles p.S539W and p.D536G treated with GLP-1R agonist. We show here for the first time that genetic variation in PAM has effects on incretin signaling that alters response to medication used commonly for treatment of T2D.

P-36-006
The oncogenic role and clinical significance of RNA N6-methyladenosine regulator METTL3 and IGF2BP3 in hepatocellular carcinoma

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RNA N6-Methyladenosine (m6A) methyltransferase METTL3 plays a major function on m6A installation process to control the half-life of mRNA. In addition, IGF2BP3 acted as a m6A-binding protein has been reported to be an essential m6A reader for recognizing numerous METTL3-methylated m6A modification mRNAs. In the current study, we seek to investigate the oncogenic role and clinical value of m6A writer METTL3 and m6A reader IGF2BP3 in hepatocellular carcinoma (HCC). First, we find that knockdown of METTL3 results in impaired cell growth of HepG2. Consistently, cell formation is also markedly declined in HepG2 cells by lentivirus expressing METTL3 sgRNA-dependent CRISPR/Cas9 construct. Next, we analyze clinical significance using GEPIA and Kaplan Meier plotter web-based tool database, and find that METTL3 mRNA is significantly upregulated in liver tumor tissue compared to their counterpart normal tissue and its high expression correlates with poor overall survival in HCC patients. Expectedly, IGF2BP3 also promotes tumor growth, upregulates in liver tumor tissue, and associates with worse overall survival in patients with HCC. Furthermore, both of high-levels METTL3 and of IGF2BP3 significantly indicates poor correlation in overall survival, suggesting that clinical value is potentially observed between METTL3 and IGF2BP3. To detect the transcriptome-wide regulation of m6A RNA modification by METTL3, we conduct MeRIP assay using anti-m6A antibodies in METTL3-depleted HepG2 cells, followed by RNA-seq analysis. The results show that m6A peak primarily enriches in 3'UTR near the stop codon, and METTL3 depletion shows a decrease in abundance of m6A peak distribution in 3'UTR compared to control. Overall, RNA m6A regulator METTL3 and IGF2BP3 exhibit the oncogenic role and clinical significance in HCC, and further investigation will be warranted to identify potential mRNA targets coincidentally regulated by METTL3 and IGF2BP3 for elucidating the pathogenesis of HCC.

P-36-007
Uncovering a novel functional interaction between adult hepatic progenitor cells, inflammation and EGFR signaling during bile acids- induced injury

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During chronic cholestatic damage, bile acids accumulate in the liver causing cell death. In this context, expansion of hepatic progenitor cells (HPCs) is observed as part of the so-called ductular reaction. Epidermal growth factor receptor (EGFR) signalling plays a relevant role during liver injury and as regulator of HPCs. Based on this, the aim of this study was to characterize the HPC response to bile acids and clarify whether the HPCs have an active role during the cholestasis-induced inflammatory response. Furthermore, the relevance of EGFR signaling in this scenario was evaluated. Here, we report that some bile acids trigger a cytotoxic effect in HPC. EGF impairs bile acids-induced cytotoxicity while synergizes with bile acids to promote an inflammatory response. Thus, co-treatment of HPC with bile acids and EGF synergistically activate inflammatory signaling and NLRP3 activation whereas EGFR inhibition blocks bile acids-induced inflammatory signaling. Aiming at understanding the impact of this HPC specific response on the liver microenvironment we run a proteomic analysis of HPC secretome. Data show an enrichment in immune and TGF-β regulators, ECM components and remodeling proteins in HPC secretome. In line with this, HPC-derived conditioned medium significantly impacts on the hepatic stellate cell (HSC) activation and/or proliferation, as well as on macrophage M1-like polarization. Strikingly, EGF and bile acids co-treatment leads to profound changes in the secretome composition, illustrated by an abolishment of HSC activating effect and by promoting macrophage M2-like polarization. Collectively, we provide new specific mechanisms behind HPC regulatory action during cholestatic liver injury, with an active role in cellular interactome and inflammatory response regulation. Moreover, findings prove a key contribution for EGFR signaling jointly with bile acids in HPC-mediated actions.

P-36-008
Hepatoprotective effect of GDF11 in acute liver injury

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Drug-caused hepatotoxicity, viral hepatitis, alcohol consumption, and other gastrointestinal tract comorbidities contribute to liver injury. Current scientific data prove that transforming growth factor-β (TGF-β) superfamily plays a significant role in hepatitis development. The novel member of the TGF-β superfamily, Growth differentiation factor 11 (GDF11), is a key molecule in regeneration and rejuvenation. It is assumed that GDF11 modulates inflammation and can affect liver conditions. The aim of our study was to determine the role of GDF11 in an animal model of acute liver injury. Liver injury was induced by D-galactosamine (D-GalN) sensitization (0.7 mg/g) and TNF-α (0.1 μg/per mouse) intraperitoneal injection (i.p.) in C57/B6 mice. After 6 hours, mice were sacrificed; liver samples and serum were collected. Firstly, we validated the animal model of liver injury and assessed the expression of *Gdf11* in the liver. Next, we injected GDF11 i.p. at the doses of 0.1 and 0.6 mg/kg 15 minutes prior to the D-GalN. We found that TNF-α at the dose of 0.1 μg/per mouse is sufficient to induce liver injury and significantly increases Gdf11 expression. GDF11 administered at the dose of 0.1 mg/kg caused a decrease in the expression of inflammatory molecules: *Il1b*, *Il4*, *Il6*, *Il18*, *Nos2*, and *Ptgs2*, but did not affect *Tnfa* and *Il10* expression at mRNA level. We stained liver sections using hematoxylin and eosin. We observed that necrotic and inflammatory lesions are reversed after injection of GDF11 at the dose of 0.6 mg/kg. The role of GDF11 in hepatic inflammation seems to be noteworthy in the context of liver regeneration and cellular signaling.

P-36-009
Proteomic characterization of extracellular vesicles released by the human accidental parasite, *Anisakis simplex*

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Anisakis simplex is a parasitic nematode of marine organisms. Humans can be an accidental host for this species, and the disease caused by the parasite is called anisakiasis. The secretion of EVs as signaling molecules by parasitic nematodes has been poorly studied. This prompted us to characterize the cargo of EVs from *A. simplex* (Anis-EVs). The Anis-EVs were isolated from the postculture medium by ultracentrifugation. The size distribution and number of Anis-EVs were analyzed using a NanoSight 300 device. The morphology of Anis-EVs was analyzed by transmission electron microscopy (TEM). Proteins were extracted from Anis-EVs (n = 6) and analyzed by nLC-MS/MS using a Proxeon EASY-nLC II liquid chromatography system coupled to an LTQ-Orbitrap Elite mass spectrometer (Thermo Fisher Scientific, USA). All acquired MS/MS spectra were analyzed with SEQUEST-HT (Proteome Discoverer 2.4 package) against a reference proteome of *A. simplex* (ID: UP000267096). The NTA analysis revealed a population of particles smaller than 150 nm. The mean size of the Anis-EVs was 85.3 nm (2.22×10^{12} particles/mL; 98.7% of all particles/mL). TEM examination also revealed uniform particles with recognizable lipid bilayer membranes, circular cross-sections, and a characteristic "cup shape". Proteomic characterization of Anis-EVs revealed that 23 proteins were enriched in the isolated vesicles (e.g. Argonaute-1, RNA-dependent RNA polymerase, nematode eukaryotic translation initiation factor 3). The identified proteins play a central role in RNA silencing processes, which makes them interesting from the perspective of parasite-host interactions. Funding: This work was funded by the National Science Centre of Poland, grant no. 2019/33/N/NZ6/01353, and 2018/31/B/NZ9/01683. R. S. also received a scholarship from the UE, grant no. POWR.03.05.00-00-Z310/17. This work was also supported by the GAIN-Xunta de Galicia Project (IN607D2017/01) and the Spanish AEI/EU-FEDER PID2019-103845RB-C21 project.

P-36-010
Glomerular podocytes accumulate glycogen under high glucose conditions

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Hyperglycemia plays a pivotal role in initiating the pathological process underlying the development of diabetic kidney disease (DKD). The main energy substrate for podocytes is glucose, with anaerobic glycolysis converting glucose to pyruvate and then to lactate. Increasing evidence suggests that in diabetes, high glucose concentrations (HG) dysregulate insulin signaling and significantly impact podocyte metabolism and function. The aim of this study was to examine effects of high glucose concentrations on glycogen metabolism in podocytes. Cells were exposed to normal (NG, 5.6 mM) or high glucose medium (HG, 30 mM) for indicated periods. The quantitative PCR (qPCR) analyses were performed to determine the genes expression. Immunodetection methods were used to detect the proteins expression. Colorimetric methods were employed to assess glycogen content. TEM analysis was conducted to visualize glycogen granules in podocytes. Glucose accumulation was measured using 2-deoxy-(1,2-^{3}H)-D-glucose. This work was supported by grant from the National Science Centre (2021/41/B/NZ5/02611). Our results revealed increased glucose accumulation in podocytes exposed to HG milieu. We demonstrated the expression of genes involved in glycogen metabolism, including glycogen synthase 1 (GYS1), glycogenin 1 (GYG1), and the liver and brain isoforms of glycogen phosphorylase (PYGL and PYGB, respectively), at both the mRNA and protein levels. We also observed that the glycogen content in podocytes exposed to HG conditions increased with prolonged exposure time. Additionally, HG led to increased expression of genes encoding GYS1 and GYG1, accompanied by a concomitant decrease in the expression level of PYGL. Our results suggest that podocytes accumulate excess glucose in the form of glycogen. While the exact role of glycogen accumulation in podocyte dysfunction and injury is not yet fully understood, it

is important to note that it cannot be excluded as a potential contributing factor.

P-36-011
Glycolysis inhibition regulates mammalian heart regeneration

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Heart failure is a leading cause of death globally, significantly contributing to healthcare costs worldwide. A major cause of systolic heart failure is the poor regenerative capacity of adult mammalian hearts following injury, the most common being infarction. We previously discovered that neonatal mice have the ability to regenerate their injured hearts, a capability that is lost seven days after birth. A major event during the maturation of cardiomyocytes from neonatal to adult stages is the metabolic shift in energy utilization. While embryonic and neonatal cardiomyocytes primarily generate energy through glycolysis, adult cardiomyocytes rely on fatty acid oxidation for energy production. Thus, our research aimed to investigate the regulation of cardiac regeneration in neonatal mice through the inhibition of glycolysis. To investigate the role of 6-phosphofructo-2-kinase (PFK-2) inhibition in neonatal heart regeneration, we utilized neonatal mice at postnatal day 1 (P1) for myocardial infarction (MI) surgery. Mice were then treated with a selective PFK-2 inhibitor after the MI for a week. We performed various analyses, including immunohistochemistry for cardiomyocyte proliferation and trichrome staining to assess heart regeneration. Our results showed that inhibition of PFK-2 post-MI inhibited cardiomyocyte proliferation, as measured by the mitosis marker phosphohistone 3 (pH3). Furthermore, PFK-2 inhibition led to scar formation and incomplete regeneration, as revealed by trichrome staining. These findings suggest that PFK-2 plays an important role in regulating cardiac regenerative capacity, which would be an important therapeutic target for human heart failure.

P-36-012
Investigating the protective role of CD44 and microRNA-146a in tendinopathic tenocytes: a signaling axis regulating apoptosis

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Tendinopathy refers to the clinical condition characterized by pain in a tendon accompanied by significant dysfunction, with preference given to pathological terms such as tendinitis and tendinosis. It is influenced by multiple factors, including chronic inflammation, senescence, and apoptosis. CD44 serves as a principal cell-surface receptor for hyaluronan, a constituent of the extracellular matrix. Previous studies have shown that inhibition of the CD44 signaling pathway induces apoptosis, inflammation, and expression of matrix metalloproteinases in interleukin-1β-stimulated tendinopathic tenocytes. Furthermore, microRNA-146a (miR-146a) blocks IL-1β-induced senescence in tendinopathic tenocytes by targeting IL-1β receptor-associated kinase 4 (IRAK-4) and TNF receptor-associated factor 6 (TRAF6), thus inhibiting NF-κB activity. Therefore, the aim of this study is to investigate the signaling axis between CD44 and miR-146a in tendinopathic tenocytes, which finely regulates apoptosis. Overexpression of CD44 and miR-146a in rat primary tendinopathic tenocytes was achieved through lentiviral vector-mediated transfer of CD44 cDNA (LVCD44) and precursor miR-146a (LVmiR-146a). TUNEL staining was performed to evaluate apoptosis. Additionally, LY294002, a PI3K inhibitor, and OX-50, an antagonizing antibody that blocks CD44 activity, were used to examine the CD44-AKT-miR-146a signaling axis in tendinopathic tenocytes through immunoblotting. Tendinopathic tenocyte transfectants overexpressing CD44 and miR-146a exhibited lower apoptotic cell numbers compared to transfectants treated with control vectors. Furthermore, the CD44-AKT-miR-146a signaling axis was shown to alleviate apoptosis in IL-1β-stimulated tendinopathic tenocytes by inhibiting SMAD4 expression. In this study, we demonstrate that overexpression of CD44 and miR-146a protects tendinopathic tenocytes from apoptosis through the CD44/AKT/miR-146a/SMAD4 signaling pathway. *The authors marked with an asterisk equally contributed to the work.

P-36-013
Renal ischemia and reperfusion alter cardiolipin metabolism

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Cardiolipins are unique phospholipids that are essential for mitochondrial structural and functional integrity. Ischemia/reperfusion can induce cardiolipin oxidation and quantitative alterations but also pathological remodeling, which further contributes to tissue damage. Different organs and tissues have specific cardiolipin fatty acid composition that, if altered, may cause mitochondrial dysfunction. There is a lack of evidence about renal cardiolipins, especially their alterations during ischemia/reperfusion. We used two models of renal ischemia/reperfusion [*in vivo* using adult male Wistar rats and *in vitro* using immortalized human renal proximal tubule epithelial cells (RPTEC)] to investigate cardiolipin alterations in the mitochondria. For Wistar rats: 30-, 40- and 60-minute *in vivo* renal ischemia was induced, followed by 30-minute reperfusion; renal mitochondria were isolated, and lipids were extracted. For RPTEC: *in vitro* 24 h hypoxia was induced (2% O_2) followed by 24 h reoxygenation, then lipids were extracted. Lipids were used for qualitative and quantitative cardiolipin analysis using UPLC-MS/MS. It was found that ischemia/reperfusion decreased cardiolipin levels, however, after 60 min ischemia/reperfusion there was a tendency for cardiolipins to increase, compared to 30 and 40 min ischemia/reperfusion. A more significant increase was observed in RPTEC after hypoxia/reoxygenation. Gene expression assay of cardiolipin synthesis and remodeling enzymes showed a significant increase in taffazin expression by 1,8-fold after 60 min ischemia without reperfusion compared to control levels in rat kidneys, while in RPTEC cardiolipin synthase and LCLAT1 expression was significantly increased by 2.9- and 2.7-fold after hypoxia respectively, and remained increased after reoxygenation

by 2.8- and 3.7-fold, respectively, compared to control levels. Our results show that ischemia/hypoxia initiates preservation of cardiolipin through remodeling and/or resynthesis mechanisms. *The authors marked with an asterisk equally contributed to the work.

P-36-014
UVB-mediated cytotoxicity and premature senescence of dermal fibroblasts: The protective role of the JNKs/ATM-p53 loop and of Cockayne syndrome B protein

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Although UVB radiation is mainly absorbed by the epidermis, approximately 5-10% of its photons reach the upper part of the dermis, leading enclosed dermal fibroblasts to cell death and premature senescence. We have recently described the major biochemical pathways activated in human dermal fibroblasts (HDFs) towards their protection against UVB-induced cell death. Our findings provide evidence that the main viability-regulating UVB-triggered biochemical pathways act synergistically towards the protection of HDFs, with EGFR/Akt and Nrf2 serving as auxiliary anti-apoptotic machineries, while JNKs/ATM-p53 activation and interplay being overriding and indispensable for the perpetuation of cellular defense and the maintenance of cell viability. On the other hand, repetitive exposures to non-cytotoxic doses of UVB radiation resulted in the emergence of a mixed population of senescent and resistant to UVB-induced senescence HDFs. RNA-seq analysis revealed that resistant HDFs constitute a separate population from both young and senescent cells that tolerate significantly better their treatment with a high dose of UVB irradiation. ERCC6 encoding Cockayne syndrome B (CSB) protein was shown to be exclusively up-regulated in resistant cells, while its siRNA-mediated loss-of-expression abolished their UVB-tolerance in accordance to the vulnerability exhibited by a dermal fibroblast cell strain from a Cockayne syndrome patient. Furthermore, resistant cells express a catabolic and inflammatory phenotype (similar but not identical to that of senescent cells) and promote the growth of tumors formed by A431 epidermoid carcinoma cells when co-injected subcutaneously in the back of SCID mice (although to a lesser extent in comparison to senescent cells). Accordingly, these UVB-induced resistant cells, although they avoid senescence, they may still negatively affect skin homeostasis.

P-36-015
DCLK1 mediated TGF-β-stimulated CTGF expression and differentiation of human lung fibroblasts through Smad3 activation pathway

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Airway fibrosis is a common pathological feature in patients with severe asthma. Literature suggests that TGF-β and CTGF are involved in the process of pulmonary fibrosis. Previous studies showed that increased expression of doublecortin-like kinase 1 (DCLK1) in lung tissue samples from idiopathic pulmonary fibrosis patients. Additionally, previous reports indicated the involvement of the TGF-β/Smad3 pathway in pulmonary fibrosis. However, whether DCLK1 is involved in TGF-β-stimulated CTGF expression and differentiation of human lung fibroblasts through Smad3 activation remains unclear. We found that increased expression and phosphorylation of DCLK1 in lung tissues of ovalbumin (OVA)-induced asthmatic mice. TGF-β induced expression and phosphorylation of DCLK1 in human lung fibroblasts. Moreover, DCLK1siRNA inhibited TGF-β-induced expression of CTGF, fibronectin, collagen I, α-SMA, and Smad phosphorylation in human lung fibroblasts. We also found that TGF-β-induced DCLK1 and Smad3 complex formation, and DCLK1 translocation from the cytosol into the nucleus. Dominant negative of Akt (AktDN) reduced TGF-β-induced DCLK1 phosphorylation and the expressions of CTGF and fibronectin. Furthermore, DCLK1 knockout mice reduced inflammatory cell infiltration in lung tissues by OVA-treated mice. In summary, these results suggest that TGF-β stimulates CTGF expression and differentiation of lung fibroblasts through the Akt/DCLK1/Smad3 pathway. Our findings reveal a potential therapeutic strategy for treating airway fibrosis in severe asthma.

P-36-016
Regulatory role of miR-9-5p disturbed in nucleoli-enriched fractions in protein expression and oncogenic pathways in clear cell renal cell carcinoma

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The prevailing type of kidney cancer, clear cell renal cell carcinoma (ccRCC), exhibits a noteworthy association between changes in nucleolar morphology and the advancement of the tumour. In contrast to healthy renal proximal tubule epithelial cells (RPTEC), aberrant expression of miR-9-5p in the nuclei of ccRCC-derived cell lines (786-0, Caki-1) has been identified in our prior research. This finding underscores the potential role of miR-9-5p in the pathogenesis of ccRCC. Employing synthetic miR-9-5p miRNA mimic transfection in Caki-1 cells, we conducted a proteomic analysis to observe changes in protein levels. Additionally, we assessed cell proliferation using the BrdU assay, viability through the MTT assay, and adhesion in both Caki-1 and 786-0 cell lines after miR-9-5p transfection. The results revealed that this miRNA stimulated proliferation in both cell lines and enhanced the viability of Caki-1 cells, with an increased proportion of cells entering early apoptosis following

transfection. Proteomic analysis after transfection of Caki-1 cells with miR-9-5p showed an upregulation of 69 proteins and downregulation of 71 proteins, engaged in key oncogenic pathways such as ferroptosis, adherens junction, ErbB signalling pathway, and HIF-1 signalling pathways as demonstrated by KEEG analysis. These findings suggest that miR-9-5p plays a significant regulatory role in ccRCC tumorigenesis, affecting cancer cells proliferation, viability, and apoptosis probably through its impact on key oncogenic pathways, underscoring the potential of miR-9-5p as a therapeutic target in ccRCC treatment strategies. Financed by National Science Center, Poland grant 2019/35/B/NZ5/00695.

P-36-017
Finding the IronICProt, an undisclosed infection-induced iron binding protein

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During infection, pathogens try to take control over the host's iron reserves, thus impacting iron homeostasis and leading to iron-related disturbances. In basal conditions, serum iron (SI) is mostly bound to transferrin and stored in ferritin nanocages. However, our data from size-exclusion chromatography indicate that, as the infection progresses, SI of infected mice is partially bound to an unknown protein entity, the IronICProt, other than transferrin. We call this alteration the "iron-shift". We aimed to describe the kinetics of the iron-shift and clarify its effects on immune response during infection. Moreover, we intend to identify, isolate, and characterize the IronICProt. Resorting to our animal model of chronic infection, C57BL/6 or genetically deficient mice for IFNg, TNFa, and CCL2 were intravenously infected with *Mycobacterium avium* (1 to 8 weeks) and serum was collected. Serum proteins were separated using high-performance liquid chromatographies, based on the proteins' size, charge, and hydrophobicity. The iron-shift was observed starting three weeks post-infection in wild-type, IFNg-, and CCL2-deficient mice. Our results suggest an important role of TNFa to trigger the iron-shift during infection, since in TNFa knock-out mice the iron-shift only occurred at four weeks post-infection. After liquid chromatography-mass spectrometry analysis of the iron-rich fractions, we suggest that ferritin and/or haptoglobin might play important roles regarding iron redistribution during infection. The full description of the iron-shift and the IronICProt may allow the identification of important pathways relating immune response and iron biology and inspire new and crucial host-targeted therapeutics. This work was financed by Portuguese national funds through FCT – Fundação para a Ciência e Tecnologia, within the projects EXPL/BIA-BQM/1170/2021 and 2022.03635.PTDC, and PhD fellowship to ÓF.

P-36-018
Endocannabinoids and eicosanoids lipid signaling molecules in chronic and acute inflammatory joint diseases

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Eicosanoids (ES) and endocannabinoids (EC) are two families of signaling lipids involved in inflammatory and immune responses in numerous tissues. ES regulate inflammatory processes among others, while EC are produced in response to specific signals from the external environment. There are few studies regarding their involvement in the onset and progression of chronic and acute inflammatory orthopedic diseases. Involvement of EC in the pathogenesis and progression of osteoarthritis has also been highlighted. However, present studies focus only on the much more known EC as AEA, 2-AG and PEA, neglecting other. Here we present, for the first time, the HPLC-MS/MS quantification of 20 EC and 12 ES molecules belonging to EC and ES families in the synovial fluid of patients undergoing knee arthroplasty aiming at highlighting differences in the involvement of EC and ES in inflammatory or autoimmune pathological conditions. Patients have been recruited at the Orthopedics and Traumatology Operational Unit of L. Sacco Hospital, 11 with autoimmune conditions such as rheumatoid arthritis (RA), and 18 with inflammatory conditions such as osteoarthritis (OA). From the 32 analytes only 5 ES and 10 EC were quantifiable in synovial fluid, and six of these showed statistically significant different in the two groups. In particular the EC 2-arachidonoylglycerol, palmitoyl-glycine and docosaexaenoyl-ethanolamide and the ES 5-hydroxyeicosatetraenoic acid and 15-Hydroxyeicosatetraenoic acid were higher in RA while linolenoyl-ethanolamide was higher in OA. Obtained data suggest a different modulation in ECs and ESs due to inflammatory of autoimmune conditions.

P-36-019
Serpins: novel players in regulation of podocyte immune response

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Podocytes are terminally differentiated cells which constitute the key functional part of the renal filtration barrier. Their injury underlies various kidney diseases associated with proteinuria resulting from morphological alterations, inflammation and podocyte loss. Although podocytes are epithelial cells, they possess some features typical for immune cells. Recently, we showed the presence of neutrophil serine proteases (NSPs; elastase, proteinase 3, cathepsin G) in podocytes and showed their role in cellular response to various immunostimulants. The aim of our next studies was to elucidate the regulation mechanisms of these NSPs, specifically the potential role of serine protease inhibitors, called serpins. We detected and identified three markedly expressed serpins, SerpinA3, SerpinE1 and SerpinB1 in human podocytes. We observed a significant elevation of mRNA expression of SerpinA3 (~50-fold) and SerpinB1 (by 43%) in podocytes

treated with immunostimulant phorbol 12-myristate 13-acetate (PMA; 100 nM, 24 h), whereas SerpinE1 mRNA was decreased by ~50% upon PMA stimulation. Interestingly, protein levels of SerpinA3 and SerpinE1 were diminished by 30% and 50%, respectively, with no changes in SerpinB1 protein level. A secretory (glycosylated) form of SerpinA3 has been detected in cell culture medium and its levels significantly increased upon PMA treatment, which may explain the reduced intracellular quantity of this protein. SerpinA3 has an ability to bind DNA and is found in NETosis-related complexes secreted by immune cells. In podocytes treated with PMA we observed almost 2-fold increase in extracellular DNA levels indicating enhanced cell-free DNA secretion in which process SerpinA3 can be involved. Our findings provide a novel knowledge on the podocyte physiology crucial for understanding their role in mediating the inflammatory response during renal diseases. This work was supported by grant 2021/41/B/NZ4/02797 from the National Science Centre, Poland.

P-36-020
Role of neutrophil serine proteases in mediation of inflammatory response in podocytes

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Podocytes are specialized cells of the glomerular filtration barrier, exhibiting various features typical for other cell types, e.g., muscles, neurons, and also immune cells. The aim of this study was to characterize the immunological properties of podocyte and elucidate potential mechanism of inflammation response in these cells. Firstly, we detected and identified three neutrophil serine proteases (NSPs): proteinase 3 (PR3), neutrophil elastase (NE), cathepsin G (CtsG) in immortalized human podocytes and primary rat podocytes using real-time PCR, western blot (WB) and immunofluorescence. The cells were treated by phorbol 12-myristate 13-acetate (PMA; 100nM, 24 h), which belongs to pathogen associated molecular patterns (PAMPs) and is capable of inducing the immune response. PMA increased NADPH oxidase activity 1,7-fold and reactive oxygen species production 1,5-fold in podocytes, which indicate an elevated oxidative stress associated with immune reaction. PMA treatment caused the increase of PR3 mRNA expression over 2-fold, NE by 48%, and CtsG 2-fold. However, WB analysis revealed a 14% decrease in PR3 level and no effects on NE and CtsG protein levels upon PMA stimulation. Additionally, we observed a decreased activity of PR3 and NE in cell lysates. Because inflammation induces NPSs secretion from neutrophil cells, we verified whether this mechanism can be activated also in podocytes. We detected all three NPSs in culture medium, specifically in the extracellular vesicle (EV) fraction. Moreover, in cell culture medium from podocytes treated with PMA we observed an increase in PR3 and NE activity. For the first time, we demonstrated NSPs presence in podocytes and their involvement in the response to inflammatory stimulation. These processes may play a key role in pathological changes in the course of kidney disease accompanied by inflammation, such as diabetes nephropathy. This work was supported by grant 2021/41/B/NZ4/02797 from the National Science Centre, Poland.

P-36-021
Glomerular podocytes are able to *de novo* synthesis of glucose – true or false?

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Gluconeogenesis (GNG) is a metabolic pathway essential for glucose synthesis from non-carbohydrate substrates, such as lactate. In diabetic patients, renal GNG is stimulated, excessively contributing to whole body glucose production. Podocytes are continuously supplied with energy to fulfill their crucial role in the regulation of filtration barrier permeability. However, podocytes exposed to hyperglycemic conditions are characterized by the energy imbalance, which may affect their metabolism and function. Therefore, we investigated whether podocytes gluconeogenic activity was altered under high glucose conditions. Cells were cultured in normal (NG, 5.6 mM) or high glucose medium (HG, 30 mM) for indicated periods. Real-time PCR analyses were performed to determine genes expression. Immunodetection methods were used to detect proteins expression and their cellular localization. Colorimetric method was employed to assess PEPCK activity. Glucose uptake was measured using 2-deoxy-(1,2-^{3}H)-D-glucose. This work was supported by a grant from the National Science Centre (2021/41/B/NZ5/02611). Our investigation revealed the presence of gluconeogenic enzymes such as pyruvate carboxylase (PC), both isoforms of phosphoenolpyruvate carboxykinase (mitochondrial: PEPCK-M and cytosolic: PEPCK-C), fructose 1,6-bisphosphatase (FBPase) and glucose 6-phosphatase (G6Pase) at both the mRNA and protein levels. We have also demonstrated that PEPCK-C activity constituted 40% of total PEPCK activity. Under HG conditions, the total PEPCK activity was altered in podocytes. These results suggest that podocytes are able to perform gluconeogenesis, and HG-induced alterations of GNG could contribute to podocyte disfunction and impairment of renal filtration barrier.

P-36-022
The modulatory role of high glucose and lipopolysaccharide on cystatin C expression and secretion in human podocytes

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Diabetic-related hyperglycemia and inflammation are the foremost causes of damage to podocytes, an essential component of the renal filtration barrier to urinary protein excretion. In nephritis, podocyte injury and loss with the urine are linked to the low extracellular levels of cystatin C (CysC), the secretory cysteine protease inhibitor. CysC is an early marker of renal dysfunction, but its involvement in podocyte injury in diabetes is unknown.

Therefore, this study aimed to determine the expression and secretion of CysC by podocytes under hyperglycemia- and inflammation-mimic conditions. Immortalized human podocytes were cultured in standard (11 mM) or high (HG, 30 mM, 5 days) glucose medium in the presence of lipopolysaccharide (LPS, 0.5 mg/mL, 24 h). Glomeruli were isolated from streptozotocin-induced (STZ, 65 mg/kg i.p.) diabetic Wistar rats. The qPCR and Western blot methods were used to determine the expression of CysC at mRNA and protein levels, respectively. To visualize CysC, immunostaining techniques were employed. A significant decrease in both CysC protein levels by 35% ($n = 18–24$, $p < 0.001$) and protein expression by 36% ($n = 5$, $p < 0.01$) was observed in both immunostained renal and isolated glomeruli of STZ Wistar rats. The expression of CycC mRNA in human podocytes was detected. Podocytes exposed to HG exhibited significantly decreased amounts of intracellular CysC, whereas LPS did not affect it. However, LPS, similarly to HG, reduced secretion of CysC by 17% ($n = 9–10$, $p < 0.05$) into the culture medium. Confocal images of CysC confirmed diminished protein levels in HG-cultured podocytes. These results suggest that HG and LPS modulate the secretion of CysC in cultured human podocytes. In diabetes, reduced expression and secretion of CysC by podocytes may result in augmented cysteine protease activity, leading to podocyte injury and consequently to impairment of glomerular filtration function. This work was supported by MMRI PAS statutory funding S-491.

P-36-023
PKA regulates MCT1 endocytosis in primary rat podocytes

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Lactate is a crucial energy substrate for podocyte metabolism. To maintain lactate and glucose homeostasis, lactate transporters are present on the apical membrane of the cell. Monocarboxylate transporter 1 (MCT1) is one of lactate transporters, which works in a proton-dependent manner and was found to be potential biomarker for diabetes complications. However, MCT1 regulation mechanisms remain to be elucidated. It was also recognized that oxidative stress (stimulation of H_2O_2 production) has been implicated in the progression of diabetic nephropathy (DN) and podocyte apoptosis. We hypothesize, that alterations in MCT1 translocation may be induced by H_2O_2 overproduction involved in the podocyte diabetic phenotype. We postulate, that MCT1 endocytosis is managed in protein kinase A (PKA)-dependent manner in primary rat podocytes. Surprisingly, PKA inhibition with H89 (10 μM, 1 h; $p < 0.005$) induced MCT1 endocytosis, which was determined by MCT1 cell surface biotinylation assay, endosomal vesicles staining with pHrodo Green Dextran and immunostaining of early endosomes markers (Rab5a and caveolin-1) colocalized with MCT1 protein. H_2O_2 (100 μM, 5 min) treatment also induced MCT1 internalization ($p < 0.05$). Our results suggest, that MCT1 internalization may be involved in podocyte dysfunction in DN and oxidative stress appears to initiate this process. This work was supported by grant 2019/33/B/NZ4/02407 from the National Science Centre.

P-36-024
FLT3L-STAT3-DKK1 axis promotes osteolytic bone lesions in advanced multiple myeloma

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Osteolytic bone lesions significantly impact the quality of life and prognosis of multiple myeloma (MM) patients, yet the precise molecular mechanisms driving this process remain poorly understood. Fms-like tyrosine kinase 3 ligand (FLT3L) has been noted to exhibit elevated levels in both the bone marrow and blood of patients with advanced MM. Here, we aimed to elucidate the functional relationship between FLT3L and the osteolytic process in MM. Our study enrolled 86 MM patients, 306 acute myeloid leukemia (AML) patients, and 52 acute lymphoblastic leukemia (ALL) patients. FLT3L levels were quantified in bone marrow-derived plasma samples from these hematologic malignancy patients. Remarkably, FLT3L levels were significantly higher in MM patients compared to those with AML or ALL, conditions rarely associated with osteolysis. Furthermore, FLT3L levels were notably elevated in MM patients with bone lesions compared to those without such lesions. *In vitro*, cell-based assay demonstrated that administration of FLT3L to HEK293T, HeLa, and U2OS cells resulted in an increase in DKK1 transcript levels through STAT3 phosphorylation at tyrosine 705. Additionally, WNT reporter assays indicated that FLT3L treatment suppressed WNT signaling and inhibited the nuclear translocation of β-catenin. These findings collectively suggest that the FLT3L-STAT3-DKK1 pathway inhibits WNT signaling-mediated bone formation in MM, thereby contributing to the development of osteolytic bone lesions. Moreover, transcriptomic analyses revealed a predominant elevation of FLT3L and DKK1 expression in the hyperdiploidy subtype of MM. Taken together, FLT3L can serve as a promising biomarker for predicting osteolytic bone lesions and also a potential therapeutic target to prohibit the progression of osteolytic processes in MM with hyperdiploidy.

P-36-025
Cytokine profile of inflammatory bowel diseases patients

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Inflammatory bowel disease (IBD) is a chronic inflammatory disorder of the gastrointestinal tract that comprises Crohn's disease (CD) and ulcerative colitis (UC). IBD etiopathogenesis is partly understood and includes both genetic and environmental factors, inducing an abnormal immune response[1]. Understanding immune dysregulation, exploring novel biomarkers, and advancing therapeutic strategies contribute to better managing IBD and improving patient outcome. The identification, quantification and/or validation of biomarkers is primarily performed in tissue, blood and fecal samples but only few studies have been done with saliva[2], which reflects the same type of biomarkers, allowing non-invasive sample collection. This work aims to study the inflammatory profile of IBD patients, exploring new biomarkers for monitoring diseases status, identifying high-risk patients allowing target interventions. In this observational cohort study,

unstimulated whole saliva was collected from adult IBD patients and healthy controls, after given informed consent. Salivary inflammatory proteins were quantified through multiplex immunoassay technology. Due to the high incidence of oral pathologies in IBD, namely periodontal disease (PD), oral health status of the patients and the control group were also assessed. The results showed that salivary inflammatory profile of IBD patients is different from patients without IBD (with and without PD). All IBD patients presented increased levels of IL-1β in both diseases' forms. Moreover, CU patients presented high levels of the anti-inflammatory IL-4, that could be considered a possible biomarker for this form of the disease. This study generates new insights on IBD, opening new perspectives for future works aiming to develop adequate therapeutic protocols for each patient, towards a precision medicine.References: 1. Alghoul, Z et al. (2022) Biomedicines, 10, 1492. 2. Majster, M et al. (2019) Arch Oral Biol 107, 104528.

P-36-026
Alterations of some functional protein expression in the hippocampus induced by diabetes mellitus: effect of nicotinamide

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Diabetes mellitus (DM) induced cognitive dysfunctions are associated with alterations in brain and its areas, especially in hippocampus, which plays an important role in learning, memory, and emotion. The present study aimed to assess whether hippocampus can be targeted for treatment of DM and whether nicotinamide (NAm) may exert beneficial effects on investigated protein expression changes in this area. Type 2 DM model was induced by a high-fat diet combined with a low-dose STZ (25 mg/kg, b.w., i.p.) in male Wistar rats (≈ 220 g, b.w.) treated for 2 weeks with or without NAm (100 mg/kg, b.w., i. p.). Levels of caspase-3 (Cas-3), nuclear transcription factor kB (NF-kB), poly-ADP-polymerase-1 (PARP-1), neuronal nitric oxide synthase (nNOS), mammalian/mechanistic target of rapamycin (mTOR), phospho-tau and neurofilament heavy chain (NF-H/pN-H) were assessed by immunoblotting followed by densitometric analysis. Development of DM was confirmed by hyperglycemia, insulin resistance testing, and increasing body weight. NAm administration affected neither blood glucose nor body weight in DM. Increased levels of NF-kB, Cas-3, and PARP-1 in hippocampus induced by DM may suggest that apoptosis was developed. NAm partially inhibited apoptosis by downregulating levels of NF-kB, Cas-3, and cleaving of PARP-1. We established normalizing effect of NAm on nNOS level (its level was increased), thereby improving synaptic transmission, neurogenesis, memory, etc. Moreover, mTOR signaling was deregulated in diabetic rats, but normalized by NAm. Phospho-tau level, major microtubule associated protein of mature neuron, and NF-H/pN-H, contributing to structural and functional integrity of neurons, neither changed in hippocampus at DM nor in treatment. Thus, the changes in expression of investigated proteins in hippocampus in DM indicate that these proteins may be markers of cognitive dysfunction development and potential therapeutic targets for the treatment of DM and its complications.

P-36-027
Iron-dependent lysosomal LDL oxidation induces expression of scavenger receptor A in human THP-1 monocytes

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Atherosclerosis leading to cardiovascular diseases remains a dominant medical problem. Little attention has been paid to the processes in the early stages of this disease. At the beginning of atherosclerosis, the interaction between monocytes and the endothelium is crucial. Monocytes express scavenger receptor A (SR-A) that mediates cell adhesion, and later also uptake of oxidised low-density lipoproteins (LDL). Next, high iron stores in monocytes or macrophages predispose to atherosclerosis, for poorly understood reasons. Our study aimed to test whether iron together with LDL given to cultured THP-1 monocytes induces SR-A expression and leads to potentially pro-atherogenic changes. We found that native LDL, but not transferrin, markedly induced SR-A expression in cultured THP-1 cells. The addition of oxidised LDL did not further increase the SR-A expression. Both splice isoforms SR-AI and SR-AII were upregulated. The expression of SR-A was present also at the protein level, and we confirmed it increased cellular adhesion. The induction of SR-A by LDL was inhibited by lipophilic antioxidant BHT, or by a thiol WR-1065, which accumulates inside the lysosomes. The usage of fluorescent probes BODIPY C11 and lysosome-targeted FOAM-LPO proved an increased lipid peroxidation inside lysosomes after LDL administration. These data suggest that the induction of SR-A by LDL is mediated by an endogenous iron catalysing intralysosomal lipid peroxidation. Final evidence for the involvement of endogenous iron was obtained by RNA interference: The induction of SR-A by LDL could be blocked by siRNA against the nuclear coactivator receptor NCOA4, the cargo receptor necessary for the autophagy of ferritin. Altogether, these results point to a new pathogenetic mechanism of early-stage atherosclerosis: high iron stores in circulating monocytes may, via more lysosomal lipid peroxidation, lead to an increased expression of SR-A, which makes the cells more adhesive and hence more atherogenic.

P-36-028
Kappa opioid receptors in experimental colitis

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Kappa opioid receptors (KOP) are known pharmacological target in treatment of nociceptive disorders that are combined with the central nervous system. KOP agonists can exert a substantial influence in colitis. The aim of our study was to determine whether the KOP agonist U50488 – can become potential anti-inflammatory agent in colitis. *In vitro* studies were performed on the RAW 264.7, macrophage cell line, with usage of the lipopolysaccharide (LPS) to mimic inflammation. Griess test and MTT assay were performed to verify the impact of U50488 on the cell viability and cytotoxicity. Dextran sulfate sodium (DSS) was given to mice for 5 days, following 3 days of tap water to induce colitis. U50488 was given at different doses (1-5 mg/kg body

weight, intraperitoneally) and in different time points. After macroscopic scoring, colonic tissues were collected for molecular analysis. We found that U50488 reduced a release of nitric oxide (NO) *in vitro*, suggesting its anti-inflammatory action. U50488 influenced cell viability. *In vivo*, U50488 improved colitis as indicated by macroscopic score as well as decreased mRNA expression of the IL-1B, IL-6, NOS2 and PTGS2 as compared to inflamed mice. Histological staining confirmed that U50488 improved mucosal architecture of the colon. Activation of the kappa opioid receptors might represent a novel therapeutic target for the treatment of colitis.

P-36-029
Adipogenic differentiation and adipocyte metabolism: a focus on protein kinase CK2

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Adipose tissue (AT) is able to rapidly expand by both increase in size of existing adipocytes (hypertrophy) or formation of new adipocytes through differentiation of resident precursors (hyperplasia). We previously identified CK2 up-regulation as a hallmark of AT pathological expansion in patients with obesity and diabetes. CK2 is a constitutively active Ser/Thr protein kinase, composed of 2 catalytic (α/α') and 2 regulatory (β) subunits. CK2 has been studied in the adipogenic differentiation of human mesenchymal stem cells, where CK2 expression and activity are high at the beginning of the process and decrease in mature fat cells. We aim to further characterize the role of CK2 in adipogenesis, as well as its involvement in the regulation of adipocyte metabolism. We exploited a double approach of genetic and pharmacological targeting of CK2 using the 3T3-L1 murine preadipocyte cell line differentiated by culturing in adipogenic medium as standard model of *in vitro* adipogenesis. In these cells, we targeted CK2 subunits by RNAi or CRISPR/Cas9, or we performed cell treatments with the specific CK2 inhibitors CX-4945 and SGC-CK2-1. The effects on the adipogenic differentiation were evaluated by morphological analysis, OIL-RED-O staining and western blot for early and late adipogenic markers. The comparison of the effects produced by the different CK2 targeting approaches allowed to disclose a role for CK2 during the first differentiation steps, with a possible specific function of the β regulatory subunit. In addition, we treated fully differentiated 3T3-L1 cells with the CK2 inhibitors and analyse lipids and polar metabolites by liquid chromatography/mass spectrometer system, to detail the role for CK2 in specific metabolic pathways of mature fat cells.

P-36-030
Mitochondrial dysfunction contributes to germ cell apoptosis via the JNK/p53/ survivin pathway

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The aim is to study whether the c-Jun N-terminal kinases (JNK) signaling is a regulator oxidative DNA damage, germ cell apoptosis (GCA) and mitochondrial dysfunction during testicular ischemia reperfusion injury (tIRI) using the JNK inhibitor SP600125. Male Sprague-Dawley rats (n = 36) were equally divided into three groups: sham, tIRI only and tIRI + SP600125. Testicular ischemia was induced for 1 hour followed by four hours reperfusion prior to animal sacrifice. Spermatogenesis was evaluated by light microscopy using the Johnsen's scoring system. Expression of oxidative stress and GCA genes and proteins were evaluated by real-time PCR and colorimetric assays, respectively. Activation of the JNK/p53/survivin signaling pathway was detected by immunofluorescence (IF) staining. Indicators of mitochondrial dysfunction were examined by western blot and colorimetric assays. In comparison to sham group, the tIRI testes showed a significant decrease in the antioxidant activity of superoxide dismutase, which was associated with increased lipid and protein oxidation products. Oxidative DNA damage was reflected by a significant increase in the number of single and double DNA strand breaks and increased concentration of 8-OHdG DNA adducts. Spermatogenic damage was associated with the activation of pro-apoptosis caspase 9, caspase 3 and an elevated Bax to Bcl2 ratio. This was also accompanied by a significantly heightened IF expression of the phosphorylated forms of JNK and p53 parallel with the down-regulation of survivin. Mitochondrial dysfunction was reflected by NADH depletion, overexpression of uncoupling protein 2 and increased cytochrome c protein levels. Such tIRI-induced modulations were all attenuated by SP600125 treatment prior to reperfusion. In conclusion, mitochondrial dysfunction could contribute to GCA during tIRI under the control of the JNK/p53/survivin signaling pathway.

P-36-031
Novel thyroid hormone receptor-β (TRβ) agonist as a promising multitarget agent for the treatment of interlinked diseases such as obesity and neurodegenerative disorders (NDD)

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Selective TRβ-agonists show beneficial effects on metabolic and neurologic alterations, giving hope for the effective treatment of interlinked diseases such as obesity and neurodegenerative disorders (NDD). Among them, TRβ-selective agonist TG68 emerges as a promising multitarget agent for the treatment of obesity-associated diseases. In this study, we investigated the potential neuroprotective effects of TG68 in *in vitro* models of neuroinflammation and β-amyloid (Aβ) neurotoxicity. Then, we

performed *in vivo* studies on high fat diet (HFD) obese mice to investigate TG68 effects on bodyweight (BW), energetic metabolism and neuroinflammation. Treatment of human microglia cells (HMC3) with TG68 (0.1-10 μM) followed by inflammatory stimulation (LPS/TNFa), significantly decreased pro-inflammatory IL6, while increasing anti-inflammatory IL10. Aβ oligomers activate microglia enhancing the release of pro-inflammatory factors. Exposure of HMC3 cells to 10μM Aβ25-35 led to a significant increase of TNFα and IL6 release. In Aβ-treated cells, TG68 pretreatment (10 μM) reduced TNFα and IL6 and increased IL10 levels. Next, we demonstrated that in HFD-mice (CD-1 mice; HFD C1090-60; 10 weeks) treatment with TG68 (10 mg/kg/day; 2 weeks) produced a 12% BW loss and a significant decrease of blood glucose and lipids levels. qPCR analysis on serum, adipose tissue, and hypothalamus, revealed that TG68 administration efficiently counteract HFD-induced transcriptional changes on metabolic (SIRT6, PPARγ, ADIPOQ, LEPTIN, APOD, GLUT1 and GLUT5) and inflammatory (TNFα and IL6) markers. Elevated serum levels of TNFα and IL6 and decreased hypothalamic expression of BDNF were detected in HFD-mice. Administration of TG68 significantly reduced TNFα and IL6 serum levels, while increasing BDNF expression, further supporting neuroprotection. Taken together, our findings highlight the ability of TG68 to modulate multiple pathological pathways involved in complex networked diseases, such as obesity and NDD.

P-36-032
Diagnostic algorithm for the assessment of hemostasis system condition

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Hemostasis system imbalance leads to thrombosis or bleeding. Therefore, developing of accurate diagnostics is an important task in medical biochemistry. Determining one or two parameters for diagnosis is not sufficient. That is why the aim of our work was to create an algorithm for hemostasis diagnosis based on the obtained results. We analyzed blood samples of pregnant women (n = 400), post-COVID (n = 199), and burned patients (n = 25). Soluble fibrin and D-dimer were detected using sandwich ELISA with monoclonal antibodies 1-3C and III-3B; platelet function was determined using aggregometry on the Solar AP 2110. Protein C, Factor Xa, and Antithrombin III levels were determined using snake venom activators and chromogenic substrates: S2366, S2765, and S2238, respectively. Clot structure was assessed by overall hemostatic potential method; plasmin activity was determined using the substrate S2251. We have selected 3 stages of hemostasis assessment: at 1st stage, we propose to determine D-dimer and soluble fibrin and platelet function. If disorders are detected, we proceed to 2nd stage using other platelet aggregation inducers, determine protein C, Antithrombin III to characterize anticoagulant link and define concentration of fibrinogen as an acute phase protein. If it is necessary to research the mechanism of pathological changes, we go to the 3rd stage of testing – determination of the overall hemostatic potential, thrombin and plasmin activity; detection of the content of Factor X and determination of tissue-type plasminogen activator and plasminogen activator inhibitor-1. We have proposed and tested an algorithm for the diagnostics of the hemostatic system in various pathological conditions, which includes 3 phases of research: in the first phase we assess the risk of thrombosis or bleeding, in the second we deepen knowledge and prescribe therapy, and in the third we determine the cause of the pathological condition. *The authors marked with an asterisk equally contributed to the work.

P-36-033
Counteracting the interleukin-8-induced senescence in a model of lung tumour microenvironment

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The senescent associated secretory phenotype (SASP) is a hallmark of senescence that, besides physiological function, might induce a tumour-sustaining microenvironment. Among other stimuli, cancer associated fibroblasts (CAFs) become senescent following the activation of the Interleukin (IL)-8 receptor CXCR2, acquiring SASP to promote cancer growth and survival. Lymphangioleiomyomatosis (LAM) is a rare pulmonary low-grade disruptive metastasizing neoplasm caused by the infiltration of LAM cells in the lung parenchyma. LAM cells disrupt the lung parenchyma and metastasize but, differently from other cancers, they do not form solid masses in the lung. For this reason, LAM can model the capability of cell secreted factors to promote the establishment of a tumour favouring microenvironment devoid of cancer cells. LAM cells feature the deregulation of the mechanistic target of rapamycin (mTOR), a master regulator of senescence. In a novel *in vitro* LAM model of human pulmonary microenvironment, we demonstrated that primary LAM/TSC cells are senescent depending on mTOR hyperactivation and induce senescence in healthy pulmonary lung fibroblasts (PLFs) through their conditioned medium (CM). Here, we prove that PLFs grown in the presence of IL-8, highly secreted by LAM/TSC cells, increase their positivity to senescence markers (e.g. SA-β galactosidase and p21 expression). PLFs induced to be senescent by LAM/TSC CM acquire CAFs features, as vimentin and α-smooth muscle actin expression, and increase motility in wound healing and Boyden chamber assay. The inhibition of CXCR2 with a monoclonal antibody or with the non peptidic molecule SB225002 reduces LAM/TSC cell senescence and counteracts the capability of LAM/TSC CM to induce senescence on PLFs. In the context of our results, the modulation of senescence through CXCR2 is intriguing to dissect the communication in the pathological lung microenvironment, allowing to identify targets for early diagnosis and novel therapies.

P-36-034
Calcium-activated nucleotidase 1 affects proteoglycan processing

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Desbuquois dysplasia type 1 (DBQD1) is a recessive chondrodysplasia caused by mutations in *CANT1* gene, encoding for a Golgi calcium-activated nucleotidase 1 (CANT1) that hydrolyses UDP, the by-product of glycosyltransferase reactions. To study CANT1 involvement in DBQD1 pathogenesis, a *Cant1* knock-out mouse ($Cant1^{-/-}$) was generated and validated. Using the animal model, the role of CANT1 in proteoglycan (PG) biosynthesis was demonstrated, since reduced PG synthesis and oversulfated glycosaminoglycans (GAGs), with reduced hydrodynamic size, were observed in $Cant1^{-/-}$ chondrocytes. Moreover, a pulse chase experiment demonstrated a reduced PG secretion in mutant cells, suggesting the possible retention of PGs in $Cant1^{-/-}$ chondrocytes [Paganini C et al (2018) Matrix Biol 81, 70-90]. The aim of this work was to further investigate the role of CANT1 impairment on PG processing and glycanation in chondrocytes, cartilage and skin from mutant mice. Western blot analysis of PG from chondrocytes cultures, digested with chondroitinase ABC in order to unmask the aggrecan core protein, demonstrated that only glycanated aggrecan was secreted by both wild-type and $Cant1^{-/-}$ chondrocytes. The same results were observed for decorin that is secreted only after glycanation both by mutant and wild-type chondrocytes. However, even if glycanated, the decorin GAG chain showed reduced hydrodynamic size in $Cant1^{-/-}$ cells compared to wild type. Interestingly, the same defect was also observed in cartilage and skin biopsies from mutant mice. In conclusion, although PG synthesis and secretion are reduced in mutant cells, $Cant1^{-/-}$ chondrocytes secrete in the extracellar matrix only glycanated PGs even if of reduced hydrodynamic size. Work supported by PRIN2022 (grant no. 2022ETJJES).

P-36-035
Repetitive transcranial magnetic stimulation induces long-term molecular changes in a treatment resistant depression animal model

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Repetitive transcranial magnetic stimulation (rTMS) is a neuromodulation technique that has recently gained interest for its use in treatment-resistant depression (TRD). The mechanism of rTMS, still poorly understood, might induce a dynamic regulation of brain circuits, presumably through biochemical and molecular mechanisms (1). We here investigated the gene expression of key genes (brain derived neurotrophic factor (*BDNF*) and cannabinoid receptor 1 (*CNR1*)) in the prefrontal cortex (PFC) and hippocampus (HIP) of a TRD animal model (2). A group of Wistar-Kyoto rats, subjected to chronic mild stress for four weeks (TRD), and a group of non-depressed Wistar rats (CTRL), have been exposed to a standard rTMS protocol for 8 consecutive days (10 Hz, 110% RMT, 40 pulses per train, 1600 pulses per daily session). Corresponding TRD and CTRL sham groups were exposed to the same manipulation and acoustic effect. Animals were sacrificed 3 days (short-term) or 16 days (long-term) after the last rTMS session. In the long-term groups, we found that both *BDNF* and *CNR1* mRNA levels resulted significantly increased in the PFC of TRD rats exposed to active compared to sham rTMS. Following long-term active rTMS, *CNR1* mRNA levels were also significantly increased in TRD compared to CTRL rats. Moreover, the expression levels of these genes in the PFC are significantly directly correlated. No significant changes were observed either in the HIP or in the short-term groups. Preliminary studies are ongoing to evaluate the role of epigenetic mechanisms (gene promoters DNA methylation levels and miRNAs expression). The results we report here further suggest the interactions between BDNF and endocannabinoid system via modulation of CB1 receptor (3) and the potential role of rTMS treatment in anti-depressant therapeutic effect. References: (1) Weiler M et al. (2023) Front Hum Neurosci 17 (2). Willner P et al. (2019) Behav Pharmacol 30, 239-250. (3) Zhao L and Levine ES (2015) eNeuro 2.

P-36-036
Effect of methylglyoxal on intestinal cells: possible molecular mechanisms

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Methylglyoxal (MG) is endogenously produced under physiological conditions as a by-product of glycolysis and by auto-oxidation of glucose and lipid peroxidation. The digestive system can

take up MG from exogenous sources, which include dietary MG and MG formed by the gut microbiome. MG is a highly reactive molecule, able to react with macromolecules forming covalent adducts resulting in advanced glycation end-products formation. MG can also enter the cell nucleus and reacts with nucleic acids resulting in MG-nucleic acid adducts formation. The MG-adducts show changes in molecular stability and function. Several studies have demonstrated a key role of MG in diabetes and other diseases (such as cancer, and cardiovascular diseases). Aim of the study was to investigate the effects of MG in intestinal cells; in fact, the intestinal epithelium is highly exposed to dietary and endogenous harmful stimuli, including MG. Using Caco-2 cells, we demonstrated that MG treatment induced an increase in cytosolic and mitochondrial reactive oxygen species. MG-induced oxidative stress was associated with activation of NF-KB pathway, with consequent increased expression of pro-inflammatory molecules such TNFα. Moreover, a higher phosphorylation of Ser-139 residue of the histone variant H2AX, forming g-H2AX, was observed in MG-treated cells, suggesting that MG induced DNA damage. Our results demonstrated a decrease in histone deacetylases (HDAC1/2/8) levels, consistent with the increase in acetylated histone H4 levels, in MG-treated cells. These results suggest an additional mechanism for MG-induced cellular damage through epigenetic perturbation. These molecular alterations reflected in impairment of intestinal barrier functions, evaluated by trans-epithelial electrical resistance (TEER). The study of MG-induced alterations in intestinal cells deserves of further investigation to better understand its potential role in the onset of inflammatory intestinal diseases.

P-36-037
Global profiling of differential genes and proteins expression in alternative forms of Borrelia

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Lyme borreliosis is a tick-borne disease that can be successfully cured by antibiotics at the early stages of infection, targeting the replicative form of the causative agent of disease, spirochetes from *Borrelia burgdorferi* sensu lato complex. However, up to 20% of patients still have persisting symptoms even after the treatment. Signals that spirochete receives from hostile environments, particularly antibiotic pressure, evoke formation of alternative morphologies (round bodies, cysts, biofilms, etc), keeping the pathogen alive, infective and refractory to elimination by antibiotics. Persistent or dormant forms of spirochetes can be hidden in tissues of patients, representing phenotypically heterogeneous metabolically quiescent bacteria with multidrug tolerance. Persisters survive by regulation of the expression of genes involved in pathogenicity, survival and the mechanism of persisters' formation. Identification of genes and proteins, differentially expressed in alternative forms of *Borrelia* is like obtaining a "fingerprint" of the agent that causes the damage to the infected hosts, including humans. The use of newly identified differentially expressed markers of *Borrelia* cysts, round bodies or biofilms in samples of human origin could be used either for development of novel detection methods, a new diagnostic tool directed against "hidden in the body" spirochetes or as the basis for developing of therapeutic vaccines. This research was supported by grant NV19-05-00191 from Ministry of Health and by grant INTER-COST LUC23151 from Ministry of Education, Youth and Sport of the Czech Republic. *The authors marked with an asterisk equally contributed to the work.

P-36-038
Neutral lipid storage disease with myopathy: first report of a patient with a novel mutation localized in the putative LC3-interacting region of ATGL

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Neutral lipid storage disease with myopathy (NLSDM) is an autosomal recessive disorder characterized by abnormal triacylglycerol accumulation in lipid droplets (LDs), leading to progressive skeletal muscle myopathy and sometimes cardiomyopathy. NLSDM is due to mutations in the PNPLA2 gene, which encodes adipose triglyceride lipase (ATGL), a crucial enzyme for neutral lipid catabolism. In the present study, we describe an Italian patient with unusual phenotype carrying a novel PNPLA2 homozygous in-frame deletion (c.45_47delCGG). The patient was a 42-year-old male with proximal muscle weakness in the upper limbs, particularly on the right side, without cardiac involvement. Specifically, MRI analysis revealed atrophy of the supraspinatus and infraspinatus muscles. Notably, for the first time, involvement of the orbicularis oculi muscles was observed, presenting with slight weakness. The identified mutation consists of a single in frame amino acid deletion localized in the first LC3-interacting region (LIR) motif of ATGL protein. LIR motifs mediate ATGL-LC3 interaction. This bond plays a key role in targeting ATGL to LDs, enhancing their degradation through a combined mechanism of lipolysis and lipophagy. Bioinformatic analyses, using I-Tasser software, predicted alterations in the secondary and tertiary structure of the mutated protein (p.Gdel16) that could impair both ATGL enzymatic and lipophagic function. Indeed, most changes were forecasted to occur in the patatin domain, which encompasses the catalytic dyad and LIR motifs. Our investigation suggests that the deletion of 16th amino acid, by altering ATGL interaction with LC3, could affect lipophagy beyond lipolysis, highlighting a complex relationship between LD biology and autophagic processes. These findings not only expand the genetic landscape of NLSDM but also confirm the clinical heterogeneity of the disease. Funding: This work was supported by European Union – Next Generation EU, PRIN 2022 PNRR (P2022AWA84). *The authors marked with an asterisk equally contributed to the work.

P-36-039
Characterization of adult individuals with Cornelia de Lange syndrome

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Cornelia de Lange syndrome (CdLS, OMIM #122470, #300590, #300882, #610759, and #614701) is a multisystem developmental disorder characterised primarily by distinctive facial features, intellectual disability, variable growth retardation, and upper limb abnormalities. It is caused by variants in genes related to the cohesin complex, with variants in the NIPBL gene being the most frequent. Although the clinical and molecular characterisation of the syndrome is increasingly comprehensive and exhaustive, there is a profound lack of understanding regarding the evolution and implications of pathogenic variants at systemic and cellular levels in adult individuals, as well as during physiological processes such as ageing. With the aim of understanding and predicting the syndrome's evolution, this study presents the phenotypic characterisation at clinical, cellular, and molecular levels of six adult patients with CdLS. All of them exhibit a classic phenotype of the syndrome and de novo pathogenic variants in the NIPBL gene. Growth curves performed on primary fibroblasts show significant differences between controls and patients. In addition, RNA-seq and qPCR studies on primary fibroblasts confirm the dysregulation of genes mainly involved in development. Furthermore, Gene Set Enrichment Analysis (GSEA) shows significant enrichment of processes related to mRNA regulation and maintenance, thus providing a link between molecular and biological processes. Lastly, the specific study of dysregulated genes during the ageing process shows a trend for premature dysregulation in these adult patients with CdLS. *The authors marked with an asterisk equally contributed to the work.

P-36-040
The effect of novel triazine-based TRPA1 agonists on TGF-β-induced lung fibroblast-to-myofibroblast transition

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Airway remodeling (AR) refers to various structural changes occurring in the airway wall during respiratory diseases. One of the phenomena that takes place during AR is transforming growth factor type β (TGF-β)-induced fibroblast-to-myofibroblast transition (FMT). Resulting myofibroblasts are characterized by increased proliferation, migration, contraction, and increased expression of profibrotic markers. Due to the lack of effective remodeling therapy, transient receptor potential ankyrin 1 (TRPA1) channel seems to be an interesting molecular target. Latest reports indicate that its activation may promote several antifibrotic effects in fibroblasts. Recently, a new series of triazine-based derivatives, representing TRPA1 agonists has been designed and synthesized in our team. Here we investigated the effect of two prominent compounds from this group (1 and 2) on TGF-β-induced FMT in human lung fibroblasts (MRC-5 cell line). On the mRNA level, 1 and 2 decreased TGF-β-induced expression of profibrotic genes (*ACTA2*, *COL1A1*, *SM22*). However, TGF-β alone led to TRPA1 down-regulation and studied compounds were not able to prevent it. Furthermore, both 1 and 2 diminished total α-smooth muscle actin (α-SMA), a known myofibroblast marker, which was confirmed by in-cell ELISA analysis. Immunofluorescence staining revealed that only compound 1 was able to inhibit TGF-β-induced α-SMA-positive stress fibers, as well as translocation of p-Smad-2 to the nucleus. Similarly, it turned out to be more effective in collagen gel contraction assay. The obtained data revealed preferential activity of both tested triazine-based derivatives in limiting TGF-β-induced FMT. We believe that these compounds are worth further studies, also in other cellular models of AR. Acknowledgements: This study was supported by the Jagiellonian University Medical College, Poland, funded grant No. N42/DBS/000323 and by the National Science Centre, Poland, funded grant No. 2018/29/B/NZ7/00285.

P-36-041
Impaired myoblast differentiation and muscle IGF-1 receptor signalling pathway activation after N-glycosylation inhibition

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The role of N-glycosylation in the myogenic process remains poorly understood [1]. Here, we evaluated the impact of N-glycosylation inhibition by Tunicamycin (TUN) or by *phosphomannomutase 2* (*PMM2*) gene knockdown, which encodes an enzyme essential for catalysing an early step of the N-glycosylation pathway, on C2C12 myoblast differentiation. The effect of chronic treatment with low-dose of TUN (0.1 mg/kg for 15 days) on tibialis anterior (TA) and extensor digitorum longus (EDL) muscles of WT and MLC/mIgf-1 transgenic mice, which overexpress muscle *Igf-1Ea* mRNA isoform, was also investigated. TUN-treated and *PMM2* knockdown C2C12 cells showed reduced ConA, PHA-L and AAL lectin binding and increased ER-stress-related gene expression (*Chop* and *Hspa5* mRNAs and *s/uXbp1* ratio) compared to controls. Myogenic markers (*MyoD*, *myogenin* and *Mrf4* mRNAs and MF20 protein) and myotube formation were reduced both in TUN-treated and *PMM2* knockdown C2C12 cells. Body and TA weight of WT and MLC/mIgf-1 mice were not modified by TUN treatment, while lectin binding slightly decreased in the TA muscle of WT (ConA and AAL) and MLC/mIgf-1 (ConA) mice. The ER-stress-related gene expression did not change in the TA muscle of WT and MLC/mIgf-1 mice after TUN treatment. TUN treatment decreased *myogenin* mRNA and increased *atrogen-1* mRNA, particularly in the TA muscle of WT mice. Finally, the IGF-1 production and IGF1R signalling pathways activation was reduced due to N-glycosylation inhibition in TA and EDL muscles. Chronic TUN-challenge models can help to elucidate the molecular mechanisms through which diseases associated with aberrant N-glycosylation, such as Congenital Disorders of Glycosylation (CDG), affect muscle and other tissue functions. References: [1] Blazev R. et al., Mol Cell Proteomics. 2021;20:100030.

P-36-042
Glucosylceramide lysosomal accumulation leads to metabolic alterations that could underlie neuronal degeneration

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β-glucocerebrosidase (GCase) is a lysosomal enzyme responsible for the catabolism of glucosylceramide (GlcCer) into glucose and ceramide. The deficiency of this enzyme causes the lysosomal accumulation of the uncatabolized GlcCer, leading to a neurodegenerative phenotype. To investigate the molecular mechanism linking GCase deficiency and the consequent GlcCer accumulation with neurodegeneration, we developed an in vitro model represented by iPSCs-derived dopaminergic neurons, obtained from a healthy subject, chronically treated with conduritol B epoxide (CBE) to inhibit GCase activity. We observed that CBE-treated neurons presented a massive lysosomal GlcCer accumulation followed by a lysosomal impairment. Considering the central role of lysosomes in the recycling of macromolecules, we investigated the effect of the lysosomal impairment on the metabolic flow through LC-MS/MS. We observed that, following the accumulation of GlcCer, both glycolysis and the TCA cycle are affected, with a reduction in glucose content and an increase in lactate, suggesting an increased consumption of glucose due to an increased energy demand of CBE-treated neurons. We also observed in CBE-treated neurons, an overall increase in amino acid content, particularly in branched-chain amino acids, probably to fuel energy production, entering the TCA cycle, or to replenish protein synthesis, since lysosomal protein degradation and recycling are impaired. Metabolomics analysis also revealed that acylcarnitines undergo changes with CBE treatment. In particular, the increase in short-chain acylcarnitines suggests that there may be an increased activation of β-oxidation, which would support our hypothesis of increased energy demand in CBE treated neurons. The obtained data let to speculate that GCase loss of function impairs the lysosomal compartment, leading to severe alterations in the energetic metabolism of the neurons, switching from a glucose-centered metabolism to a protein-centered one.

P-36-043
Analyzing conformational changes in the potassium channel Kv 4.2 to investigate the role of pathological variant associated with early myoclonic encephalopathy (EME)

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Early myoclonic encephalopathy (EME) is a severe epileptic encephalopathy frequently beginning in the first several months of life. Genetic mutations are acknowledged as important contributors, even if their etiology is yet unknown. These mutations mostly impact proteins essential for brain development and function, neurotransmitter receptors and ion channels. Among these, Kv4.2 channels are essential for controlling synaptic transmission and neuronal excitability in the central nervous system. EME has been linked to specific mutations of Kv4.2 affecting critical residues for channel gating, including Arg305 and Pro403. We investigated the structural and dynamic characteristics of WT Kv4.2 channel, phosphorylated Kv4.2 channel, and Kv4.2 channel with mutations at Arg 305 (Cys) and Pro 403 (Leu) residues, using molecular dynamics simulations and computational modeling approaches. We have been able to clarify the effects of phosphorylation and mutations on channel stability, conformation, and

function. Different structural and dynamical changes are linked to each channel variant, according to our findings. Phosphorylation and mutation at specific sites affect the conformational dynamics of Kv4.2 channel, altering their gating kinetics and ion permeation properties. We offer a better understanding of the molecular processes that underlie the control and malfunction of Kv4.2 channels. Our computational approach provides a greater knowledge of the intricate interaction between structure and function in Kv4.2 channels, complementing experimental findings. This study advances our knowledge of how phosphorylation and mutations control Kv4.2 channels. The knowledge gathered from our *in-silico* modeling, is essential for elucidating the pathophysiology of EME and developing targeted therapeutic strategies for this condition and also create new treatment for neurological conditions linked to Kv4.2 channel malfunction.

P-36-044
Precision targeting and regulation of epigenetic protein HDAC6 enable advanced potent therapeutics for Alzheimer's disease

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Alzheimer's disease (AD) is the primary aging-related neurodegenerative disorder lacking effective therapeutics. The neuropathology of AD is multifaceted and primarily consists of β-amyloid plaques and phosphorylated-tau consisted neurofibrillary tangles that are closely regulated by neuroinflammation. Emerging evidence suggests that the epigenetic regulatory protein histone deacetylase 6 (HDAC6) underlies in the pathogenesis of AD, and offers as a potential drug target for AD. We conducted further proof-of-concept and mechanistic investigations regarding HDAC6 as a suitable drug target for AD through a rational design of specific, potent, and BBB-penetrant HDAC6 inhibitors as well as our analysis of Alzheimer's neuropathological alternations in AD models. Herein, we report a precise development of a small molecule inhibitor of HDAC6, PB131, that displays unprecedented potency and strong specificity of HDAC6 which significantly elevates Aβ phagocytosis, preserves tubulin/microtubule network integrity, notably reduces NLRP3-related innate immunity activation, inflammatory biomarkers in cell and animal models of AD and lowers phosphorylated-tau levels in a 3D-AD neural culture model. These findings support the pivotal role of HDAC6 in regulating AD neuropathology and position PB131 as a potential therapeutic with pleiotropic effects against AD.

P-36-045
Fate mapping of peripherally-derived macrophages after traumatic brain injury

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Traumatic brain injury (TBI) represents a critical health problem for our society, given its increasing occurrence, complex pathophysiology, challenging diagnosis, and long-term neurological disabilities. It is well established that TBI-induced neuroinflammation engages resident microglia as well as infiltrating monocytes (CCR2+) recruited from the periphery which both contribute to long lasting cognitive deficits. After brain engraftment, peripherally derived macrophages stop expressing their signature marker CCR2, thus making discrimination from reactive microglia cells elusive. To overcome this issue, we took advantage of CCR2-creERT2::Ai14D mice, where CCR2+ cells are permanently labeled even after *in situ* reprogramming. Adult CCR2-creERT2::Ai14D male and female mice were injured using the controlled cortical impact (CCI) model of TBI. Injury-induced cognitive deficits were measured using the radial arm water maze and infiltrated CCR2+ macrophages were traced at 7, 30 and 240 days post injury. We characterized localization, transcriptomic signatures and functionality of peripherally derived macrophages focusing on how these features change from sub-acute and chronic time points using flow cytometry, imaging, RNA sequencing and *in vivo* synapses phagocytosis assays. This unprecedented knowledge on long lasting infiltrated macrophages will help future studies focused on promoting brain resilience against injury. *The authors marked with an asterisk equally contributed to the work.

P-36-046
Drosophila chitin lectins control intestinal homeostasis and tumorigenesis

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In humans, chitinase-like proteins (CLPs) are biomarkers in inflammatory and malignant conditions of gastrointestinal tract, yet their physiological and biological functions are still unclear. *Drosophila melanogaster* is an exceptional model to investigate intestinal inflammation and tumorigenesis, due to the extensive conservation in the signals that regulate pathophysiology and regeneration between flies and humans. By performing comparative transcriptomics of *Drosophila* intestines carrying tumors induced by *Ras1** oncogene expression or by inactivation of the *Notch* tumor suppressor, we selected *CG13309, CG7298* and *CG10154*, which encode chitin-binding domain (CBD) proteins, and significantly reduced Ras tumorigenesis when silenced, for further analysis. We found that, in non-tumorous midguts, intestinal stem cell (ISC)-specific silencing of *CG13309* and *CG10154* compromised damage-induced ISC-mediated midgut regeneration, whereas *CG13309* and *CG10154* ISC-specific overexpression was sufficient to drive midgut mitosis in the absence of damage. Also, we observed that CG13309 protein was closely associated with intestinal progenitors and its punctate localization was extracellular. Mosaic clonal analysis and marker co-expression in *CG13309*-silenced guts showed that *CG13309* was necessary for ISC mitosis but not maintenance or differentiation. Moreover, time-course RT-qPCR analysis of *CG13309*-silenced tumorous and non-tumorous intestines showed alterations in expression of ISC mitosis regulators. Last, mRNA-Seq transcriptomics of control vs. ISC-specific *CG13309*- and *CG10154*-silenced midguts in baseline and infection conditions showed enrichment in genes with lysozyme activity and transmembrane domains upon CBD silencing. Since the fly CBD genes encode small, secreted peptides associating with tumors with sequence similarity to human CLPs, our research can provide mechanistic insights into the action of human CLPs, which are upregulated in colon cancer and inflammation. *The authors marked with an asterisk equally contributed to the work.

P-36-047
Remodeling tumor immune microenvironment through FKBP5 triggering: a dual effect approach against melanoma

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FKBP51 plays a relevant role in melanoma resistance and invasion. FKBP51 is a marker of melanocyte malignancy, correlating with the vertical growth phase and lesion thickness. It promoted the typical features of epithelial to mesenchymal transition and sustained apoptosis resistance, in *in vitro* and *in vivo* melanoma models. Recently, we identified the splicing isoform of FKBP5, termed FKBP51s, and in the peripheral blood of melanoma patients related to an immune-tolerant phenotype. With respect to FKBP51, FKBP51s lacks the TPR domain, but they share the N-terminal domains target of a new selective and powerful small molecule, called SAFit. Aim of the present study was to assess the efficacy of FKBP5 inhibition in inducing both tumor cell apoptosis and boosting antitumor immune response. Our results show that SAFit increased the doxorubicin and dacarbazine cytotoxicity. These agents were effective in impairing NF-κB activity of melanoma cell and turning off pro-survival genes triggered by the tumour. Moreover, SAFit reduced levels of TGF-β and its receptor TβRI, thus hampering phosphorylation of Smad2/3 transcription factors. In line with this finding, the invasion assay showed a reduced capability of melanoma cells to migrate in the presence of SAFit. Interestingly, SAFit treatment of melanoma cell also had effects on the immune system. The reduced secretion of the immunosuppressive cytokine, indeed, drastically reduced the tolerance of the immune system in both in vitro and in vivo systems. In particular, the FKBP51 inhibiting molecule changed the ratio Tregs/Teff and the type of macrophages infiltrating the tumor. In conclusion, our study shows that selective FKBP51 targeting agents act as anti-cancer drug promoting anti-tumor immunity thus providing preclinical elements in support of the efficacy of recently generated small molecules. Such compounds appear to be novel promising agents for therapeutic strategies against melanoma for partnering with immunotherapy.

P-36-048
EWS participates in the DNA damage response through the stabilization of double-strand breaks repair complexes

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Cells experience thousands of DNA lesions per day, with double-strand breaks (DSBs) being the most cytotoxic ones. To deal with this threat, cells rely on repair pathways comprehensively known as DNA damage response (DDR). RNA-binding proteins (RBPs) are essential effectors of the DDR and, among them, FET (FUS, EWS, TAF15) proteins are emerging as important players. Notably, mutations in FET proteins and impaired DDR have been implicated in the pathogenesis of neurodegenerative diseases such as amyotrophic lateral sclerosis. We recently demonstrated that FUS-dependent liquid-liquid phase separation is necessary for the initiation of the DDR, whereas the function of EWS is still unclear. Therefore, the aim of this work is to investigate the role of EWS in the DDR, through the characterization of knockout (KO) HeLa cells and with a focus on DSBs response. Laser-microirradiation experiments confirmed that EWS is promptly recruited to DNA damage sites in a PARP1-dependent manner. Viability and proliferation assays showed that EWS-KO cells are more sensitive to etoposide, a topoisomerase II inhibitor which causes DSBs. Accordingly, analysis of the DSB marker γH2AX (H2AX phospho-Ser139) showed that EWS-KO cells have reduced repair ability. To detail this defect, we analysed the formation of DSBs-associated foci, showing that EWS-KO cells form less MDC1 and 53BP1 foci after treatment with etoposide. In addition, microirradiation experiments showed that DDR mediators FUS and RNF8 were recruited normally to DNA damage sites but were released earlier in EWS-KO cells compared to WT ones. Notably, EWS has been previously found in the interactome of both RNF8 and MDC1, suggesting that EWS may directly interact with these proteins at DNA damage sites. Overall, our results strengthen the notion that EWS participates in the DSB response and suggest a role in the stabilization of the members of the MDC1-RNF8-RNF168 axis, which are crucial for the formation of DSB repair complexes. *The authors marked with an asterisk equally contributed to the work.

P-36-049
Identifying genes involved in geroconversion

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Senescent cells are stable growth arrested and accumulate with age in multiple tissues. While we know the mechanisms by which many stressors induce cell cycle exit, how signaling pathways affect lysosomal biology, and how the senescence associated secretory programs (SASP) are activated, we know little about this developmental pathway. To elucidate changes that occur as cells transition from reversible cell cycle exit into stable arrest, a process called geroconversion, we developed a synchronized system using CDK4/6 inhibitor (CDK4/6i) therapy-induced senescence and performed RNA sequencing at different time points. Approximately 150 non-SASP-related transcripts are regulated coincidentally with stable arrest before the elaboration of the inflammatory SASP. These encode various nuclear, cytosolic, membrane-associated, and secreted proteins, which are not similarly regulated in quiescent cells. We are looking at the expression of these transcripts in multiple therapy-induced senescence models representing diverse tissue types to determine if any might have a broadly conserved function and provide general insight into the process. Additionally, we will perform a shRNA screen to understand their regulation in the process of geroconversion. Mapping the landscape of cellular senescence under physiological or pathological conditions will undoubtedly aid the targeted development of therapeutic approaches for senescent cells.

P-36-050
ALS-linked mutations at amino acid position 93 in superoxide dismutase 1 (SOD1) lead to aggregation and disrupt the mitophagy process

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Amyotrophic lateral sclerosis (ALS) is a neurodegenerative disease characterized by progressive demise of motor neurons. A major known cause of familial ALS is the mutation of the gene encoding superoxide dismutase 1 (SOD1) that leads to abnormal protein aggregates. Six mutations have been identified at amino acid position 93 in SOD1, notably G93A, which has been extensively studied in transgenic mice showing protein aggregation. In this study, we generated 19 mutations at the glycine residue of position in SOD1, situated in a loop structure, and their ability to form aggregates was examined in N2a neuronal cells using fluorescence microscopy. All mutants, except wild-type SOD1 G93, formed aggregates, regardless of any amino acid substitutions including those with non-polar, polar, basic, and acidic amino acids, indicating the critical role of glycine's structural flexibility in SOD1 folding The mechanism by which SOD1 aggregation contributes to ALS pathogenesis remains unclear. Our next study reveals that G93A mutant SOD1 (mSOD1) proteins interact with and sequester optineurin essential for mitophagosome formation, into aggregates in N2a cells, leading to reduced mitophagy flux. This suggests that ALS-linked mutations in SOD1 interfere with mitophagy by sequestering optineurin, potentially implicating the accumulation of damaged mitochondria in ALS pathology.

P-36-051
Circulating cell-free mitochondrial DNA as a proinflammatory DAMP for inherited hemolitic anemia

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Circulating cell-free mtDNA (ccf-mtDNA) is perceived as Damage Associated Molecular Patterns (DAMPs) by Pattern Recognition Receptors (PRRs). Recently, ccf-mtDNA has been widely used as a biomarker of mitochondrial function and is increasingly proposed to study disease-associated changes in blood samples. The pathophysiology of diseases that cause inherited hemolytic anemia, such as thalassemia and sickle cell anemia (SCA), is driven by chronic inflammation fueled by DAMPs. This study aimed to evaluate ccf-mtDNA levels in thalassemia and SCA by comparing them with mitochondrial disease as positive control and healthy negative control groups. Fifty-seven plasma samples from thalassemia, SCA, mitochondrial disease patients, and age/gender-matched 12 healthy controls were included in this study. Cell-free DNA from plasma samples was performed with Quick-cfDNA™ Serum-Plasma Kit. Quantitative PCR was performed in Q2000b Real-Time PCR with an SYBR green I master mix, 20 ng of cfDNA, and specific primers designed for the genes encoded in mtDNA, ND1, and COXI. The two housekeeping genes GAPDH and B2M expression levels were analyzed for normalizations. For each gene, the mean Ct value of the two replicates for each sample was calculated. Statistical comparisons between groups were analyzed using Student's t-test. A value of $p < 0.05$ was considered to indicate statistical significance. When compared to the control group, both thalassemia and SCA patients had significantly higher levels of ccf-mtDNA ($p < 0.01$ and $p < 0.001$ respectively). Mitochondrial disease patients also had a higher level of ccf-mtDNA compared to the control group ($p < 0.05$). ND1 and COX1 results were similar and thus showed consistency, as expected. ccf-mtDNA is an erythrocytic DAMP that highlights the role of mitochondria in the pathology of inherited hemolytic anemias. It may be a predictive biomarker in the follow-up of antioxidant therapies. *The authors marked with an asterisk equally contributed to the work.

P-36-052
Adult zebrafish brain as a demyelination model and role of Wnt signaling in remyelination

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Wnt/β-catenin signaling is an evolutionarily conserved pathway from primitive metazoans to complex mammals and controls cell fate determination during embryonic development, maintenance of tissue homeostasis, and tissue regeneration. Canonical Wnt signaling is known to contribute to the decision/fate of oligodendrocyte progenitor cells (OPCs) to differentiate during both development and remyelination after demyelination. Recent experimental findings present conflicting evidence regarding the precise function of the Wnt signaling pathway in the processes of myelination and remyelination. In the present study, we aimed to explore the activity and functional role of Wnt signaling in the adult zebrafish brain demyelination/remyelination model. For this purpose, we combined the cerebroventricular microinjection (CVMI) method with Lysolecithin (LPC) to develop

demyelination and investigated remyelination. We administered 10 μM IWR-1 to the fish water starting a day before the CVMI procedure to complete the inhibition Wnt/β Catenin signaling pathway. Brain samples were collected at 1-, 3-, and 7- days post injection (dpi) as we determined stages as demyelination, debris clearance/transition, and remyelination, respectively. Gene expression changes related to oligodendrocyte lineage and myelination, immune response formation, neuronal and glial cell markers, apoptosis, and proliferation were determined by using qPCR and immune staining. Our results indicate that inhibition of Wnt signaling alters the remyelination process. Furthermore, quantitative proteomic analyses were conducted to assess differentially expressed proteins and the Wnt targetome during these processes. We believe our study will contribute to the role of the molecular mechanisms underlying demyelination and remyelination of the brain as well as the involvement of Wnt/β-catenin signaling in these processes.

P-36-053
Effect of probiotics on vascular calcification promoted by *Porphyromonas gingivalis* in vascular smooth muscle cells

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Vascular calcification is the pathological precipitation of phosphate and calcium in the vasculature, closely associated with an increased risk of cardiovascular events and mortality. In a previous study, we demonstrated that infection with *Porphyromonas gingivalis* (*P. gingivalis*), a major periodontal pathogen, increases inorganic phosphate-induced vascular calcification through phenotype transition, apoptosis, and matrix vesicle release in vascular smooth muscle cells. Additionally, *P. gingivalis* infection accelerated phosphate-induced calcium deposition in cultured rat aorta *ex vivo*. Overall, our findings confirm that *P. gingivalis* may play a role in vascular diseases associated with periodontal infection and vascular calcification. We are further exploring the relevance of various probiotics in diseases triggered by periodontal bacteria. Specifically, we are investigating the impact of probiotics on vascular calcification promoted by *P. gingivalis*. Previously published in: Hyun-Joo Park et al. (2020) Cells 9(12) 2694.

P-36-054
Adenosine in neutrophil-fibroblast interplay: effects of the neutrophil extracellular trap release on a cellular model of lung fibrosis

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Lung fibrosis is characterized by the accumulation of myofibroblasts and the deposition of extracellular matrix boosted by immune system activation, which progressively leads to respiratory failure. Recently, besides the central role played by fibroblasts, other mechanisms such as neutrophil activation and the release of neutrophil extracellular traps (NETs)1 have emerged. Herein, the effects of extracellular stimuli, such as adenosine (ADO), on the neutrophil-fibroblast cross-talk were deeply investigated. ADO acts as a signalling molecule through adenosine receptors (ARs) but also as a genetic and metabolic regulator. Thus, the AR expression and the purinome machinery (ADO production (CD39, CD73, ADK), transport (ENT1, ENT2), and degradation (ADA)) were investigated in differentiated HL-60 cells. ADO was also able to modulate NET release. In parallel, a cellular model of myofibroblast to lipofibroblast differentiation was set up. Human lung fibroblasts, IMR-90, were treated with TGF-b alone or in the presence of rosiglitazone to prompt the switch. The lipofibroblast phenotype was revealed by the decrease of a-SMA expression and the increase of lipid droplet content. A specific expression pattern of AR and purinome machinery was evident in myo- vs lipofibroblasts. Finally, to investigate the neutrophil-fibroblast interplay, NETs were isolated, characterized and used to treat fibroblasts. NETs per se were not able to affect the fibroblast viability, however, a modulation of myofibroblast phenotype was evidenced. Overall, the results pave the way for the identification of new potential therapeutic targets able to modulate different mechanisms involved in fibrosis progression. This study received funding from European Union-NextGenerationEU-National Recovery and Resilience Plan (PNRR)–MISSION 4 COMPONENT 2, INVESTIMENT N. 1.1, PRIN 2022–2022NAFK8C CUP I53D23004290006 1. Previously published in: Suzuki, M. et al. Am J Respir Cell Mol Biol. (2020) 63, 806-818.

P-36-055
Exploring IsdB/hemoglobin complex to develop new antimicrobial strategies against *Staphylococcus aureus*

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Staphylococcus aureus has the ability to develop resistance to a great number of antibiotics used in clinics, posing significant challenges in clinical management. *S. aureus* relies on iron to grow and induce infections, therefore the bacterium developed different strategies to acquire iron from the host. In mammals, the main iron source is represented by hemic iron, which is extracted from cell-free hemoglobin (Hb) by two cell wall-anchored proteins named IsdB and IsdH, the former being the most relevant for virulence. In order to develop new antimicrobial strategies for *S. aureus* based on iron starvation, we undertook a virtual screening-driven drug discovery campaign, aiming at targeting cell-free Hb released by hemolysins upon infection, at the IsdB-Hb interacting region, thus preventing heme extraction. The most promising compound that we have identified, C35, binds Hb with a low micromolar dissociation constant. X-ray crystallography revealed that the compound binds Hb at a binding site among alpha-subunits, in a well-known allosteric site involved in a quaternary transition to a relaxed high-affinity conformation. However, the compound was also able to directly interact with IsdB as observed by STD-NMR, thus revealing a

second mechanism for inhibiting the formation of the IsdB-Hb complex and slowing down heme extraction. The development of more potent C35 derivatives will enable testing on *S. aureus* with the aim to find effective inhibitors of iron acquisition that might find application as antimicrobials or enhancers of antimicrobial therapy, thus contributing to contrast the insurgence of resistance in *S. aureus*. Project funded by: PRIN2020AE3LTA "Defeat antimicrobial resistance through iron starvation in *Staphylococcus aureus* (ERASE)."

P-36-056
Conformational dynamics and susceptibility to proteolysis of human transthyretin oligomers unravel their possible role in the mechanism of fibril formation

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Human transthyretin (TTR) is a homo-tetrameric protein responsible for the onset of an acquired form of systemic amyloidosis, which is caused by the deposition of fibrils of TTR in different organs, especially in the heart. In most patients, fibrils consist predominantly of protein C-terminal fragments (e.g., 49-127). Fibrils are not the only aggregated form of TTR that has been detected *in vivo*. Indeed, in a hereditary form of TTR amyloidosis, circulating soluble oligomers of the protein were identified as a diagnostic biomarker and driver of the disease. Even if proteolysis of TTR appears to modulate the amyloidogenic potential of the protein, the mechanism of amyloid formation by proteolytic cleavage of TTR is not completely understood. In an attempt to delineate a mechanistic explanation to the production of amyloidogenic TTR fragments leading to transthyretin amyloidosis, in this study we examined the susceptibility to proteolysis of human TTR oligomers using digestive, coagulative and fibrinolytic proteases. The conformational features of TTR oligomers were elucidated by hydrogen/deuterium exchange mass spectrometry (HDX-MS), an emerging structural biology technique providing valuable insights into the dynamics of proteins in solution. HDX-MS analysis showed that oligomers display an enhanced structural flexibility compared to native TTR that is localised mainly at the level of regions involved in tetramer stabilization. Importantly, oligomers were found to be more susceptible to proteolysis by trypsin, plasmin, α-thrombin and factor XIa than the native tetrameric protein, leading to the formation of the more amyloidogenic C-terminal fragments. These findings identify two novel TTR-cleaving proteases along the pathway to blood coagulation (i.e., α-thrombin and factor XIa) and provide a new pathogenic mechanism of fibril generation in which oligomer formation may drive TTR proteolysis and amyloid formation *in vivo*.

P-36-057
Metabolic reprogrammed primary skin fibroblasts as patient-specific cellular models for Parkinson's disease

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Mounting evidence shows a causal link between mitochondrial dysfunction and Parkinson's disease (PD). The lack of the proper mitochondrial disposal by mitophagy seems to be an early and leading event in the pathogenesis. Mutations in mitophagy-related PRKN gene, which encodes the E3 ubiquitin ligase Parkin, have been linked to autosomal recessive juvenile PD (PARK2). Recently Rab proteins, especially Rab5, Rab 7 and Rab9, have been linked to the mitophagic pathway, and mutations in RAB32 and RAB39B are cause of familial PD. In order to elucidate the interplay between Rab proteins and dysfunctional mitophagy in PD, we exploited PRKN-mutated human skin fibroblasts and controls to evaluate levels and sub-cellular localization of a Rab proteins subset. Fibroblasts are easily accessible subject-specific cellular models for neurodegenerative diseases. However, they are less sensitive to mitochondrial insults because of their glycolytic metabolism. To render fibroblasts more sensitive to mitochondrial damage, we induced a metabolic reprogramming toward oxidative phosphorylation by substituting glucose with galactose in the medium. The increase of mitochondrial markers suggested that the protocol succeeded in increasing the mitochondrial mass. As regards Rab proteins, we observed a general increase of Rab5, Rab7 and Rab 9, hinting their role in mitochondrial biogenesis. We then exploited mitochondrial toxins related to PD, Rotenone and 1-methyl-4-phenylpyridinium (MPP+), to induce mitophagy, after the set-up of the proper experimental conditions in cells other than neurons. Metabolic reprogrammed fibroblasts resulted more sensitive to both rotenone and MPP+. Both mitochondrial dynamics and Rab proteins' level and localization resulted to be different in PRKN-mutated human skin fibroblasts, suggesting Rab proteins involvement in mitochondrial dynamics related to PD and the possible use of reprogrammed fibroblasts as patient-derived cell lines for PD investigation.

P-36-058
Rare disease mediated by endoplasmic reticulum glycoprotein quality control

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Endoplasmic reticulum (ER) retention of misfolded glycoproteins is mediated by the ER-localized eukaryotic glycoprotein secretion checkpoint, UDP-glucose glycoprotein glucosyl-transferase (UGGT). The enzyme recognizes a misfolded glycoprotein and flags it for ER retention by re-glucosylating one of its N-linked

glycans. In the background of a congenital mutation in a secreted glycoprotein gene, UGGT-mediated ER retention can cause rare disease, even if the mutant glycoprotein retains activity ("responsive mutant"). We shall present the results of our subcellular localization study of human Trop-2 mutants, which cause gelatinous drop-like corneal dystrophy (GDLD). Compared with the wild-type Trop-2, which is correctly localized at the plasma membrane, these Trop-2 mutants are retained in the ER. The membrane localization of the Trop-2 mutants was successfully rescued in UGGT1$^{-/-}$ cells. UGGT1 also efficiently reglucosylated one of the Trop-2- mutants *in cellula*. The study [1] supports the hypothesis that UGGT1 modulation would constitute a novel therapeutic strategy for the treatment of pathological conditions associated to misfolded membrane glycoproteins (whenever the mutation impairs but does not abrogate function). The results encourage the testing of modulators of ER glycoprotein folding quality control as broad-spectrum rescue-of-secretion drugs in rare diseases caused by responsive secreted glycoprotein mutants. Reference: [1] Tax G, et al. Traffic. 2024 Jan;25(1): e12927. doi: 10.1111/tra.12927.

P-36-059
Sex-dependent effects of hyperlipidemia and HSPB1-overexpression on cardiac function in transgenic mice

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Metabolic syndrome is a complex disease involving obesity and hyperlipidemia increasing the risk of cardiovascular diseases. Heat-shock proteins (HSP) have been found to have protective functions in several chronic diseases, however, their role in metabolic disorders is not yet fully understood. Therefore, we studied the effects of HSPB1 in high-fat diet-fed APOB-100-overexpressing strain, a mouse model of hyperlipidemia, by crossing the disease model animals with HSPB1-overexpressing mice (APOB/HSP). In both sexes, the bodyweights of the APOB animals were higher compared to the healthy mice. Interestingly, APOB females showed a further increase in bodyweight when HSPB1 was overexpressed. The serum triglyceride and LDL-cholesterol levels were significantly higher in APOB males compared to the female group. HSPB1 did not affect serum triglyceride levels, however, it significantly decreased LDL-cholesterol levels in APOB males. In contrast, APOB/HSP females showed a higher LDL level compared to APOB females. Transthoracic echocardiography revealed significantly decreased left-ventricular end-diastolic diameter, increased wall thickness, and higher E/e' in the APOB males than in the healthy animals, suggesting the development of mild hypertrophy and diastolic dysfunction induced by hyperlipidemia. In contrast, these symptoms were not pronounced in females. HSPB1 overexpression did not affect these parameters in APOB mice, however, it induced a physiological form of hypertrophy in healthy animals. According to our data, male APOB mice show more severe hyperlipidemia and cardiac dysfunction after 7 months of a high-fat diet compared to females. Additionally, HSPB1 overexpression influenced serum LDL-cholesterol levels and weight gain in a sex-dependent manner suggesting its role in the regulation of obesity-related disturbances. This work was supported by NKFIH FK138390, ÚNKP-23-5-SZTE-708, and the János Bolyai Research Fellowship of the Hungarian Academy of Sciences. *The authors marked with an asterisk equally contributed to the work.

P-36-060
The serine-rich repeat glycoprotein Srr2 from *Streptococcus agalactiae* acts as an adhesin for the human fibronectin

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Group B *Streptococcus* (GBS) is a colonizer of the healthy population and is accountable for severe diseases in elderly, pregnant women and newborns. GBS expresses several virulence factors that promote host colonization and invasion. Among these, the cell-wall anchored protein Srr2 binds fibrinogen (Fbg) through a dock, lock and latch (DLL) mechanism. The DLL is a mechanism based on conformational changes of the N2 and N3 subdomains of Srr2 upon ligand binding. In this study, we investigated GBS ability to bind fibronectin (Fn), a multidomain glycoprotein ubiquitously present in the human tissues, where it promotes cell adhesion and migration. We demonstrated that GBS exploits the adhesin Srr2 to bind Fn. We observed through ELISA assay that deletion of *srr2* strongly decreased GBS BM110 hypervirulent strain ability to bind Fn, while *srr2* complementation restored bacterial Fn binding ability. We investigated Srr2 mechanism of Fn binding through ELISA assay using recombinant wt or mutated binding regions (BR) of Srr2, purified through affinity chromatography. Mutated Srr2-BRs defective in Fbg binding were not able to bind Fn as well, suggesting the same DLL mechanism of binding. We identified the Fn domain bound by recombinant Srr2-BR at the level of the central cell-binding domain of Fn through dot-blot assay and western-blot of recombinant Srr2-BR-conjugated microspheres incubated with Fn or Fn fragments. Finally, we observed that GBS ability to adhere to epithelial cervico-vaginal HeLa cells' monolayers is strongly increased upon addiction of exogenous Fn. In conclusion our data suggest that Srr2 plays an important role in GBS adhesion to Fn and demonstrate the importance of fibronectin in mediating GBS adhesion to the host. In light of this, our results may be useful to develop new alternative strategies to prevent GBS colonization of host epithelia and control GBS infections.

P-36-061
Integrative structural characterisation of the pathogenic ordered aggregation pathway of alpha-1-antitrypsin

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We have integrated single-particle EM, biomolecular NMR, X-ray crystallography and mass spectrometry, conformationally selective monoclonal antibodies and clinical samples to structurally elucidate a disease-associated protein aggregation pathway. α_1-Antitrypsin is a member of the serpin family of protease inhibitors, expressed at high levels by hepatocytes and abundant in the plasma where it protects tissue from damage by neutrophil elastase. In its active state, α_1-antitrypsin is in a kinetically stable, but thermodynamically unstable, configuration. This makes it susceptible to inappropriate conformational change: it is one of the causative agents of a class of conformational pathologies termed serpinopathies. In the presence of certain mutations, α_1-antitrypsin accumulates in the liver as dense intracellular deposits; these are the consequence of a non-amyloid ordered aggregation that yields unbranched protein polymers, that are both extremely stable and functionally inactive. This in turn leads to a circulating deficiency and a corresponding protease-antiprotease imbalance, predominantly in the lung, predisposing affected individuals to emphysema and COPD, whilst their hepatic accumulation can lead to liver disease. Using orthogonal structural and biophysical techniques applied to protein extracted from human-derived samples, we have defined molecular details of the serpin polymerisation pathway. Our data support a mechanism whereby α_1-antitrypsin progresses through distinct states as it transitions from a mildly-perturbed monomeric intermediate to a hyperstable polymeric form whose subunits have undergone an intra- and inter-molecular reconfiguration. This has allowed us to draw conclusions regarding transient species on the pathway and the likely mechanism by which the barrier to conformational change is circumvented. The commonalities between mechanistic function and pathological malfunction reveal a molecule that is poised between these opposing outcomes. *The authors marked with an asterisk equally contributed to the work.

P-36-062
Bi-allelic inactivating variants of lactosylceramide synthase B4GALT5 responsible for a novel congenital disorder of glycosylation involving glycosphingolipids

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A 7-year-old girl of Italian ancestry presenting with developmental delay, intellectual disability, microcephalia, and bilateral cataract was found to carry compound heterozygous variants in the *B4GALT5* gene (deposited in Decipher: ID 412221) encoding a galactosyltransferase producing lactosylceramide (LacCer), the precursors of the bulk of glycosphingolipids. By LC/MS, an accumulation of ceramide monohexoside and a relevant loss of LacCer were detected in patient's serum compared to her parents and age-matched healthy controls. The human B4GALT5 cDNA was cloned in an expression vector and by site directed mutagenesis each of the two patient's variants were introduced. The constructs were then transfected into HEK-293T cells to measure the enzyme activity *in vitro*. In contrast to the wild-type construct, cells transfected with constructs carrying each of the variants resulted in an enzyme activity indistinguishable from the background, suggesting almost no residual activity. B4galt6 has been also reported as LacCer synthase in studies performed using knock-out mouse models, which suggested that b4galt5 is the major LacCer synthase that can be rescued by b4galt6 in the mouse brain. We generated HEK-293T cells where *B4GALT5* is knocked out and found that glycosphingolipid biosynthesis is impaired. We compared the ability of human and mouse B4GALT6, as well as of B4GALT5 variants, to restore the cell phenotype. Our findings indicate that human B4GALT6 and B4GALT5 variants very poorly rescue wild-type B4GALT5, probably not enough for rescuing specific human brain functions. Modelling studies are in progress to assess the structural basis of the relevant ortholog differences. Although further cases need to be identified to confirm the nature of this new disorder and to define the spectrum of clinical manifestations, our data suggest that we have identified a novel disease B4GALT5 congenital disorder of glycosylation (B4GALT5-CDG).

P-36-063
Molecular interactions of Nsp14 of SARS-CoV-2 with the human enzyme IMPDH2

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The emergence of the COVID-19 pandemic prompted global efforts in the search for anti-coronavirus drugs and vaccines. Host-pathogen interactome studies revealed the interaction between the non-structural protein Nsp14 (an RNA proofreading and capping protein) and the human enzyme inosine 5′-monophosphate dehydrogenase 2 (hIMPDH2), essential for the

synthesis of purine bases. This interaction is shared across MERS, SARS-CoV-1, and SARS-CoV-2, making it a potential target for pan-coronavirus antivirals. We recombinantly produced and purified both proteins in *E. coli* and analysed the enzymatic activity of hIMPDH2 in the presence of Nsp14. The results demonstrated a linear increase in hIMPDH2 activity with rising concentrations of Nsp14. This aligns with previous findings of enhanced production of nucleotides and purine-base precursors in *in vitro* cellular experiments. The binding affinity of Nsp14 with the untagged hIMPDH2, as measured using surface plasmon resonance, was in the low micromolar range. However, binding was lost when an N-terminal His-tagged construct of hIMPDH2 was used. Furthermore, contrary to the untagged hIMPDH2, the enzymatic activity of His-tagged hIMPDH2 was not enhanced by Nsp14, suggesting that an intact N-terminus of hIMPDH2 is necessary for Nsp14 binding and hIMPDH2 enzymatic activity enhancement. Consistent with these observations, structure prediction of the Nsp14-hIMPDH2 complex using AlphaFold Multimer revealed a model where the N-terminus of hIMPDH2 binds to a central groove of Nsp14, thus rationalizing the loss of binding and the enzymatic activity enhancement when His-hIMPDH2 is utilized. Our functional and *in silico* structural prediction analysis provide a model of the Nsp14-hIMPDH2 complex, to be validated through experimental structural analysis. Additionally, this model reveals a binding interface to be targeted for the design of pan-coronavirus antivirals.

P-36-064
Targeting lipid peroxidation in cystic fibrosis as a new approach to assist current therapy

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Cystic fibrosis (CF) is an inherited disorder caused by mutations in the gene encoding for the cystic fibrosis transmembrane conductance regulator (CFTR), a chloride/bicarbonate channel located in different tissues and notably in the lungs. Here, CF is responsible for airways obstruction by viscous mucus and chronic inflammation. Approved therapies are addressed to a limited numbers of mutations consequently the search for new therapeutic proposals is still urgent. The most common variant, F508del, is responsible for misfolding, ER accumulation and premature degradation of the channel preventing its trafficking to the plasma membrane. This results in ER stress, persistent inflammation and oxidative imbalance related to an overproduction of ROS. Specifically, a marked depletion of glutathione (GSH), as well as of its synthesis regulator NF-E2-related factor 2 (Nrf2), is observed in CF cell lines which fosters the oxidative damage of polyunsaturated fatty acids (PUFA) via lipid peroxidation (LPO). LPO may evolve in ferroptosis, an iron-mediated form of cell death. We detected high ROS and LPO levels in cells expressing F508del-CFTR (CFBE41o- parental) compared to WT (16hBE). Based on that, we aim to improve mutated CFTR proteostasis and function by restoring the redox homeostasis. Indeed, we analysed molecules with specific radical-trapping activity demonstrating a decrease of LPO, as observed through C11-Bodipy technique, and a protection of CF cell lines from erastin-induced ferroptotic cell death. Furthermore, these compounds, in specific combinations with current therapies, increase channel trafficking at the PM and reinstate CFTR-mediated ion transport in CFBE epithelia providing an enhanced effect in Ussing chamber experiments. The obtained data suggest a novel approach that could implement existing clinical therapies. Being not directed against the mutated CFTR, this proposal holds the potential to be translated to orphan mutations.

P-36-065
Effect of D-ribose on glycation of human high-density lipoprotein

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Advanced glycation end products (AGEs) have significant role in the pathophysiology of diabetes. Glucose, fructose and methylglyoxal trigger glycation of plasma lipoproteins as we previously demonstrated. D-ribose is a naturally occurring pentose monosaccharide present in all living cells and their microenvironments and is a key component of numerous biomolecules involved in many important metabolic pathways. It also participates in the glycation of proteins, producing AGEs that lead to cell dysfunction and death. Recent studies show that incubation with D-ribose causes protein aggregation and alterations of their functional activities. No report has been previously published to investigate D-ribose induced glycation (ribosylation) of high-density lipoprotein (HDL). The aim of the study was to investigate the effect of *in vitro* ribosylation on human HDL composition and functions; in particular, the activity of the HDL surface-associated enzyme paraoxonase-1 (PON1) and HDL antioxidant properties, were investigated using the dihydrorhodamine 123 (DHR) assay. The significant increase in fluorescent AGEs after incubation with D-ribose, confirms that human HDL is susceptible to ribosylation. The decrease of free amino groups and of intrinsic fluorescence of tryptophan demonstrates HDL apoprotein modifications in ribosylated lipoproteins. The compositional changes are associated with a significant decrease of PON1 activity and impaired HDL antioxidant properties. Our results provide evidence that ribose triggered glycation of HDL interferes in their normal physiological functions and might contribute to diabetic complications such as atherosclerosis and other cardiovascular diseases. *The authors marked with an asterisk equally contributed to the work.

P-36-066
Insulin signaling is critical for sinoatrial node maintenance and function

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Insulin and insulin-like growth factor 1 (IGF-1) signaling regulate cellular growth and glucose metabolism in the myocardium. However, their physiological role in the cells of the cardiac conduction system has never been explored. Therefore, we sought to determine the spatiotemporal function of insulin/IGF-1 receptors in the sinoatrial node (SAN). We generated cardiac conduction cell–specific inducible IGF-1 receptor (IGF-1R) knockout (KO) (CSIGF1RKO), insulin receptor (IR) KO (CSIRKO), and IR/IGF-1R double-KO (CSDIRKO) mice and evaluated their

phenotypes. Telemetric electrocardiography revealed regular sinus rhythm in CSIGF1RKO mice, indicating that IGF-1R is dispensable for normal pacemaking. In contrast, CSIRKO and CSDIRKO mice exhibited profound sinus bradycardia. CSDIRKO mice showed typical sinus node dysfunction characterized by junctional rhythm and sinus pauses on electrocardiography. Interestingly, the lack of an insulin receptor in the SAN cells of CSIRKO and CSDIRKO mice caused sinus nodal fibrosis. Mechanistically, hyperpolarization-activated cyclic nucleotide-gated channel 4 (HCN4) protein expression significantly decreased in the CSIRKO and CSDIRKO mice relative to the controls. A patch-clamp study of the SAN cells of CSIRKO mice revealed a significant decrease in the funny current, which is responsible for spontaneous diastolic depolarization in the SAN. This result suggested that insulin receptor loss reduces the heart rate via downregulation of the HCN4 channel. Additionally, HCN1 expression was decreased in CSDIRKO mice, explaining their sinus node dysfunction. Our results reveal a previously unrecognized role of insulin/IGF-1 signaling in sinus node structural maintenance and pacemaker function.

P-36-067
N-acetylaspartate promotes glycolytic-to-oxidative fiber-type switch in myotubes

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N-acetylaspartate (NAA) is an N-acetylated derivative of aspartate and stands as the second most abundant metabolite in CNS, reaching concentrations of approximately 10 mM. NAA is almost exclusively synthesized in neurons by the mitochondrial enzyme aspartate N-acetyltransferase 8-like (NAT8L) starting from aspartate and acetyl-CoA. It is widely used as a marker for neuronal integrity/function, and its brain concentration promptly decreases in neurodegenerative disorders while increasing in the circulatory blood system [Previously published in: Schuff N. et al. (2006) Adv Exp Med Biol. 576: 241–363]. Although NAT8L is primarily expressed in CNS, aspartoacylase (ASPA), which catabolizes NAA into free acetate and aspartate, is more widely distributed in most peripheral tissues [Previously published in: Bogner-Strauss JG. (2017) Front Endocrinol (Lausanne) 8:240]. We focused our attention on the skeletal muscle, which is frequently altered in patients affected by neurodegenerative disorders, such as motor neuron diseases. In particular, amyotrophic lateral sclerosis (ALS) induces a change in muscle metabolic phenotype passing from glycolytic-to-oxidative fiber types [Previously published in: Scaricamazza S. (2020) iScience 23 (5):101087]. Our results indicate that treatment of differentiated myotube with NAA induces this type of metabolic switch by enhancing lipid turnover. The importance of NAA catabolism was demonstrated by the use of CRISPR-Cas9 ASPA knockout muscle cells. Overall, we hypothesize that NAA accumulation in the blood of patients suffering neurodegenerative diseases may contribute to brain-body signalling and impact the metabolism and adaptive response of the skeletal muscle.

P-36-068
COVID-19 induces cell surface-associated fibrinolysis shutdown

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Hypercoagulation, thrombosis, and ARDS are typical complications in patients with COVID-19. The thrombotic tendency is often linked to increased plasminogen activator inhibitor-1 (PAI-1) levels and a concomitant suppression of fibrinolysis. In the present study, we examined changes in cell- and fibrin-associated fibrinolysis markers, their correlation with inflammatory factors and determined the predictive impact of urokinase and tissue-type plasminogen activators (uPA and tPA) and PAI-1 as a prognostic biomarker for COVID-19 severity. The cohort included 69 Japanese adults admitted to hospitals due to COVID-19 and 20 healthy controls. We found elevated free, non-complexed PAI-1 antigen, low circulating uPA, and uPA/PAI-1 but not tPA/PAI-1 complex levels associated with COVID-19 severity and ARDS development. This biomarker profile was typical for patients in the complicated phase. There was a lack of PAI-1 activity in circulation despite free, non-complexed PAI-1 protein. Furthermore, plasmin/α2antiplasmin complex – known to indicate active plasmin – correlated with soluble uPAR and soluble VCAM levels, markers indicating endothelial dysfunction. uPA/PAI-1 complex levels positively correlated with TNFα, a cytokine reported to trigger inflammatory cell death and tissue damage. Those levels also positively correlated with the pro-inflammatory factors interleukin1b (IL1b), IL6, and C-reactive protein, markers associated with the anti-viral inflammatory response. Reductions in uPA, a crucial protease for cell-associated plasmin production, and uPA/PAI-1 complex, alongside increased plasmin/α2antiplasmin complexes, indicate activation of fibrinolysis in circulation, but suppression of pericellular plasmin generation in individuals diagnosed with COVID-19. The finding that the disease severity correlates with cell-associated fibrinolysis shutdown argues for using uPA and uPA/PAI-1 as novel biomarkers to detect patients at risk of developing severe COVID-19, including ARDS.

P-36-069
Development of methods and tools in NPCs and zebrafish towards modeling of DNA sequence variants in patients with pachygyria

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The main bottleneck in identification of DNA variants that cause neurogenetic diseases is functional analysis of VUS. The aim of this study was to develop a methodology for modelling candidate causative variants observed in patients with pachygyria by using CRISPR/Cas9 genome editing in NPCs and zebrafish. DNA from 20 patients with pachygyria/lissencephaly were analyzed by aCGH and WES, and variants were prioritized. Mutant lines were generated in NPCs and Zebrafish by using CRISPR/Cas9 genome editing, and compared to models where one of three key genes (TUBG1, LIS1, DAB1) that are known to play role in pachygyria/lissencephaly. Characterization of NPCs were performed with a 3D matrigel chamber system (IC-Chip) and phenotypic changes were observed in developing zebrafish at 3 dpf and 5dpf. The comparison of target mutant lines and selected variant lines was made with qPCR. A delay in migration was observed in mutant NPC lines of three selected genes compared to control group. WES identified two candidate variants, CGREF1 and NOL9. Expression changes of lissencephaly and microcephaly-related and neuronal differentiation genes in CGREF1-KO-zebrafish and CGREF1-KO-NPCs were observed. A severe phenotype including small-head and eyes, and abnormal liver/gut development was observed in Tubg1 mutant zebrafish. Our results provide evidence that variants that cause defects associated with NPC migration can be tested using NPC and zebrafish models in a time- and cost-efficient manner. Multi-omic analysis could further expand the use of this approach to other groups of neurogenetic defects. This Project was supported by TUBITAK-COST Action with 217S944 code number.

P-36-070
Proteomics and metabolomics of lysosomal alterations in methylmalonic acidemia

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Methylmalonic acidemia (MMA) is an autosomal recessive inherited metabolic disorder whose pathogenesis involves defects in the catabolism of propionyl-CoA. It is caused by defective activity of methylmalonyl-CoA mutase (MUT) enzyme, responsible for the mitochondrial conversion of the methylmalonyl-CoA into succinyl-CoA. The enzymatic defect leads to accumulation of toxic metabolites such as methylmalonic acid and methylcitric acid in body fluids due to alterations in the degradation of odd-chain fatty acids, cholesterol side chain, and branched-chain amino acids. Actually, the exact mechanisms that drive the cell stress in MMA are not completely elucidated and the therapeutic options available are not sufficient for patients' management. In order to understand the molecular mechanisms of cellular damage in the MMA disease, we employed a MUT-knockout (MUT-KO) HEK-293cellular model by CRISPR/Cas9 and MMA patients-derived fibroblasts. The cellular proteomic and metabolomic profiles were characterized using mass spectrometry-based approach and bioinformatics, showing alterations in autophagy- and lysosomal-related pathways. Functional experiments demonstrated a strong compromission of the lysosomal/autophagic machinery in both MUT-deficient cell lines. The biochemical alterations found in the proteome of the MMA cell models are in part reverted in a MUT-rescued HEK-293 cell line, reporting a significant connection between the MMA-induced damage and the compromission of the lysosomal and autophagic cellular functions. Finally, the findings obtained in the cellular models may provide novel potential pathways to be targeted for therapeutic intervention in MMA.

P-36-071
Characterization of alpha-1 antitrypsin mutants with an alternative polymerisation mechanism

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Mutations in the SERPINA1 gene can cause alpha-1 antitrypsin deficiency (AATD), where Z (Glu342Lys) represents the most common severe mutation. The formation of ordered Z alpha-1 antitrypsin (AAT) polymers in hepatocytes is associated with liver disease and a deficiency of circulating AAT, predisposing to early onset emphysema. Current evidence suggests that the Z mutation induces a mildly perturbed intermediate conformation (M*) which likely undergoes a substantial structural rearrangement, resulting in polymers mediated by inter-molecular domain swap. The polymers formed by Z AAT, found in the plasma and within liver tissue, expose a cryptic epitope recognised by a monoclonal antibody (2C1), and their formation can be inhibited in cellular and animal models by a small molecule (c716). This molecular chaperone acts by maintaining the molecule in a monomeric state stabilising the M* intermediate. Here, we present the biochemical characterisation of naturally occurring AATD-associated mutants that do not expose the 2C1 epitope, and additionally show resistance to the activity of the molecular chaperone c716, indicating differences in structure and/or kinetics of their formation. These observations have led us to hypothesize an alternative polymerisation mechanism for these variants. Moreover, to study the formation and accumulation of such polymers, we have also generated a new monoclonal antibody capable of selectively recognising a wider range of polymers. Taken together, our data could represent an important bridge to clarify how different AAT mutations may be linked to various pathologies associated with serpin aggregation.

P-36-072
Development of CRISPR/Cas9-edited HEK293 cells to study the impact of N-glycosylation defects on IGF1 production and IGF1 receptor signaling activation

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Deficiencies of N-linked and other glycosylation pathways lead to a clinically heterogeneous group of inherited diseases called congenital disorders of glycosylation (CDG) [1]. Growing evidence suggests that insufficient levels and/or activity of growth factors, including the insulin-like growth factor 1 receptor (IGF1R) signaling, might contribute to some of the clinical manifestations in CDG including developmental delay and intellectual disability [2,3]. Here, we use the CRISPR/Cas9 system to inactivate individual glyco-genes (*PMM2*, *GMPPB*, *ALG3*, *ALG8*, *STT3B*, *VPS13B*, *MGAT2*, *CLCN2*) in HEK293 cells. The gene editing efficiency was checked with GeneArt® Genomic Cleavage Kit and Sanger sequencing. The IGF1Ea prohormone production and IGF-1R expression and signaling pathway activation were quantified by western-blotting and ELISA assays in CRISPR/Cas9-edited HEK293 cells and patient-derived fibroblast. Western blot analyses showed that WT HEK293 mainly expressed IGF-1Ea prohormones with two immunoreactive bands of ~17 (glycosylated) and ~12 kDa (unglycosylated), while the mature IGF-1 (~7 kDa) was barely detectable. We demonstrated that the *PMM2*, *GMPPB*, *ALG3*, *ALG8* and *STT3B* knockout causes an hypoglycosylation of the IGF1Ea prohormone in HEK293-edited cells associated with a reduction of IGF1 secretion in the culture media. Similar results were obtained in patient-derived CDG fibroblasts. The quantification of cell membrane IGF1R showed a reduction of protein expression in HEK293-edited cells and partially disrupts IGF1-induced IGF1R signaling pathway activation. This study provides new evidence of a direct link between N-glycosylation defects found in CDG and the impairment of IGF1 system components, suggesting an association between clinical CDG features and IGF1/IGF1R signaling abnormalities. References: [1] Freeze, H. et al. J Inherit Metab Dis 2022; 45:383. [2] Chan B. et al. Hum Mol Genet 2016; 25:2182. [3] Di Patria L. et al. Cellular and Molecular Life Sciences 2022; 79:150.

P-36-073
Insights into tick-borne encephalitis virus packaging process

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Tick-borne encephalitis virus (TBEV; *Orthoflavivirus encephalitidis*, *Flaviviridae*) poses a significant threat as one of the most serious tick-borne viral pathogens. It leads to severe neurological damage, known as tick-borne encephalitis, affecting thousands annually in the Eurasian continent. In recent years the expansion of the TBEV endemic areas, coupled with the absence of specific treatment, made it a focal point for the research. For targeted treatment to be developed, a comprehensive understanding of TBEV pathogenesis within the host cell is essential. However, several knowledge gaps persist regarding the TBEV. One of such blank spaces is an understanding of the production process of new viral progeny – referred to as "packaging" – and specific interactions of the TBEV capsid protein (C), the main structural block of the nucleocapsid, within the host cell. Specifically, we lack insight into how the correct (viral genomic) RNA is selected, transported, and packaged into newly formed virions. In our research, several approaches have been adopted to gain initial insight into the packaging process of TBEV, focusing on TBEV C protein. Firstly, we analysed the RNA content of the viral particles. Then, using mass spectrometry, we investigated the TBEV C interacting proteins in the host membrane compartments. Also, the role of TBEV non-structural protein NS2A in the assistance with the packaging process was studied. While our findings provide initial pieces to the puzzle, it is important to note that our data are still in the early stages of research. Further investigations are needed to unravel the complexities of TBEV packaging and contribute to a more comprehensive understanding of this critical topic.

P-36-074
Subcellular fraction-specific interactomes of the tick-borne encephalitis virus capsid protein

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The *Orthoflavivirus encephalitidis* (*Flaviviridae* family; *Orthoflavivirus* genus), previously known as tick-borne encephalitis virus (TBEV), is an arthropod-borne virus transmitted by ticks. This virus attacks the central nervous system causing a disease called tick-borne encephalitis whose incidence has been steadily increasing in Europe and Asia in recent years. Since there is a lack of a specific treatment for TBEV infection, attempts have been made to identify prospective candidates for drug targeting. One of these is the capsid protein (TBEV C), responsible for the formation of nucleocapsids. Its ability to bind the viral RNA and function in promoting the proper assembly of infectious particles makes it a crucial regulatory viral protein in infected cells. Even more attention has been paid to this protein since its nuclear localization was documented. Almost all TBEV C functions take

place in the cytoplasm or endoplasmic reticulum, and to this day, only a few details are known about the role of TBEV C in the nucleus. For these reasons, the primary aim of this work was to identify the TBEV C interaction partners in subcellular fractions, with an emphasis on the nuclear, organellar, and cytoplasmic binding partners using co-immunoprecipitation and mass spectrometry. To eliminate the identification of false-positive nucleic acid-mediated binding partners, benzonase was used to ensure the degradation of all DNA and RNA in samples. This protein was found to bind histones amongst others during the infection process which indicates an important role for TBEV C in the nucleus; however, further research is needed to verify our findings.

P-36-075
Structural basis of glucocorticoid receptor multimerization

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The glucocorticoid receptor (GR) is a ubiquitously expressed ligand-regulated transcription factor essential for life and one of the most targeted proteins in drug discovery due because of its powerful anti-inflammatory actions. The functional oligomeric state of the full-length receptor, which is essential for its transcriptional activity in cells, remains disputed. Here we present a new crystal structure of agonist-bound GR-LBD in a large cell, along with a thorough analysis of previous structural work. The building block of the current structure is a homodimer we previously identified in GR-LBD crystals and its biological relevance has been verified by studying a battery of GR point mutants including crosslinking assays in solution and quantitative fluorescence microscopy in living cells. Several mutually exclusive multimeric assemblies of this dimer in the crystal highlight the versatility of GR-LBD for self-association and reveal implications for the conformation of the active full-length receptor. Our results underscore the relevance of non-canonical dimerization modes for GR-LBD, especially of contacts made by key residues such as Tyr545, Pro637 and Asp641. Of note, a non-conservative mutation of the latter, p.Asp641Val, causes Chrousos syndrome in humans. Revealing relevant quaternary assemblies of the GR is pivotal not only to understand and predict the therapeutic outcome of major blockbuster drugs but also to lessen their deleterious side effects and open new avenues for drug design.

P-36-076
Exploring the role of AEBP1 protein in skeletal muscle fibrosis: insights from cellular models and cytokine regulation

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Skeletal muscle is maintaining heat homeostasis, mobility and circulation. Apart from the contractile myocytes, neuromuscular junction, circulation and a rich connective tissue is required for the tissue maintenance. Chronic injury repair cause excessive accumulation of extracellular matrix elements and particularly collagens lead to fibrosis lead to functional impairment. Maintenance and regeneration of skeletal muscle is concerted by the action of multiple cell types, cytokines and growth factors. Myoblasts, myo-fibroblasts and other stromal cells are majorly regulated by IGF1 and TGFβ. These two major cytokines involved in tissue regeneration also control the synthesis and organization of ECM elements. Adipocyte enhancer binding protein 1 (AEBP1) is a secreted ECM-associated protein expressed in smooth muscle, adipocytes and vascular cells, has emerged as a – functionally yet unknown – potential actor in fibrotic processes. Here in this study, C2C12 myoblast, 3T3-L1 pre-adipocytes and OP9 stromal fibroblasts are selected to model skeletal muscle stromal compartments to model fibrosis. These mesenchymal cells are treated with IGF1 and TGFβ across proliferation, confluence, and differentiation to investigate AEBP1 expression. IGF1 treatment did not influence AEBP1 expression while upregulating mitotic ratio. TGFβ treatment inhibited differentiation while upregulating AEBP1 expression, along with the upregulation of ECM production. In myoblasts, AEBP1 expression is upregulated along with myogenic differentiation. This is interpreted as an induction parallel to the ECM formation along with myotube development. AEBP1 expression is observed in proliferation and undifferentiated preadipocytes but expression is downregulated along with differentiation. This observation shows that AEBP1 expression is accompanying ECM components that are expressed in niche building. AEBP1 is expressed in different amounts on variant cell types and regulated via TGFβ but not IGF1.

P-36-077
Alternative model of protofibrils branching in fibrin polymerization

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Protofibril branching is a pivotal process in the formation of the three-dimensional polymerized fibrin network crucial for blood clot structure. While the mechanisms governing fibrin fibril branching are well-understood, those governing protofibril branching are more intricate, yet crucial during the initiation stages of fibrin self-assembly, significantly influencing the overall fibrin network structure. We hypothesized a novel mechanism of protofibril branching orchestrated by additional protein-protein interactions within the D-E-D complex in the protofibril. D-E-D interactions were modeled by forming a complex of fibrin desAB and D-dimer, with the D fragment serving as a D-region of monomeric fibrin or a fibrin from the sticky-end of neighboring protofibril. Size-exclusion chromatography confirmed the formation of a ternary complex comprising one molecule of desAB fibrin, one D-dimer, and one D fragment. Coarse-grained molecular dynamics simulations of the desAB fibrin-D-dimer complex revealed positional changes of the D-dimer relative to the fibrin molecule. After 40 ns, the D-dimer began to lean toward one of the coiled-coil regions of the fibrin molecule, elucidating why the complex of fibrin desAB with D-dimer can accommodate one additional D fragment. Study of the action of the D fragment on the inhibitory effect of D-dimer on fibrin polymerization, using turbidity studies and electron microscopy, confirmed the suggestion of intercalation of one D monomeric unit in D-E-D

interactions during the protofibril formation. Ternary complex of fibrin desAB, D-dimer and D fragment serves as a model for a potential branching point in protofibril formation. In this model, the central desAB fibrin molecule interacts with two other fibrin molecules end-to-end in the protofibril, while the D region of an external desAB fibrin molecule interacts with the central desAB molecule through the individual 'B-b' contacts. *The authors marked with an asterisk equally contributed to the work.

P-36-078
Multi-omics dissection of neurometabolic involvement in Mucopolysaccharidosis type IIIB

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Mucopolysaccharidosis (MPS) IIIB is a lysosomal storage disease due to the deficiency of α-N-acetylglucosaminidase (NAGLU), required for heparan sulfate (HS) degradation. The enzymatic defect leads to the accumulation of HS in liver, kidney, spleen, heart and in the central nervous system, resulting in multiple organ dysfunctions including hepatosplenomegaly, cardiac defects and mental degeneration with behavioral abnormalities. To dissect the molecular basis of neuropathological mechanisms in MPSIIIB, metabolomics and proteomics were performed to identify the brain signature of the disorder. To this aim, the murine model of the disease (NAGLU ko), exhibiting an increased HS levels in organs and biofluids, was used. Omics analyses revealed defects of mitochondrial activity accompanied by anaerobic metabolism, acting on autophagy and lysosomal defects. In addition, as NK1 (natural spliced variant of hepatocyte growth factor) is capable of reducing HS accumulation in MPS IIIB patient-derived fibroblasts, we used this compound to rescue MPS phenotype in NAGLU-silenced cells. Specifically, we investigated whether the mechanism of action of NK1 might involve autophagy regulation. The results provide insights into the molecular mechanisms of MPS IIIB physiopathology, supporting the development of new promising approaches based on autophagy inhibition and metabolic rewiring to correct lysosomal pathology in MPSs [1]. Finally, lipidomics reveals an unbalance in lipid cerebral metabolism highlighting the accumulation of specific lipid classes as lysophosphatydylcholines, hexosyl ceramides and di-tri-acylglicerols. Reference: [1] Scarcella et al. iScience. 2024 Jan 29;27(3):108959. doi: 10.1016/j.isci.2024.1089.

P-36-079
BCR-ABL1 chronic myeloid leukaemia (CML) transgenic fish: a new model to understand the pathogenesis of CML and a rapid and reliable test of tyrosine kinase inhibitors (TKIs)

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Philadelphia-positive (Ph+) chronic myeloid leukemia (CML) is a neoplastic myeloproliferative disease. In hematopoietic stem cell, the leukemogenic event is induced by the BCR:ABL1 oncogene derived from the translocation between chromosomes 9 and 22 and encoding for the BCR::ABL1 P210 protein which constitutively expresses a tyrosine kinase activity. To date, few animal models are mainly used to investigate the pathogenesis of CML. Conventionally, the pre-clinical studies for testing different tyrosine kinase inhibitors (TKIs), used for treatments of Ph+CML patients, are conducted using *in vitro* models as leukemic cell lines, but there are several limitations. To deepen our understanding of CML mechanism, we generated a new transgenic model expressing human BCR-ABL1 using a GAL4/UAS/hsp70 technique named tg(BCR-ABL1pUAS-CFP/hsp70-Gal4) that regulates gene expression in a cell-specific and temporally restricted manner. To test the robustness of the model, we treated our transgenic line with different TKIs of first, second and third generation (imatinib, dasatinib, nilotinib, bosutinib, ponatinib, asciminib). By whole-mount *in situ* hybridization, we observed that tgBCR-ABL1 zebrafish line had an altered expression of myelopoietic markers (pu1, L-plastin and mpx) during embryonic development. Moreover, at 48 hpf tgBCR-ABL1 show high proliferating hematopoietic cells in the caudal hematopoietic tissue. All tested TKIs induce cardiac toxicity (presence of edema) and related cardiac parameters alteration (artery blood flow, heart rate) and antiangiogenic effects. TKIs-treated tgBCR-ABL1 show a downregulation of all hematopoietic markers (analyzed by qRT-PCR) and cell proliferation rate (analyzed by immunofluorescence and bromouridine assay). These results suggest that our model resume Ph+ CML-disease and the answer to TKIs treatments will be useful for further investigations such as to explore different combinations of compounds for new therapeutic strategies assessment.

P-36-080
Reduced expression of cardiac sodium channel Nav1.5 promotes premature senescence

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SCN5A encodes the voltage-gated sodium channel Nav1.5 is recognized for its role in cardiac conduction. *SCN5A* deficiency is associated with increased reactive oxygen species (ROS). Increased ROS and senescence are hallmarks of cardiovascular diseases including heart failure (HF). However, it remains elusive whether reduced *SCN5A* expression may lead to cellular senescence. We recently identified the downregulation of Nav1.5 in left ventricular (LV) tissue of rats with severe HF. LV tissues from HF rats were characterized by increased p-53 signaling and ROS

production. Given the increased ROS and activation of p-53, a key regulator of senescence/cell cycle in end-stage HF. We evaluated *SCN5A* knockdown human cardiac fibroblast (*SCN5A* KD-fib), generated using shRNA-lentivirus, and found reduced culture growth, increase in ROS, and decrease in total ATP as compared to wildtype (WT-fib). Immunoblot analysis showed that *SCN5A* knockdown induces overexpression of p-53, p-21. Mechanistically, small RNA-sequencing revealed differential expression of miR-34a-5p in SCN5A KD-fib than WT-fib confirmed via qRT-PCR, creating a positive loop p-53/miR-34a-5p/p-21, and induce differentiation of *SCN5A*-KDfib attenuating injury due to cardiac fibrosis. However, the p-53/p21 axis does not exhibit apoptosis, indicating the compensatory response to *SCN5A* deficiency-induced pathological conditions. Furthermore, Pifithrin alpha, a bona fide inhibitor of p-53 significantly rescued the senescence phenotype by decreasing the p-53/miR-34a-5p/p-21 axis and ROS in *SCN5A* KD-fib after 24 hours of treatment, without altering Nav1.5 expression. These results demonstrated premature senescence is secondary to defective Nav1.5 channel and can be treated via intervention in the p-53 pathway. In conclusion, the reduced *SCN5A* levels might lead to the accumulation of ROS and trigger premature senescence through the p53/miR-34a-5p pathway, potentially worsening outcomes in heart failure.

P-36-081
Danon disease: histological and immunohistochemical characterization of the affected tissues

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Danon disease is a rare X-linked disorder caused by defects in the *LAMP2* gene encoding for the lysosome-associated membrane glycoprotein 2 (LAMP2) involved in lysosome biogenesis and autophagy. Due to the X-linked dominant inheritance, males manifest a complex and severe phenotype characterized by hypertrophic cardiomyopathy (HCM), skeletal muscle involvement, and cognitive impairment. The HCM typically progresses through heart failure that leads to heart transplantation in the second or third decade of life. Females manifest a later onset phenotype characterized by milder HCM. In this work, we describe the myocardial histopathological, and immunophenotypical features observed in Danon disease, both males and females, carriers of the pathogenic variants in *LAMP2* gene: p.Val310Ile (1 M and 1 F, sibs), p.His260fs (1 M) and p.Gln91* (1 F). Histopathology showed myocyte hypertrophy with optically empty sarcoplasms, suggesting intracellular accumulation, and extensive interstitial fibrosis. The ultrastructural study showed intra-myocyte accumulation of neutral lipid bodies, and osmiophilic, electron-dense bodies, all suggesting features of autophagy impairment. Anti-LAMP2 immunostains showed abnormal lysosome biogenesis and autophagy in cardiac tissue compared with controls. In one prematurely died female patient we extensively investigated both cardiac and non-cardiac tissues demonstrating a ubiquitous representation of the same pathology features. These findings demonstrated that Danon disease is a systemic disorder, irrespective of the major clinical manifestations affecting the heart, muscle, and central nervous system. Our preliminary study provided a precise histopathological and immunohistochemical characterization of the cardiac and extra-cardiac damage in Danon disease suggesting autophagy impairment as a possible mechanism of tissue damage. Only the full elucidation of the pathogenic mechanisms may provide the basis for future developments of target treatments.

P-36-082
SIRT7 deacetylation activity prevents interferon signalling activation due to endogenous RNA sensing

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In mammals, sirtuins are a family of seven enzymes that play crucial roles in cellular stress response through their deacetylase and/or ADPribosyltransferase activities. Using unbiased quantitative proteomics, we have identified a novel target of sirtuin 7 (SIRT7) deacetylase activity. The identified substrate is a critical pattern recognition receptor (PRR) that upon detection of misplaced or aberrant RNA activates interferon signalling. In the absence of stress, SIRT7 deacetylates this PRR, preventing interferon signalling activation. We have also observed that endogenous RNA accumulates in the cytoplasm after treatment with chemotherapy drugs. These treatments also promote degradation of SIRT7 in the cytoplasm, allowing for activation of interferon signalling due to misplaced RNA. Moreover, we have also observed that SIRT7 can restrict interferon signalling in a paracrine manner, hence controlling amplification of interferon signalling in neighbouring non-stressed cells. Previous studies in our lab have shown that SIRT7 KO mice exhibit a premature ageing phenotype and SIRT7 protein levels decrease with age in mice in different tissues. Using publicly available RNA-sequencing data of ageing tissues from mice, we have identified interferon signalling as one of the most prominent signatures that increase across several tissues during organismal ageing. We observe that SIRT7 KO mice exhibit higher levels of transcriptional activation of interferon signalling. In all, we propose a new molecular mechanism by which SIRT7 helps maintain cellular homeostasis. Upon stress, accumulation of cytosolic RNA and degradation of SIRT7 leads to activation of interferon signalling. In the context of organismal ageing, we propose that decreased levels of SIRT7 and consequent insufficient SIRT7 deacetylase activity may be a new mechanism that allows aberrant detection of endogenous RNA followed by interferon signalling activation, overall contributing to inflammation.

P-36-083
Generation of 2D and 3D iPSC-derived neuronal models for the study of neurodevelopmental disorders: focus on congenital central hypoventilation syndrome (CCHS)

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The biggest limitation in the study of the pathogenesis of neurodevelopmental disorders is the generation of a valid model that can recapitulate the complex adult human neurological phenotype and possible defects occurring during embryonic neurodevelopment. Congenital central hypoventilation syndrome (CCHS) is a rare genetic disorder affecting the autonomic nervous system (ANS) and central chemosensitivity. It is caused by mutations on *PHOX2B*, a master transcription factor whose role is essential during development of the ANS and central structures that participate in breathing control. Given PHOX2B's crucial role, obtaining a viable animal model has not been possible yet and only conditional models that recapitulate partial CCHS phenotypes have been generated. Here, we used reprogramming technology to generate induced pluripotent stem cells (iPSC) from patient's fibroblasts carrying different *PHOX2B* mutations. We conducted a characterization of all iPSC lines by karyotyping, immunocytochemistry, and qPCR analysis to confirm the expression of pluripotency markers (NANOG, OCT4 and SSEA4) [Previously published in: Cuadros et al. (2022) Stem Cell Res. 61:102781]. To give insight into possible developmental defects at both peripheral and central level, we generated 2D autonomic neurons and 3D cerebral organoids with cytoarchitectures resembling central chemoreceptors from CCHS patients and controls. We adapted a protocol for the differentiation to autonomic neurons based on the generation of intermediate cell populations, neural crest cells and sympathoadrenal progenitors, mimicking the embryonic development and demonstrated that the protocol can be successfully applied to CCHS patients iPSCs. These new personalized disease-in-a-dish models of CCHS open numerous possibilities to identify molecular and cellular defects induced by the mutations as well as modelling for drug discovery/screening for therapeutic perspectives. *The authors marked with an asterisk equally contributed to the work.

P-36-084
Insights into innate immunity derived from evolutionarily-conserved DEDDh exonucleases

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The cGAS-STING signaling axis plays a key role in the interferon based innate immune response. This response is negatively regulated by DEDDh exonuclease TREX1. TREX1 mutations that prevent DNA binding, catalytic function, or TREX1 dimerization are implicated in human autoimmune diseases such as Aicardi-Goutières syndrome and familial chilblain lupus. There are no identified TREX1 homologs in fish, but recently the PML-like exon 9 (Plex9) gene in zebrafish was found to encode a DEDDh exonuclease that resembles TREX1 [as previously published in: Mathavarajah S et al. (2023) Nucleic Acid Res 51(7), 3185-3204]. Akin to its homolog TREX1, Plex9.1 can digest pathogenic DNA and suppress LINE-1 retrotransposable elements that cause genome instability and subsequent cGAS-STING activation. In this study, we determined the binding and catalytic mechanism of Plex9.1 to deduce the evolutionary conservation of DEDDh exonucleases and innate immunity. While the crystal structure and oligomerization state of Plex9.1 is appreciably different from TREX1, we demonstrate that the active site and key DNA binding features are conserved. We characterized the DNA binding affinity and exonuclease activity of several Plex9.1 variants with cognate autoimmune disease related mutations in TREX1 to assess the importance of evolutionarily conserved residues in these exonucleases. Combined with *in vivo* cell assays monitoring LINE-1 retrotransposition, our data suggests the exonuclease activity of Plex9.1 is independent of its LINE-1 retroposition suppression functionality. This study of Plex9.1 and TREX1 uses structural elucidation, fluorescent activity assays, and microscale thermophoresis to highlight an evolutionarily conserved link between LINE-1 suppression, DNA binding, and autoimmune pathogenesis.

P-36-085
A multi-approach characterization of glycosphingolipids disorders: the case of ST3GAL3 variants

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ST3GAL3 is a member of the sialyltransferase family able to transfer sialic acid to the C-3 position of galactose, terminating the oligosaccharide chain of both glycoproteins and gangliosides. It was supposed to be mainly dedicated to the biosynthesis of histo-blood antigens such as sialylated Lewis a, but the recent discovery of a rare disease caused by inactive variants of the enzyme revealed patients presenting predominantly neurological symptoms and expressing circulating CA19.9. Moreover, enzymatic studies *in-vitro* showed that ST3GAL3 prefers glycosphingolipid substrates presenting both the Galb1,3GlcNAc (lacto-series) and the Galb1,3GalNAc (ganglio-0 series) sequences. This

evidence led to the hypothesis that ST3GAL3 may be crucial for the generation of a definite pool of minor brain gangliosides that could be essentials for the function of specific subsets of neurons. To prove this theory, we created a new method to assess enzyme activity using liquid chromatography coupled with mass spectrometry, which allowed us more sensitive detection avoiding the use of radioactive compounds. Then, plasma samples from patients have been analyzed by ultra-sensitive LC-MS/MS to study if and how the global levels of glycosphingolipids produced were affected by the variants. Results obtained for the enzyme activity assay conducted using LC-MS confirmed that most of the known pathogenic variants of ST3GAL3 lack enzymatic activity. The semi-quantitative LC-MS/MS analysis allowed us to detect lower levels of the putative main reaction products, sLc4 (Siaa2,3Galb1,3GlcNAcb1,4Galb1,4Glc-Cer)/GM1b (Siaa2,3Galb1,3GalNAcb1,4Galb1,4Glc-Cer), in plasma patients when compared to controls. Considering the promising results obtained, further studies are required to study the distribution of glycosphingolipid in brain, in order to be able to connect the biochemical changes in patients to the clinical outcome.

P-36-086
Within-day variation of cell-free mitochondrial and nuclear DNA in blood plasma of patients with tuberculosis: associations with drug exposure and patient-related factors

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Circulating cell-free (ccf) mitochondrial DNA copy number (mtDNA CN) is a biomarker of cellular injury or cellular stress, and was associated with inflammation, the response of innate immunity, and mortality in individuals with severe infections. In addition, ccf nuclear DNA (nDNA) levels are related to inflammatory cytokines and tissue damage. In this study, blood plasma samples were collected from patients with drug-susceptible tuberculosis (TB) receiving standard four-medication regimen (isoniazid, rifampicin, ethambutol and pyrazinamide) at three time points: before medication intake (0 st), and 2- and 6-hours after medication administration. DNA extraction was performed and absolute CN (per microliter of plasma) of both mtDNA and nDNA were quantified using the QX200 ddPCR System (Bio-Rad) through separate amplifications targeting the *MT-ND1* gene region for mtDNA and the *B2m* gene region for nDNA. The QuantaSoft software version 1.0.596 was utilized for the analysis of reaction data. Statistical analysis was conducted using GraphPad Prism 5.0 software and XLSTAT, with a significance level set at $\alpha = 0.05$. Ccf-mtDNA CN levels significantly changed after medication administration ($p = 0.03$). The main factors determining this change were the exposure to ethambutol and isoniazid ($p = 0.03$ and $p = 0.04$, respectively). In contrast, ccf-nDNA CN remained stable after TB drug consumption. However, ccf-nDNA CN levels in blood samples collected before medication administration showed a significant association with drug-related liver injury ($p < 0.0001$), patient smoking status ($p = 0.007$), and, to a lesser extent, with patient age and isoniazid exposure ($p < 0.05$). In conclusion, this study demonstrates the potential of ccf-mtDNA and ccf-nDNA as biomarkers for drug exposure, drug-induced liver injury, and several TB patient-related factors. Additional studies in larger cohorts are necessary. This study was supported by "Recovery and Resilience Facility" PhD Research Grant No. 5.2.1.1.i.

P-36-087
Fatty acids accumulation in intrahepatic cholangiocarcinoma-derived tissue and sera

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Intrahepatic cholangiocarcinoma (iCCA) is the second most common primary hepatic malignancy. Lipids are crucial for cell survival as components of biological membranes, energy suppliers and bioactive mediators. However, information on the iCCA lipidome profile is still lacking. Fatty acid (FA) synthesis is downregulated in iCCA while active uptake by specific transporters is enhanced, even though conflicting data exist on the mechanism of FA accumulation. Proliferation of CCA cell lines rely on lipid uptake to fuel FA catabolism and to promote FA storage in lipid droplets (LD) as neutral lipids. In this study we investigated the iCCA lipidome in paired iCCA and non-tumour (NT) liver tissue and serum from iCCA patients, focusing on fatty acid metabolism. Lipid profile characterization of serum, surgically resected iCCA specimens and NT tissue was analyzed by LC-MS/MS. In iCCA specimens and NT tissue, gene and protein expression were analysed by qPCR and WB analysis. The untargeted lipidome profile showed a clear-cut separation between iCCA and NT tissues as well as between iCCA and HC sera. Indeed, FA accumulated both in iCCA tissue and patients' sera. Expression of Acetyl-CoA Carboxylase Alpha ACACA, the rate-limiting enzyme of *de novo* lipogenesis, was higher in iCCA tissue than in NT tissue. FABP5 membrane-associated transporter expression was unchanged; conversely, FABP4 and CD36 gene expression was down-regulated in iCCA tissue. Several species of phospholipids and sphingolipids, the main components of plasma membranes, were upregulated in iCCA compared with NT tissues. This result was also confirmed by an *in-vitro* metabolic labelling using deuterated palmitic acid. These findings suggest that FA accumulation could promote iCCA aggressiveness by supporting membrane biogenesis and generation of bioactive lipids. Differences in lipidome serum profile between iCCA patients and HC suggest a mean to identify individuals with iCCA by liquid biopsy. *The authors marked with an asterisk equally contributed to the work.

P-36-088
Sphingolipidoma alterations: relationship with glucose intolerance/diabetes and overweight/obesity

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The CA.ME.LI.A (CArdiovascular risks, MEtabolic syndrome, Liver, and Autoimmune disease) epidemiological study (2009-2011) aimed to identify risk factors for cardiovascular disease in a representative population in northern Italy (n = 2545, 1251 men). In this work, we characterized circulating sphingolipidoma to retrospectively correlate it with glucose metabolism and body mass index (BMI). The population was stratified into 6 categories: (1) NGT/NBW (fasting blood glucose (FG) <110 mg/dL)/ BMI ≤24.9 kg/m^2; (2) NGT/OWO (BMI ≥25 kg/m^2); (3) IFG/ NBW (FG 110-125 mg/dL); (4) IFG/OWO; (5) DM/NBW (FG ≥126 mg/dL); and (6) DM/OWO. Lipidomic analysis was performed by LC-MS/MS on a selected number of subjects in each class. The difference between groups was assessed by Kruskall-Wallis test. The most significant alterations were evident when glucose intolerance was associated with overweight or obesity conditions. In particular, dihydroceramides were found to be increased in the IFG/OWO group (0.39 ± 0.18 μM) compared with NGT/OWO (0.35 ± 0.18 μM) and also DM/OWO (0.32 ± 0.2 μM), probably being the accumulation of these molecules an early marker of glucose intolerance. In DM patients, both in association with obesity (DM/OWO, 13.9 ± 7.2 μM) and in normal-weight patients (DM/NBW 16.6 ± 6 μM), hexosylceramides were significantly lower than in the other groups (19.9 ± 6 μM NGT/NBW). S-1P, a species with potent signaling activity, is significantly reduced in IFG/OWO (1.8 ± 0.5 μM) and DM/OWO (1.8 ± 0.7 μM) patients compared with NGT/ OWO (2.2 ± 0.7 μM). The results indicate important alterations in sphingolipids in the presence of both diabetes and glucose intolerance. When intolerance is associated with overweight or obesity, the alterations are synergistic and can be diagnosed as low as ≥126 mg/dL (7.0 mmol/L).

P-36-089
In silico analyses of the structural effects of aminoacidic mutations on proteins involved in rare disease: a case study on ceruloplasmin

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In the era of big data, the available information permits a better understanding of disease for instance through improved understanding of structure-function relationships in causative proteins. Knowledge of naturally occurring post-translational modifications (PTMs) associated with such proteins available through public databases, provides opportunities for querying how these PTMs influence protein structure and function. Phosphorylation is one of the most abundant and functionally important PTMs, representing a well-studied target of pharmacological and therapeutic tools (e.g. kinase inhibitors). In proteins, the structure-function effects of phosphorylation can be studied in biological systems (cellular[1] and animal models[2]) and through *in silico* analysis of phospho-mimetic/abrogative mutations (e.g. by Alphafold2 and Molecular Dynamics Simulations, MDS). Here, how phosphorylation affects ceruloplasmin (CP) biology is studied. CP is the major plasma ferroxidase whose mutations cause aceruloplasminemia (ACP an ultra-rare neurodegenerative disease resulting in iron deposition in various organs including the brain[3]) and whose levels are also low in other more common diseases, such as Wilson disease and a subset of Parkinson's disease. Some ACP mutations are in residues of functionally important regions and close to experimentally validated phosphorylated residues. The phosphorylation of these residues, and how it affects CP expression levels, enzymatic activity, and degradation is here studied by a combination of Alphafold2, MDS, and *in vitro* functional experiments. In the human population, this approach can also be applied to generate hypotheses about how genetic variation in the CP gene associated with PTM residues affects CP biology, potentially leading to personalized approaches in treating CP deficiencies. References: 1. Cariulo C et al (2023) Life Sci Alliance. 6, 02302006 2. Gu X et al (2009) Neuron. 64, 828-40 3. Marchi G. et al (2019) Front Neurosci. 13, 325.

P-36-090
Endocan knockdown modulates the expression of fibrotic-related genes in human cardiac fibroblasts

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Cardiac fibrosis is a common pathological feature of different cardiovascular diseases, and it consists of the aberrant deposition of extracellular matrix (ECM) proteins in the cardiac interstitium, leading to fibrotic scar deposition [1].Activated fibroblast and myofibroblast play a key role, serving as the main source of matrix proteins, including proteoglycans [2]. Endocan is a small proteoglycan deeply involved in several pathological conditions, including inflammation and angiogenesis. Notably, increased serum endocan levels have been observed in patients affected by nonalcoholic fatty liver disease (NAFLD) and advanced liver fibrosis, suggesting a role of such proteoglycan in the fibrotic processes [3]. However, this hypothesis has not been fully elucidated. Herein, we investigated the effects of endocan knockdown in an *in vitro* model of TGF-β-induced fibrosis. Immortalized human cardiac fibroblasts (IM-HCF) were stimulated with TGF-β (10 ng/mL) for 24 h to induce a fibrotic phenotype. A separate set of plates was also treated with a specific siRNA to obtain endocan knockdown before TGF-β treatment. The expression of MMP3 and MMP9, IL-1β, α-SMA, SMAD3, and COL1A1 has been evaluated. Furthermore, the long non-coding RNAs MALAT-1 and HOTAIR expression was also evaluated. Results have shown that endocan, MMP3, MMP9, IL-1β, MALAT-1, HOTAIR, α-SMA, SMAD3, and COL1A1 expression was significantly up regulated by TGF- β treatment. On the contrary, the expression of such parameters was significantly affected in cells where endocan expression was inhibited. In conclusion, our data clearly show that endocan is able to modulate the expression of fibrotic related genes, suggesting its involvement in fibrotic processes. Previously published in:1. Jiang, W., et al (2021) Front

Cardiovasc Med. 8: p. 715258. 2.:Sarohi V. et al. (2022). Front Mol Biosci 9: p. 1030226. 3. Klisić, A., et al. (2020)J Med Biochem 39(3): p. 363-371.

P-36-091
High levels of double-stranded RNA in the plasma of multiple sclerosis patients with anomalous epstein-barr virus reactivation

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Multiple sclerosis (MS) is an autoimmune disease associated with Epstein-Barr virus (EBV) infection. The EBV transcription factor, EBNA1, demonstrates molecular mimicry with central nervous system (CNS) antigens, such as GlialCAM, Anoctamin 2, MBP, and CRYAB. Typically, the immune response shifts from anti-EBNA1 IgM to IgG antibodies post-viral exposure. Nonetheless, a subset of individuals maintains elevated anti-EBNA1 IgM levels, indicating a maladaptive immune response to EBV. In this study, peripheral blood samples were collected from 52 MS patients during their first clinical episode. Plasma was isolated to assess anti-EBNA1 IgG and IgM levels, categorizing EBV status into anomalous reactivation (IgG + IgM +, n = 8) and seropositive (IgG + IgM-, n = 44). Plasma dsRNA levels were measured using a sandwich ELISA. The assay utilized three antibodies: J2, a mouse monoclonal IgG2a kappa antibody; K2, a mouse monoclonal IgM antibody, both supplied by Nordic Mubio; and a peroxidase-conjugated Goat F(ab')2 Anti-Mouse IgM mu chain secondary antibody from Abcam. Poly(I:C) and Hepatitis C virus (HCV) RNA were employed as positive controls. Conversely, RNaseIII, an enzyme that selectively degrades dsRNA, was used to cleave Poly(I:C) and HCV RNA, serving as a negative control. Descriptive and inferential statistics were conducted in R, setting the significance threshold at $p < 0.05$. EBV infection was universal among the cohort. We found that patients with anomalous EBV reactivation exhibited significantly elevated plasma dsRNA levels in comparison to the seropositive group, as evidenced by two independent experiments. This suggests a disrupted immune surveillance of EBV. RNA sequencing experiments will be performed to determine the nature and potential significance of these elevated dsRNAs within the MS pathology framework.

P-36-092
Novel cell models for the study of SLC25A38-related congenital sideroblastic anemia and new therapeutic perspectives

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Mutations in the SLC25A38 gene are responsible for the second most common form of congenital sideroblastic anemias (CSA). The symptoms of this severe type of anemia are significant and closely resemble those observed in thalassemia major. Unfortunately, there is currently no cure for the SLC25A38 related anemia, and the progress of drug-screening studies for new therapies have been hindered by the lack of suitable biological models. To address this limitation, we investigated two human cell lines: K562 erythroleukemia cells exhibiting reduced expression of the SLC25A38 protein, and a lymphoblastoid cell line originating from a patient carrying a nonsense mutation in the SLC25A38 gene. Both cell lines replicated the main features associated with this anemia, including reduced heme content and respiratory defect. On the contrary, other defects were specific to K562 mutant cells such as the increase in mitochondrial iron, ROS species levels, and sensitivity to oxidative stress. Both models were employed to assess drug efficacy, identifying P2 receptors as prospective targets for addressing the cellular dysfunctions linked to this particular form of CSA. Acknowledgments: Supported by Ministry of University and Research, Progetti di Ricerca Nazionale, grant number 2017PAB8EM_005 and the Italian Ministry of Education, Universities, and Research, Scientific Independence of young Researchers grant number RBSI14LTIS. *The authors marked with an asterisk equally contributed to the work.

P-36-093
Prospects on NAD metabolism deregulation in ageing models

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During ageing, most of the metabolic pathways undergo deep modifications with compromised mitochondrial function and bioenergetics. Age-related NAD decline appears to be the effect of an unbalanced equilibrium between NAD-consuming and NAD-synthesizing enzymes. Since NAD-consuming enzymes lead to the production of nicotinamide (Nam), NAD recycling via the Nam salvage pathway represents a crucial step to maintain NAD homeostasis. In this work, we have used bioanalytical

methodologies to ascertain the profile of NAD metabolites as age-dependent molecular signature in two different models: a *Saccharomyces cerevisiae* strain harbouring a polyQ-Huntingthin (PolyQ-Htt) protein and a senescence-accelerated mouse prone-8 (SAMP8) strain, representative systems of proteotoxicity-induced cellular stress and accelerated senescence, respectively. The information acquired about the alterations in the NAD profiles of the two model systems compared to their respective controls, could allow to infer about the different metabolic adaptive strategies adopted in the presence of polyQ expansion induced cell damage and accumulation of senescent cells, during ageing. In this regard, we observed a common trend in the levels of NAD decline with accelerated ageing with a parallel accumulation of NAD precursors, in particular nicotinic acid mononucleotide (NaMN), in both polyQ-Htt yeast cells and SAMP8 mice blood. Furthermore, adenylyl pool (ATP, ADP, AMP) evaluation in the SAMP8 mice has resulted to be a meaningful panel of molecules to monitor energetic metabolism impairment associated with mitochondrial dysfunction in accelerated senescence. These findings could serve to identify frailty points in the ageing process to address via targeted pharmacological or nutraceutical interventions.

P-36-094
Phosphoproteomic approaches to highlight differences regarding the celiac cellular phenotype

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Celiac disease (CD) is an inflammatory intestinal disease, with a genetic background, caused by the ingestion of gluten-containing cereals. Recently, scientific data have allowed us to define a "Celiac Cellular Phenotype" consistent with all the constitutive differences between celiac and control cells, even evident in a site far from the main site of inflammation. In particular, CD skin-derived fibroblasts have alterations in vesicular trafficking, cell morphology, response to anti-transglutaminase 2 antibodies [Paolella et al., 2020 Int. J. Mol. Sci., 21(4), 1231]; furthermore, a difference in the protein phosphorylation pattern was observed [Nanayakkara et al., 2013 Plos One, 8(10)], indicating basal alteration in signalling pathways. In the present work, we investigated phosphoproteome differences in fibroblasts from CD and control subjects by mass spectrometry analysis. After phosphoproteins enrichment by affinity-chromatography, we found differences in protein profiles in samples from CD subjects and control ones. In particular, we identified an alteration in the level of phosphoproteins involved in various biological processes, such as proliferation, multivesicular body formation, folding and unfolded protein response (UPR). Our work contributes to understand the molecular mechanism underlying the CD cellular phenotype. *The authors marked with an asterisk equally contributed to the work.

P-36-095
Constitutive differences in Ca^{2+} homeostasis, stress response and protein expression in primary fibroblasts from celiac and healthy control subjects

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Celiac disease (CD) is an inflammatory intestinal disease caused by the ingestion of gluten-containing cereals by genetically predisposed individuals. The ubiquitous enzyme type 2 transglutaminase (TG2) increases gluten immunogenicity by deamidating specific glutamine residues. TG2 also triggers an auto-immune response by forming covalent complexes with gluten peptides. Constitutive differences between celiac and control cells regarding TG2 distribution and activation, together with differences regarding vesicular trafficking and protein phosphorylation have been reported, thus contributing to the definition of a peculiar CD cellular phenotype. Our aim was to further investigate molecular differences between CD and control cells focusing the attention on Ca^{2+} homeostasis, ER-stress and autophagic responses to a stress agent and on the differential global basal protein expression. For this study, we mainly employed skin-derived primary fibroblasts, a suitable model to study phenomena independent from gluten exposure. We found that basal concentration of Ca^{2+} ions in cytosol and ER was lower in CD cells than in control ones. We also registered a slightly higher ER-stress response to thapsigargin in CD cells than in controls and a delayed or blocked autophagy in CD cells, whereas normal cells presented a regular autophagic flux in response to thapsigargin stimulation [Previously published in: Sposito S et al. (2023) Int J Mol Sci, 24, 1495]. Finally, proteomics analyses suggested that several proteins involved in processes such as extracellular vesiculation, oligomers formation, and response to oxidative stress, are upregulated in CD samples with the respect to controls. Our findings support the idea that CD cells are probably less capable to adapt to stress conditions and, on the whole, give a contribution to the comprehension of molecular features of the CD cellular phenotype.

P-36-096
The effect of a high-fat diet and fructose supplementation on inflammation and pathological changes in obese mice

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Obesity is a global health issue associated with increased risk of chronic diseases. During obesity, elevated levels of pro-inflammatory factors are released from the adipose tissue, triggering low-grade systemic inflammation, which can lead to the development of various metabolic or even neurodegenerative diseases. However, the molecular basis of this mechanism remains unclear. Therefore, our aim was to investigate inflammation and pathological changes related to obesity in two different in mouse models. 3-months-old male mice were fed either a high-fat diet (HFD) or HFD supplemented with 30% fructose solution (HFD + FR) while control animals were fed a normal chow. Although HFD alone induced obesity-related pathology, HFD + FR mice show an even higher weight gain and hepatic steatosis, as confirmed by hematoxylin-eosin staining of liver sections. In parallel, oral glucose tolerance test verified glucose intolerance only in mice fed with HFD + FR. This was accompanied by a higher level of systemic inflammation, as indicated by the increased expression of pro-inflammatory genes in visceral white adipose tissue and by a higher serum TNFα level in the HFD + FR group. In addition, doublecortin immunostaining of brain sections revealed increased neurogenesis in the case of certain HFD + F mice. This may be a compensatory response to diet-induced brain damage, explaining the lower brain weight of these animals. Our results confirm that inflammation and metabolic changes induced by obesity have a systemic impact, with more severe consequences observed in the case of HFD + FR. In the future our research may improve understanding of the molecular and cellular mechanisms behind obesity and contribute to better therapeutic treatments for obesity-related disorders. This work was supported by NKFIH FK138390, ÚNKP-23-5-SZTE-593 and the János Bolyai Research Fellowship of the Hungarian Academy of Sciences.

P-36-097
A transcriptomic analysis of fibroblasts isolated from mice with ELOVL1 gene mutation

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ELOVL1, a fatty acid elongase 1, is an enzyme that catalyses the synthesis of very long chain fatty acids with 20-28 carbon atoms. In our previous study, a p.Ser165Phe mutation was found in two children, which was associated with ichthyotic keratoderma, spasticity, mild hypomyelination and dysmorphic features [1]. In order to better understand the role of the alterations associated with the dysfunction of ELOVL1, we have generated a mouse model of this mutation using the CRISPR-Cas method. Fibroblasts were then isolated from the skin of the tail of C57BL/6J mice that carried the mutation (homozygotes) and from wild-type (WT) mice. Total RNA was then isolated from the primary fibroblast cell culture and RNA sequencing was performed. The results showed a large number of gene expression differences between the two groups. 1195 genes were upregulated in fibroblasts from homozygous mice with the ELOVL1 mutation and over 1500 genes were downregulated. These results are an indication that a mutation in the ELOVL1 gene results in a strong rearrangement in the expression of various genes. Funding: National Science Center, grant no. 2020/37/B/NZ4/00821 Reference: [1] A. Kutkowska-Kaźmierczak et al. J. Med. Genet. 55:408-414 (2018).

P-36-098
Unravelling the pathogenic bases of Catel-Manzke syndrome using a zebrafish knock-out model

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Catel-Manzke syndrome (CMS) is a rare recessive disorder characterized by skeletal and heart malformations, due to mutations in the TGDS gene. TGDS is annotated in the databases as dTDP-glucose 4,6 dehydratase, due to its homology with the bacterial enzymes of the dTDP-L-rhamnose biosynthesis; however, this pathway was never reported in vertebrates. CMS clinical

presentation led to the proposal that TGDS could have a role in glycosaminoglycan formation, but biochemical evidence is lacking and TGDS function remains unknown. In the perspective of developing a model to study CMS, we have identified and characterized zebrafish *tgds*. When expressed in *E. coli*, Tgds protein exhibited dehydratase activity on UDP-D-glucose only. Mutants harboring the mutations observed in CMS patients showed reduced catalytic activity and stability; the damaging effects of the amino acid substitutions were further confirmed by Alphafold2 structural modeling. RT-PCR, RNA-seq, and *in-situ* hybridization analyses revealed that zebrafish *tgds* behaves as a maternal gene, with the highest expression levels in the zygote. After gastrulation, a second wave of expression occurs in specific locations, such as the brain, optic capsule, and pharyngeal cartilage. A zebrafish *tgds* knock-out model is under development in our laboratory using CRISPR/Cas9. sgRNAs targeting three different exons were designed, and they were used in the perspective to obtain F0 biallelic knock-outs. Indeed, preliminary experiments have led to embryos with abnormal phenotypes, highlighting the role of *tgds* in development. These findings suggest that transgenic zebrafish can be a valuable model for the study of the molecular events underlying CMS pathogenesis and for delineating Tgds function in vertebrates. The establishment of a heterozygous line is currently ongoing, which will allow a more precise analysis of the phenotypes in the knock-out embryos. Founded by Telethon GMR22T1065 and MUR-PRIN 2022 22ETJJES.

P-36-099
Expression of genes associated with ARDS after surfactant replacement therapy

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Acute respiratory distress syndrome (ARDS) is a serious lung condition that cause difficulty breathing and even hypoxemia. This is caused by pulmonary edema and diffuse pneumonia, which leads to low oxygen levels in the blood. Mechanisms of molecular dysregulation at the transcriptional level in ARDS are poorly understood. This can also be attributed to the extreme heterogeneity of ARDS, including various triggering stimuli influenced by several comorbidities and genetic factors. ARDS is mainly characterized by alveolar damage, inactivation of pulmonary surfactant and associated respiratory failure. Substitution surfactant therapy can contribute to the recovery of lung function and effective ventilation. The aim of the study is to analyze the expression changes of selected genes associated with ARDS and/or after exogenous surfactant therapy in an animal model of ARDS. Adult rabbits with severe ARDS were divided into groups based on surfactant treatment with the natural surfactant poractant alfa, and the synthetic surfactants CHF5633 and Combo with synthetic analogues of surfactant proteins B and C. Isolated total RNA from lungs was analyzed by qPCR. Data were corrected for the signal from two endogenous controls used as reference genes (B2M 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 (VEGFA, SFTPA1, SFTPB, SFTPD, ABCA3, EGLN1, FGF7, RAB11A, LCN2, IL-6) was performed against healthy controls. Genes associated with ARDS were regulated in the untreated animals in the same way as results already published by other authors. All surfactant therapies affected the expression of selected genes. Poractant alfa and Combo surfactant significantly downregulated VEGFA, RAB11A, LCN2 compared to ARDS. This may be related to the regulation of molecular pathological processes in ARDS. This study was supported by VEGA 1/0004/21, VEGA 1/0097/23.

P-36-100
Potential role of DSG2 as a murine receptor for entry of Coxsackie viruses

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The study of viral internalization mechanisms and cellular receptors used for its initiation is one of the most important aspects of viral biology. Coxsackie and adenovirus receptor (CXADR) is well-known receptor for group B of Coxsackie viruses and subgroup C of adenoviruses. We found that an antiserum to Coxsackie virus B5 (CVB5) obtained in common chimpanzee (Pan troglodytes) 120 times more effectively inactivates CVB5 on the human cell line HEK293T line than on the murine cell line CT2A. However, antiserum to CVB5 produced in rats (Wistar) exhibits diminished neutralizing activity on human cell lines compared to murine ones. Since the target for neutralizing antibodies is the region of the viral capsid that is responsible for interaction with cellular receptor, our findings led us to suggest that CVB5 entry into murine cells predominantly employs a receptor distinct from CXADR. Desmoglein-2 (DSG2) is the receptor for several human adenoviruses, so we subsequently hypothesized a role for DSG2 as the corresponding murine receptor facilitating the entry of Coxsackie viruses. To validate this hypothesis, we conducted experiments using the murine glioblastoma cell line CT2A as well as this cell line with hyperexpression or knockout of the murine DSG2 gene. It was confirmed that cells with enhanced DSG2 expression showed increased sensitivity to Coxsackie B3, B4, B5 and B6 viruses compared to the original CT2A line, while DSG2 knockout clones remained resistant. We also observed a significant increase in the replicative activity of Coxsackie viruses B3, B5 and B6 on cells with DSG2 hyperexpression and detected almost no replication in knockout cells. The results obtained allow us to hope that DSG2 may indeed function as a receptor for the entry of both Coxsackie viruses and adenoviruses into murine cells.

P-36-101
A new mechanism of regulation of LIMK1 And LIMK2, kinases involved in cytoskeleton remodelling

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LIMK1 and LIMK2, LIMKs, are the only two members of the LIMK family. They are dual kinases as they phosphorylate both serine/threonine and tyrosine. They are involved in cytoskeleton remodelling by promoting actin filament formation via the phosphorylation and subsequent inhibition of cofilin, an actin depolymerizing factor. They are also involved in microtubule turnover, but the molecular requirements of this action still remains to be elucidated. LIMK1 and LIMK2 play a crucial role in many physiological processes, such as migration, motility, division, apoptosis, and neurite plasticity. Their role in many diseases has been established: cancer, neurological disorders, Neurofibromatosis, pain, erectile defects, and inflammation. LIMK1 and LIMK2 belong to the Rho, Rac, CDC42/ PAK, MRCK, ROCK signalling pathway. They are phosphorylated and activated by upstream kinases on their Thr508 for LIMK1 and Thr505 for LIMK2. Here, we show that this canonical well-described activation is necessary but not sufficient for LIMK optimal activity on cofilin. Indeed, we unravel a new unsuspected mechanism of activation of these kinases via their C-terminal extremity. We point out a major role of a Tyr, required for LIMK dimerization and trans-phosphorylation, prerequisite of LIMK phosphorylation on their Thr and triggering their stability. These new mechanism of regulation of LIMKs may pave the way to new therapeutic strategies targeting these kinases as, so far, they remain undruggable proteins.

P-36-102
Secondary pathways cause the proliferation of amyloid aggregates of medin, an aortic medial amyloid peptide associated with cerebral amyloid angiopathy

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Medin, a proteolytic fragment of lactadherin, forms one of the most common senile localised amyloids, typically found in the medial layer of upper body vasculature in humans. Medin has also been shown to co-localise with, and modulate, the aggregation of amyloid-β isoforms in cerebral amyloid angiopathy (CAA). High correlation of cerebrovascular medin with cognitive decline strongly indicates a potential role of this peptide in the disruption of blood–brain barrier (BBB) integrity. Despite the discovery of medin's amyloidogenicity more than two decades ago, its mechanism of aggregation is yet to be deciphered. This study addresses this knowledge gap by monitoring the aggregation kinetics of recombinant human medin under physiological conditions *in vitro* in real time via a fluorescence-based assay using the amyloid binding dye thioflavin T. The resulting data are fitted into a kinetic model to determine the dominant mechanism and kinetic parameters of the aggregation reaction. We observed that secondary pathways dominate medin aggregation under physiological conditions. At least under the conditions that we tested, these secondary pathways are saturated, i.e., they are independent of the initial monomer concentration. Combined with electron microscopy and secondary structural analysis, this approach helped elucidate the microscopic mechanisms underlying medin aggregation. Current work focusses on determining the pathological effects of the secondary pathway intermediates on an *in vitro* brain endothelial cell model of BBB, thereby probing at the role of medin in CAA. The combination of structural, kinetic and cell viability analyses of medin aggregation will help identify the pathologically significant intermediates, and thereby provide a platform for screening potential inhibitors of pathological aggregation that can stabilise medin in its non-toxic state. This approach will also help establish how medin mechanistically influences the aggregation of amyloid-β in CAA.

P-36-103

Abstract withdrawn.

P-36-104
Oxidative stress-induced senescent model of visceral adipose stem cells (vASC) as a promising strategy for investigating visceral adipose tissue senescence in aging and diseases

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Visceral adipose tissue (VAT) is a secretory organ that maintains systemic homeostasis through its metabolic and immunological properties. In particular, visceral adipose stem cells (vASC) regulate VAT metabolic homeostasis as well as regulate immune cells functionality to prevent inflammation. However, during aging, adipose tissue is subjected to many stressors, including oxidative stress and telomere shortening, which lead to VAT dysfunction and senescence. While VAT senescence has already been reported to be associated with several age-associated pathologies (i.e. diabetes, obesity, and cancer), the underlying molecular alterations remain poorly described. Here, we investigated vASC senescence by comparing two strategies: replicative senescence (RS) achieved through extensive culturing and oxidative stress-induced senescent through hydrogen peroxide (H_2O_2) administration. Upon senescence induction, vASC displayed impaired proliferation and differentiation along with expression of several senescence features, including senescence-associated β Galactosidase (SA-β-Gal) expression, morphological alterations, and DNA damage

response pathway activation. Also, both RS and oxidative stress-induced senescent vASC displayed modifications in several metabolic and molecular pathways including increased autophagic flux, mitochondrial dysfunction, and inflammation establishment. Our untargeted proteomic approach on vASC secretome underlined the association of vASC senescence with modifications in several biological processes associated with age-associated diseases, including platelet aggregation, vesicular trafficking, extracellular matrix structure, and glycolysis. Overall, our data show that oxidative stress could be a promising model for investigating VAT senescence. Furthermore, all together, our results suggest that senescent vASCs could contribute to several age-related diseases' establishment through the paracrine and endocrine effects of their secretome.

P-36-105
New insights into liver hydroxyproline metabolism from a cell model of primary hyperoxaluria type 3

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Primary hyperoxaluria type 3 (PH3) is an autosomal recessive disorder caused by the deficit of 4-hydroxy-2-oxoglutarate aldolase (HOGA1), a mitochondrial enzyme involved in the terminal step of hydroxyproline catabolism that cleaves 4-hydroxy-2-oxoglutarate (HOG) into pyruvate and glyoxylate, the precursor of oxalate. PH3 patients display increased oxalate excretion leading to recurring kidney stones beginning in childhood. While the genetic basis of the disease is well known, how the deficit of an enzyme generating glyoxylate results in hyperoxaluria remains puzzling. The current hypothesis is that HOGA1 deficiency in tissues with active hydroxyproline metabolism (e.g., liver and kidney cortex) leads to increased oxalate production as a consequence of secondary pathways active upon HOG accumulation [previously published in Belostotsky R. et al. (2022) Int J Mol Sci. 23, 1005-17]. To investigate the metabolic role of HOGA1 and the mechanisms underlying PH3, we knocked-out HOGA1 in hepatocarcinoma (HepG2) cells via CRISPR/Cas9. Incubation of HOGA1-KO HepG2 cells with hydroxyproline resulted in increased HOG in medium and increased oxalate production, consistent with the biochemical abnormalities observed in patients. Interestingly, HOGA1-KO HepG2 cells showed increased expression and activity of alanine:glyoxylate aminotransferase 1 and glyoxylate reductase, suggesting a compensatory peroxisomal/cytosolic glyoxylate detoxification mechanisms in PH3. RNA-seq experiments on HOGA1-KO and wild-type HepG2 cells followed by gene set enrichment analysis showed increased expression of genes involved in lipid catabolism and in organic compounds detoxification. In conclusion, the results we generated in a hepatocyte cell line support broad metabolic derangements in cells deficient for HOGA1 but further studies are needed to understand the biochemical bases of PH3 as premise to develop therapeutic strategies.

P-36-106
Insight into the molecular pathology of Marinesco-Sjogren's syndrome and a potential innovative therapeutic approach

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Marinesco-Sjogren's syndrome (MSS) is an autosomal recessive early-onset genetic disorder for which there is no specific treatment. MSS patients suffer from cerebellar ataxia and muscle weakness due to degeneration of Purkinje neurons and myopathy. More than 50% of MSS patients have a loss-of-function mutation in the SIL1 gene. SIL1 encodes for a co-chaperone of the immunoglobulin heavy chain binding protein (BiP), the master chaperone of the endoplasmic reticulum (ER). Loss of SIL1 prevents the release of ADP by Bip and impairs protein folding, which in turn leads to the unfolded protein response (UPR). Long-lasting UPR and other ill-defined mechanisms contribute to MSS. Our recent proteomic analysis of patient-derived fibroblasts revealed a profound metabolic reorganisation aimed at reducing the ER workload and recovering energy from lipids and amino acids. Transcriptomic analysis showed that the patient fibroblasts have a membrane trafficking issue. Furthermore, the extracellular matrix (ECM) and cell adhesion components were found to be altered. In fact, patient fibroblasts showed a reduced capacity of ECM remodelling and reduced motility. In line with this, structural analysis of myotendinous junctions revealed a disorganisation of collagen fibres in the mouse model of MSS (woozy). With the aim of finding a treatment for MSS, we have recently shown that UPR inhibition can improve cellular disease and motor performance in woozy mice. Currently, in order to find a more effective treatment, we are evaluating a replacement therapy based on SIL1 proteins fused with cell penetrating peptides.

P-36-107
A one-two punch? Investigating the potential of phage-antibiotic combination (PAC) therapy using pleurotin and phage K in *Staphylococcus aureus*

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The current limitations on antibiotic discovery have necessitated the search for novel discovery methods and sources, as well as non-antibiotic alternatives. The basidiomycete-derived, secondary metabolite pleurotin and its congeners could be a proponent of the former, as these have been shown to be effective against Gram-positive bacteria, while bacteriophages (phages) could be the ultimate non-antibiotic alternative. A very promising solution is phage-antibiotic combination (PAC) therapy, where cocktails of phages and conventional antibiotics are employed against problematic bacterial strains. In this project, the combination of pleurotin and bacteriophages targeting *Staphylococcus aureus* was examined. Pleurotin was isolated from a bioreactor fermentation of the basidiomycete *Hohenbuehelia atrocaerulea*. Purification and structure characterisation was performed using liquid chromatography and NMR. Purified pleurotin was compared to pleuromutilin and vancomycin, in combination with Phage K against *S. aureus* NCTC-9318 in single agent and PAC time-kill assays. The cytotoxicity of pleurotin was assessed using LDH and Live/Dead viability assays in human diabetic skin fibroblasts in comparison to pleuromutilin, vancomycin and Phage K. Phage K in combination with both pleurotin and vancomycin showed a decrease in growth rates compared to treatment with phage or antibiotic alone whereas adding phage to pleuromutilin yielded an antagonistic effect. Cytotoxicity of pleurotin in human cells was not significantly different to vancomycin and pleuromutilin. This suggests that phage K can lower the working MIC for pleurotin and vancomycin. Currently, work is underway to test efficacy against clinically relevant strains and biofilms.

P-36-108
The relationship between circulating cell-free DNA and inflammatory cytokines in patients with bipolar disorder

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Circulating cell-free DNA (ccfDNA) levels increase in biological fluids during various pathological conditions. The investigation of changes in the levels of genomic and mitochondrial ccfDNA has become relevant in various psychiatric disorders, such as schizophrenia, depression, and bipolar disorders [1]. Additionally, the analysis of ccfDNA is of interest in relation to inflammatory conditions [2]. Although inflammatory cytokines are recognized biomarkers of psychiatric diseases [3], the molecular mechanism behind this is not yet understood. The objective of our study is to determine the correlation between serum ccfDNA and inflammatory cytokines in bipolar disorder. The potential use of ccfDNA as a biomarker will also be investigated. We measured the concentration of plasma ccfDNA using real-time quantitative PCR and the levels of cytokines using an enzyme-linked immunosorbent assay (ELISA). A comparison was conducted between the data of 20 patients diagnosed with bipolar disorder and 20 healthy controls. The results provide evidence of changes in ccfDNA gene expression and plasma levels in patients with bipolar disorder. Additionally, correlation analyses revealed a robust association between cfDNA and inflammatory cytokines. This preliminary study offers novel insights into potential plasma-based biomarkers that may be valuable in predicting bipolar disorder. Moreover, we have identified a specific alteration in ccfDNA expression that is linked to changes in inflammatory factors, suggesting their potential involvement in the inflammatory response. References: 1. Melamud MM et al. (2023) Int J Mol Sci 24(4), 3402. 2. Kageyama et al. (2022) J Affect Disord 299, 644-651. 3. Yuan et al. (2019) Transl Psychiatry 9(1), 233.

P-36-109
Oxidative damage and alteration of cellular bioenergetics in primary culture-based model of Alzheimer's disease

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Ongoing research in Alzheimer's disease (AD) shows the critical role of oxidative stress and bioenergetic alterations in development and progression of AD. Our previous studies on primary hippocampal cells strongly indicated that combination of pivotal factor of AD – amyloid beta (Aβ) with external oxidative damage (OD) and inhibition of internal antioxidant defense was even more toxic, impaired cellular defense systems, and might mimic the late phase of AD-associated cell damage. Analyzing the obtained results and realizing that the culture model of AD should "work" not only in the hippocampus, but also in the other AD-target brain structures, it was decided to apply Aβ1-42 to the cells of the cerebral cortex and the locus coeruleus (LC), and analyze the expression of OD and alteration of cellular bioenergetics. Primary culture-based model of AD was mimic by adding Aβ1-42 to primary hippocampal, cortical and LC cells obtained from adult rats. We measured the levels of molecules reflecting cellular metabolism (glucose and lactate), cell damage (lactate dehydrogenase), oxidative stress (protein carbonyls, advanced oxidation protein products, and thiobarbituric acid reactive substances). Cellular bioenergetics markers – mitochondrial respiration, glycolysis, and ATP production rates were assessed by measuring oxygen consumption rate (OCR) and extracellular acidification rate (ECAR). Results obtained showed that the minimum level of oxidation was recorded in the LC, and the maximum – in the hippocampal cells. The latter were characterized by lower metabolic activity (lactate, glucose) and cells number compared to those obtained from the LC and cortex. Bioenergetic data indicated that both OCR and ECAR were dramatically reduced by exposure to Aβ. The AD damage was most pronounced in the hippocampus, then cortex and the least in the LC. It is suggested that the "light" damage of LC could contribute to the protection of AD brain via LC based noradrenaline pathway.

P-36-110
Identification of disease-associated signatures in pneumonia by integrated transcriptome and proteome analysis

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Pneumonia is one of the most common acute respiratory infectious diseases caused by bacterial infection. Pneumonia patients often show patchy lung consolidations and may develop parapneumonic pleural effusion (PPE) and empyema thoracis; severe clinical stages with pleural effusions are associated with higher mortality. We have identified several cytokines and chemokines in PPE with different etiologies. However, little is known about the comprehensive profiling of whole transcriptome and secretome in pleural effusion in relation to the severity of lung damage. In order to investigate the pathological mechanisms in PPE, we performed a comprehensive transcriptome and proteome analysis to reveal immune regulatory networks in pneumonia patients with complicated PPE (CPPE) or uncomplicated PPE (UPPE). Pathway annotation by the bioinformatics analysis revealed that dysregulated genes in CPPE were associated with pathogen induced cytokine storm signaling pathway, IL-6 signaling, TREM1 signaling and IL-8 signaling by IPA canonical pathway analysis. Integrated transcriptome and proteome profiling revealed greater levels of six novel inflammatory mediators including high mobility group protein B2, bactericidal permeability-increasing protein, matrix metalloproteinase 8, elastase, ferritin heavy chain, and matrix metalloproteinase 9 for CPPE patients compared with UPPE patients. Our results indicated that Streptococcus infection stimulated the expression of these inflammatory mediators in neutrophils and macrophages. Furthermore, overexpression of one of these inflammatory mediators in macrophages enhanced IL-8 and IL-1β production via NFκB activation. Our findings provide new insights into the mechanisms underlying CPPE inflammation and possibly a novel therapeutic target for further investigation.

P-36-111
EP300 mediates alveolar type II cell–dependent chemotaxis signaling to promote pulmonary fibrosis

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Idiopathic pulmonary fibrosis (IPF) is a chronic, fatal, fibrotic, interstitial lung disease of unknown cause. Despite extensive studies, the underlying mechanism that leads to IPF development remains unknown. We found that p300 is upregulated in multiple epithelial cell types in lung samples from patients with IPF and mouse models of lung fibrosis. Lung fibrosis was significantly diminished by the alveolar type II (ATII) cell-specific deletion of the p300 gene, suggesting an ATII cell-specific contributions of p300 signaling to fibrosis. Moreover, p300 in ATII cells mediates the TGF-β1-induced transcriptional activation of Ccl2, Ccl7, and Ccl12 chemokine genes through the cooperative action of C/EBPβ, which promotes M2 macrophage polarization. Blockade of p300 in ATII cells resulted in the reprogramming of M2 macrophages into anti-fibrotic macrophages. This study demonstrates a pivotal role for p300 in the development of lung fibrosis and suggests p300 as a promising target for IPF treatment.

P-36-112
Selective glucocorticoid receptor modulator impinging on the orexigenic action of glucocorticoid receptor in AgRP neurons

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Obesity, a worldwide epidemic, leads to various metabolic disorders threatening human health. In response to stress or fasting, glucocorticoid (GC) levels are elevated to promote food intake. This involves GC-induced expression of the orexigenic neuropeptides in agouti-related protein (AgRP) neurons of the hypothalamic arcuate nucleus (ARC) via the GC receptor (GR). Here, we report a selective GR modulator (SGRM) that suppresses GR-induced transcription of genes with non-classical glucocorticoid response elements (GREs) such as Agrp-GRE, but not with classical GREs, and via this way may serve as a novel anti-obesity agent. We have identified a novel SGRM, 2-O-trans-p-coumaroylalphitolic acid (Zj7), a triterpenoid extracted from the Ziziphus jujube plant, that selectively suppresses GR transcriptional activity in Agrp-GRE without affecting classical GREs. Zj7 reduces the expression of orexigenic genes in the ARC and exerts a significant anorexigenic effect with weight loss in both high fat diet-induced obese and genetically obese db/db mouse models. Transcriptome analysis showed that Zj7 represses the expression of a group of orexigenic genes including Agrp and Npy induced by the synthetic GR ligand dexamethasone (Dex) in the hypothalamus. Taken together, Zj7, as a selective GR modulator, showed beneficial metabolic activities, in part by suppressing GR activity in non-classical GREs in orexigenic genes. This study demonstrates that a potential anorexigenic molecule may allow GRE-specific inhibition of GR transcriptional activity, which is a promising approach for the treatment of metabolic disorders.

P-36-113
DEPTOR uratylation: a new mechanism leading to pancreatic β-cell death in diabetes

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Loss of viable pancreatic β-cells is a hallmark of the development and progression of type 2 diabetes mellitus (T2DM). A compound recently recognised to be elevated in the context of T2DM is uric acid (UA), a metabolite of the purine metabolism and the main antioxidant in the blood. We aimed to determine the molecular mechanism of pancreatic β-cell death under elevated UA (hyperuricemic) conditions (HUC). The pancreatic β-cell lines 1.1B4 (rat) and MIN6 (mouse) were used to determine the molecular mechanisms of β-cell death via co-immunoprecipitation, siRNA knock-down, western blot analyses, autophagy, MTT, and Caspase 3/7 assays under HUC. Pancreatic β-cell metabolic activity was reduced and autophagy/apoptosis was

increased upon HUC, which could be reverted by benzbromarone as well as GLUT9 knock-down. AMP-activated protein kinase (AMPK) phosphorylation was increased under HUC leading to the reduction of Raptor phosphorylation, the mTOR complex-1 (mTORC1) activator indicating an inhibition of mTORC1. Also, HUC stabilised the expression of the mTORC1 negative regulator DEP domain-containing mTOR-interacting protein (DEPTOR). The increase in DEPTOR protein expression was due to changes in DEPTOR ubiquitination. We observed a down-regulation of the E3 ligase β-TrCP and upregulation of USP3, a newly identified DEPTOR-regulating ubiquitin-specific protease in cells exposed to HUC. In addition, we document for the first time that a protein is UA-modified (uratylated) in a cellular context. Interestingly, DEPTOR uratylation seems to contribute to the change of its ubiquitination. Our findings suggest a causal basis for the connection between HUC and T2DM based on the stabilisation of DEPTOR permanently inhibiting mTORC1 and ultimately resulting in the autophagy-mediated β-cell death. *The authors marked with an asterisk equally contributed to the work.

P-36-114
Dissection of the molecular mechanisms responsible for myofibroblasts to lipofibroblasts switch in pulmonary fibrosis: a role for sphingosine 1-phosphate

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Pulmonary fibrosis (PF) is a chronic and progressive pathologic condition characterized by an unescapable decline of respiratory function with a limited survival expectance. The main hallmark of PF is represented by activation of myofibroblasts (MF), responsible for extracellular matrix (ECM) deposition that leads to respiratory failure, albeit the molecular mechanisms of the onset and progression of the disease have not been clarified yet. Recently, fibroblast plasticity has been addressed as a possible strategy to improve lung tissue regeneration. Indeed, upon injury lipofibroblasts (LF) differentiate into MF, which eventually de-differentiate back to LF in the case of PF resolution. Thus, it appears to be pivotal to boost the research towards new pathways that trigger the activation of LF phenotype[1]. Sphingosine 1-phosphate (S1P) signalling has been found to be profoundly dysregulated in PF[2], although its role is so far unclear. For this reason, the specific aim of this study is to analyse the modulation of S1P signalling in fibroblast plasticity. The cellular model to study MF to LF switch was set up using IMR-90 human lung fibroblasts that differentiate into MF or transdifferentiate back into LF by the treatment with TGFβ, in the absence or presence of rosiglitazone, respectively. The expression of fibrosis and lipogenesis markers was analysed together with electrophysiological parameters and lipid droplet content during MF-LF transition. Moreover, the expression pattern of S1P metabolic enzymes and S1P receptors was measured in MF and LF. Finally, to study the involvement of sphingosine kinase and S1P receptors in the regulation of fibroblast plasticity, specific inhibitors and antagonists or RNA interference were employed. Overall, the findings could be crucial to identifying innovative and effective therapeutic strategies for the treatment of PF. The project has been founded by PRIN2022 Prot.2022NAFK8C. References: [1] Lingampally et al. (2020) Front. Bioeng. Biotechnol. 8:1-9 https://doi.org/10.3389/fbioe.2020.569865. [2] Donati et al. (2021) Cell Signal 78: 09861 https://doi.org/10.1016/j.cellsig.2020.109861

P-36-115
Immune cell infiltration and inflammatory landscape in primary brain tumours

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Primary malignant brain tumours are more than one-third of all brain tumours and despite the molecular investigation to identify cancer driver mutations, the currently available therapeutic options are insufficient. Therefore, we defined an immune and inflammatory profiling of meningioma and glial tumours. Using tissue microarrays of 158 brain tumour samples (66 meningiomas, 60 glioblastomas and 32 astrocytomas), we assessed CD3, CD4, CD8, CD20, CD138, Granzyme B (GzmB), 5-Lipoxygenase (5-LOX), Programmed Death-Ligand 1 (PD-L1), O-6-Methylguanine-DNA Methyltransferase (MGMT) and Transglutaminase 2 (TG2) expression by Immunohistochemistry (IHC). IHC expression results were correlated using a Spearman correlation matrix. Then, we tested whether RNA-seq expression could reflect the respective protein expression by analysing publicly available datasets with GEPIA2. Correlation and Overall Survival (OS) analyses on expression data were also performed in Glioblastoma (GBM) and Lower Grade Glioma (LGG). Seven out of ten markers showed a significantly different IHC expression in at least one of the evaluated cohorts whereas CD3, CD4 and 5-LOX were differentially expressed between GBMs and astrocytomas. Correlation matrix analysis revealed that 5-LOX and GzmB expression were associated in both meningioma and GBM, whereas 5-LOX expression was significantly and positively correlated to TG2 in both meningioma and astrocytoma cohorts. These findings were also confirmed with correlation analysis of TCGA-GBM and TCGA-LGG data. The results in GBM patients showed that the gene expression of GZMB (GzmB), SDC1 (CD138) and MGMT predicted a poor OS. Moreover, in LGG patients an increased gene expression of CD3D, CD3E, CD3G, CD8A, GZMB, MS4A1 (CD20), SDC1 (CD138), PD-L1, ALOX5 (5-LOX), and TGM2 (TG2) was associated with worse OS. Further evaluation is needed to understand the interplay of inflammation and immune infiltration in glioma progression. *The authors marked with an asterisk equally contributed to the work.

P-36-116
MiR-148a-3p/SIRT7 axis relieves inflammatory-induced endothelial dysfunction

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MicroRNAs (miRNAs) have emerged as versatile inflammatory mediators regulating endothelial dysfunction, the triggering factor of vascular damage and cardiovascular disease onset. In endothelial cells, MiR-148a-3p is involved in several pathological pathways, including chronic inflammatory conditions. However, the molecular mechanism of miR-148a-3p in endothelial inflammatory state is not fully elucidated. To this end, we investigated the involvement of miR-148a-3p in mitochondrial dysfunction and cell death pathways in human aortic endothelial cells (TeloHAEC) treated with interleukin-6 (IL-6), a major driver of vascular dysfunction. Results showed that during IL6-activated inflammatory pathways, including increased protein levels of sirtuin 7 (SIRT7) ($p < 0.01$), mitochondrial stress ($p < 0.001$) and apoptosis ($p < 0.01$), a decreased expression of miR-148a-3p was observed ($p < 0.01$). The employment of MiR-148a mimic counteracted the IL-6-induced cytokine release ($p < 0.01$) and apoptotic cell death ($p < 0.01$), and ameliorated mitochondria redox homeostasis and respiration ($p < 0.01$). The targeted relationship between miR-148a-3p and SIRT7 was predicted by bioinformatics database analysis and validated via dual-luciferase reporter assay. Mechanistically, miR-148a-3p targets the 3′ untranslated regions of SIRT7 mRNA downregulating its expression ($p < 0.01$). Herein, these results add knowledge on miR-148a-3p as a player in the regulation of mitochondrial damage and apoptosis during endothelial dysfunction by targeting SIRT7.

P-36-117
Conformational and dynamic properties of the KH1 domain of FMRP and its Fragile X syndrome linked G266E variant

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The Fragile X Messenger Ribonucleoprotein (FMRP) is a complex, multi-domain protein involved in interactions with various macromolecules, including proteins and coding/non-coding RNAs. The three KH domains (KH0, KH1 and KH2) within FMRP are recognized for their roles in mRNA binding and translation repression. In the context of Fragile X syndrome (FXS), characterized by CGG triplet repeats expansion, three specific point mutations have been identified, each affecting one of the three KH domains (R138Q-KH0, G266E-KH1 and I304N-KH2) resulting in the expression of non-functional FMRP. Our study aims to elucidate the molecular mechanism underlying the loss of function associated with the G266E-KH1 pathological variant. We investigated the conformational and dynamical properties of the isolated KH1 domain and the two KH1 site-directed mutants G266EKH1 and G266A-KH1. Employing a combined *in vitro* and *in silico* approach, we reveal that the G266E-KH1 variant lacks the characteristic features of a folded domain. This observation provides an explanation for functional impairment observed in FMRP carrying the G266E mutation within the KH1 domain as it renders the domain unable to fold properly. Molecular dynamics simulations suggest a pivotal role for residue 266 in regulating the structural stability of the KH domains, primarily through (de)stabilizing the a-helices of the domain rather than its β-sheet portion. Overall, these findings enhance our comprehension of the molecular basis for the dysfunction associated with the G266EKH1 variant in FMRP References: Myrick LK et al.(2015) 15;24(6):1733-40. R.J. Hagerman et al.(2017) Nat Rev Dis Primers 3 NRDP.2017.65. Santorelli D et al.(2022) Int J Mol Sci. Oct 12;23(20):12178.

P-36-118
MicroRNA-200a-3p: a promising biomarker and novel therapeutic target orchestrating amyloidogenesis and cell survival control in Alzheimer's disease pathomechanism

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Alzheimer's disease (AD) is the most common incurable neurodegenerative disorder, responsible for over 55 million dementia cases worldwide. Its molecular basis is complex, involving various molecules in the brain and peripheral tissues, including toxic amyloid peptides and genome-encoded microRNAs (miRNAs). MiRNAs regulate gene expression post-transcriptionally by binding and causing degradation of their target mRNAs. Some miRNAs can be released from cells and transmitted in the circulation. Mounting data show deregulated miRNA levels both in the AD brain and blood, suggesting their involvement in AD pathomechanism and biomarker potential. We recently found that miR-200a-3p is significantly elevated in the blood plasma of patients with early-stage AD [previously published in: Nagaraj S et al. (2017) Oncotarget 8, 16122–16143]. Now we aimed to elucidate the function of miR-200a-3p in AD's molecular mechanism. Using bioinformatics, we predicted miR-200a-3p targets and validated them in human HEK293T and neuroblastoma cells transfected with miR-200a-3p mimic using luciferase assay, RT-qPCR, and immunoblotting. We found that miR-200a-3p directly binds to and downregulates mRNA of BACE1, a key enzyme responsible for amyloidogenesis in AD. Moreover, miR-200a-3p altered levels of CDK2 and of the guardian of the genome TP53 transcripts, influencing cell survival program. These findings suggest miR-200a-3p plays an important role in AD pathomechanism by orchestrating control of amyloidogenesis and cell fate, and could serve as a novel diagnostic biomarker and therapeutic target in AD. This work was supported by the Polish National Science Centre grant OPUS 2018/29/B/NZ7/02757.

P-36-119
Transcriptomic analysis revealed aberrant extracellular matrix in SIL1-deficient human fibroblasts

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Marinesco-Sjögren syndrome (MSS) is a rare genetic neuromuscular disease that causes cerebellar ataxia, myopathy, and bilateral cataracts in children. About 60% of MSS patients carry recessive mutations in the SIL1 gene, which encodes for an ATP exchange factor of Bip, the major chaperone of the endoplasmic reticulum (ER). Loss of function of Sil1 protein affects Bip activity, resulting in ER stress and consequent activation of a complex cellular mechanism, the unfolded protein response (UPR), deputed to restore ER homeostasis. Purkinje cells and skeletal muscle fibers are the most affected by a prolonged dysfunctional UPR, but pathological hallmarks have been found in other cells, like fibroblasts. On this basis, we decided to perform a transcriptomic analysis on fibroblasts isolated from an MSS patient (HF-MSS), discovering more than 600 transcripts differentially expressed in comparison with control fibroblasts (HF-CTRL). Most of them are involved in membrane trafficking and extracellular matrix (ECM) remodeling. Several functional assays confirmed alterations in ECM of patient fibroblasts and showed reduced motility. Additionally, the different compositions of ECM between HF-MSS and HF-CTRL have been highlighted by transmission electron microscopy. Thus, we speculate that abnormal ECM might be directly involved in the pathogenesis of MSS, and this could be useful to better explain its clinical manifestations.

P-36-120
Exploring engineered SIL1 protein reintroduction to treat Marinesco-Sjögren Syndrome

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Alterations in the SIL1 gene cause the Marinesco-Sjögren Syndrome (MSS). MSS individuals display a complex phenotype, with cerebellar ataxia, early-onset congenital cataracts, and progressive myopathy as the main present symptoms among others. Analogously, the woozy (wz) mouse was selected through a spontaneous recessive mutation in the Sil1 gene, resulting in cerebellar ataxia and muscular atrophy, thus representing a valid preclinical MSS model to explore pharmacotherapeutic approaches. We explored the use of an engineered human SIL1 protein in woozy mice, investigating SIL1's ability to reach the involved tissues and reestablish the healthy muscular phenotype. We investigated two different roots of protein administration: an engineered human SIL1 protein isolated from *E. coli*, purified for affinity, and administered intraperitoneally (IP); and an AVV-carried SIL1, intravenously (IV) injected. The phenotype of IV-treated mice was monitored in cognitive-motor tests to check the ability of the AVV to reach the tissues and release SIL1 before sacrificing them after 6 weeks. Blood samples were taken from the tail of IP-treated mice, to check SIL1 concentration in the serum, before sacrificing them and freeze the tissues for molecular analysis. ELISA and western blot were used to determine the presence of SIL1 in the different tissues. The performed analysis revealed the presence of SIL1 in the quadriceps of treated mice, confirming the ability of the engineered protein to reach the peripheral muscular tissues. The presence of SIL1 in the mice skeletal muscles confirmed the good nature of the engineered protein and the roots of administration, and its ability to reach tissues mainly affected by the syndrome. Further studies are needed to deepen the pharmacokinetics of the engineered protein and whether this treatment may act by regressing the affected phenotype or slowing down the course of MSS.

P-36-121
Generating an *in vitro* model for the study of Marinesco-Sjögren Syndrome

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Marinesco-Sjögren Syndrome (MSS) is one of the many genetic neuromuscular diseases for which there is no cure yet. Most MSS patients carry homozygous or compound heterozygous SIL1 mutations. Despite the genetic variability, the pathognomonic clinical symptoms are cerebellar ataxia, bilateral cataracts and myopathy described histologically as variable muscle fiber sizes, myofibrillar degeneration and interstitial fibrosis, features shared besides with many other myopathies. Conversely, the molecular aspects are only partially known. The genetic mutation leads to a loss of function of the SIl1 protein, a nucleotide exchange factor essential for the functioning of BIP, master chaperone of the endoplasmic reticulum (ER), primarily responsible for protein folding and the activation of the so-called unfolded protein response (UPR). Although a spontaneous murine model exists, i.e. Woozy mouse, a valid *in vitro* model that can be exploited to delve deeper into the molecular aspects is instead missing, and we seek here to fill this gap. Using CRISPR Cas9 technology we stably removed SIL1 from a commercial line of murine myoblasts, C2C12. From a first validation of this line, the enormous difference that the KO clones had in differentiating from myoblasts into myotubes, compared to WT cells, was immediately evident, probably indicative of the aberrant phenotype that emerges from patients' biopsies. Furthermore, to be closer to what occurs *in vivo*, we decided to isolate in parallel primary myoblasts directly from the muscles of Woozy mice. This study therefore aims to generate an *in vitro* model for MSS which can possibly apply to other myopathies.

P-36-122
Exploring the use of host-directed miRNAs to prevent intracellular infections caused by *Staphylococcus aureus*

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Staphylococcus aureus is a facultative intracellular pathogen. Its proficiency in thriving within host cells facilitates evasion from antibiotic treatments. Many antibiotics are not able to reach the pathogen within infected cells, reducing their efficacy. Additionally, this intracellular habitat shields *S. aureus* from the host's immune response. The pathogen's ability to infect professional phagocytes and disrupt the phagosomal membrane allows it to spread in the organism to secondary points of infection, complicating its elimination. This underscores the urgent need for novel treatments targeting *S. aureus*. Recently, host-directed RNA-based anti-infectives have shown promise in the development of combinatorial antimicrobial therapies. They are particularly attractive due to their potential to prevent resistance as they are not targeting directly the pathogen. Notably, certain human microRNAs (miRNAs) have demonstrated significant antimicrobial roles, silencing crucial host genes involved in the progression of viral and bacterial infections. Despite challenges in the delivery of miRNAs to target cells, recent advancements are addressing these issues through the use of exosomes or nanoparticles, which protect the miRNAs from degradation in the bloodstream. In our study, we focused on exploring the potential of miRNAs in inhibiting the intracellular survival of *S. aureus*. We pre-treated A-549 lung epithelial cells with a miRNA mimic library before infecting them with *S. aureus* USA300. This library comprised 2,798 synthetic and double-stranded RNA molecules designed to mimic mature human microRNAs. Our preliminary results have shown encouraging outcomes with certain miRNA mimics. While further research is essential, these findings suggest that miRNA-based therapies could effectively complement traditional antibiotherapy in treating *S. aureus* host cell infections. This approach holds promise in combating the rise of multidrug-resistant superbugs. *The authors marked with an asterisk equally contributed to the work.

P-36-123
Hydrogen-deuterium exchange mass spectrometry (HDX-MS) analysis of pathogenic transthyretin (TTR) variants unveils the structural basis of genetic amyloidosis by TTR

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Amyloidoss are a group of rare diseases characterized by the abnormal *in vivo* accumulation of misfolded proteins in the form of insoluble fibrils. TTR, a homo-tetrameric plasma protein responsible of the transport of thyroid hormones, is one of the 30 proteins linked to the onset of amyloid-based diseases. Genetic amyloidosis by TTR (ATTR) is associated to single amino acid mutations in the resulting protein variants, which are much more prone to aggregate and form amyloids, compared to the wild-type protein. One common feature of TTR-amyloidosis is the presence of proteolytic TTR fragments in the amyloid deposits of patients with ATTR. However, the protease(s) responsible for this proteolytic processing have been not yet safely identified. Intriguingly, the crystallographic analysis of pathogenic TTR variants usually does not provide any reasonable structural explanation for their enhanced aggregation propensity. To better understand the pathway of amyloid formation, we produced five pathogenic mutants of TTR (i.e., V30M, S52P, L55P, V122I, E51S52dup), along with a protective TTR variant (T119M), and determined their structure and dynamics, stability and susceptibility to proteolysis in comparison to the wild-type protein. Proteolysis experiments were conducted with trypsin and subtlisin, two TTR-cleaving proteases which efficiently generate amyloidogenic fragments. Standard and pulse-labelling HDX-MS measurements were carried out to study the dynamics and the equilibrium unfolding denaturation of TTR mutants. Our results indicate that pathogenic mutations increase the conformational flexibility and reduce the stability of TTR variants, which become more prone to dissociate into monomers and even more susceptible to proteolysis, while more efficiently forming amyloid fibrils.

P-35-023

Abstract moved to talks.

POSTERS – EDUCATION

Undergraduate Teaching/ Learning

P-E-01-01
Bridging science with real-world challenges through SDGs and critical thinking

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Our 3rd year undergraduate elective course (CM3261) hosted at the NUS Department of Chemistry empowers undergraduates to bridge disciplinary divides and tackle real-world environmental challenges. Through diverse learning activities, including UN Sustainable Development Goal integration and critical source evaluation using the CRAAP framework, the course equips students with the knowledge and skills needed to combat misinformation and contribute to a sustainable future. Specifically, students had enhanced engagement with UN SDGs through project work and discussions. They developed critical thinking skills via training that fosters discernment of reliable environmental information. Our classroom observations showed that students appeared to have increased confidence in applying knowledge to address current environmental issues. This innovative approach highlights the importance of integrating real-world relevance and critical thinking skills into environmental science education, empowering future generations to navigate the complexities of information access and contribute meaningfully to a sustainable future. *The authors marked with an asterisk equally contributed to the work.

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P-E-01-02
A problem-based learning approach in molecular pathology

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This study utilizes a problem-based learning (PBL) strategy to contextualize and solidify the theoretical understanding of Molecular Pathology, a subject typically studied in the fourth year of the Health Biology degree program. This approach engages students dynamically, bridging the gap between theoretical concepts and clinical practice. Students were tasked with examining both genuine and hypothetical clinical scenarios, along with scientific literature. They had to correlate clinical parameters with symptomatology, understand the molecular basis of the pathology, and discern diagnosis and treatment options. Moreover, they were required to comprehend the targets of prescribed drugs and the underlying molecular mechanisms of their actions. This activity was conducted over eight separate days during the first semester of the 2023–4 academic year. A variety of clinical cases, drawn from either hypothetical scenarios or real cases sourced from scientific publications, were examined. Students were then prompted with a series of questions to which they were required to formulate responses. Following this period, students underwent assessment through an examination task that necessitated commenting on a case similar to the activity undertaken. They were tasked with providing commentary on the case, identifying the molecular causes underlying the pathology, and elucidating the relationship between cause and effect. The results indicated that the activity enhanced students' comprehension of the molecular foundations of pathology and their proficiency in articulating them using scientific terminology. Additionally, students demonstrated an increased capacity for critical analysis, coupled with heightened self-confidence in expressing their thoughts. The activity received high praise from students, with 95% expressing that it was both highly interesting and motivating.

P-E-01-03
Impact of 3D-printed molecular models on teaching – a compensatory research study

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The structure-to-function relationship is the hallmark of biochemistry and molecular biology. Although very useful and convenient, the two-dimensional structural formulae that students usually first encounter in the chemistry class are not able to picture the complexity of macromolecular structures such as proteins and nucleic acids. For this, animations, movies, and virtual reality are much better suited. Still, touching and handling physical models of molecules should allow students to better overcome the problems associated with the translation of 2D formulae into 3D space. To test this hypothesis, a compensatory research design was employed. Second-year bachelor students enrolled in the Molecular Biology class were randomly allocated to two groups. Both groups attended independently two lectures and were alternatively control and intervention groups. In the control group, only animations and drawings were used while in the intervention group, the same animations and drawings were replaced by 3D-printed molecular models of various amino acids and nucleotides, peptides, α-helices, β-sheets, proteins, and DNA in various representations. Models were used by the educator in front of the class but also handed to the students who were given time to interact with the models. Before and after each lecture, both groups received the same pre- and post-test consisting of a total of 23 questions evaluating the following key biomolecular visualization learning goals [Dries et. al. (2016) Biochem. Mol. Biol. Educ. 45(1), 69–75]: AR2.01; AR2.02; AG3.01; AG3.02; MA1.01; MR1.01; TC1.01; TC1.02; TC2.06; TC3.01; SF1.02. At the end of the experiment, the students were asked to fill in an anonymous feedback form. Students' scores before and after intervention as well as final feedback are currently being analyzed and statistically assessed. The experiment was approved by the Ethics Committee of the Psychology and Education Sciences Department, UAIC, Iasi (approval no 186/29.01.2024).

P-E-01-04
Promotion of intellectual and industrial property in biochemistry at the University of Bordeaux through teaching in a Master degree

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Raising awareness of intellectual property, particularly industrial property is highly recommended as soon as possible in every country and each population. Deposit patents, trend marks or design and models allow you to protect your research and is source of inventiveness, recognition and income for the inventors. These intangible assets add value to the holder even if it is an industrial company or an academic institution. In Biochemistry, the most famous example is that of the patent of Cohen and Boyer about recombinant DNA. Inventors (in the United States) benefit from a grace period of 1 year after divulgation of results to be able to decide finally to deposit a patent. It was a chance for them and for the University of Stanford in United States as they received billions of dollars through the license of their patent and royalties[1]. As a period of grace do not exist in France, it would not have been possible because a divulgation even oral stops the possibility to protect the invention by a patent. That is why it seems to be important to teach the rules of industrial properties for student's population. What is an invention? How long runs the protection? How to read the intellectual property code? What are the institutions? At the University of Bordeaux, we work during 19 hours through lectures, conference, tutorials and practical work, with students from the Master of "Biochemistry and Molecular Biology" and also from the Master "Biomaterials and Medical Devices" to teach them how to handle intellectual property assets, including copyrights, patents, trade secrets and trademarks, and how to register them legally. Whatever their future place to work (academic or private company), we assume that it is essential for them to know the rules as it will help them to take their future decisions in terms of research strategy and link it effectively with intellectual property. Reference: [1]Bera K. The story of the Cohen-Boyer patents, 2009, Current Science, 96 (6), 760-763.

P-E-01-05
Role-playing as an active learning method for postgraduates

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Role-playing in the classroom is a motivating and challenging method aiming at active learning. We held one in the workshop *Design and selection of active biological molecules*, within the *Master in Therapeutic Targets in Cell Signalling: Research and Development* at UAH. The instructors pretended to belong to a Global Health Innovation Investments company and the students (n = 21), in teams of 5-6 entrepreneurs, were part of innovative companies in the health sector. These start-ups were invited to *Funding Workshop's Investor Pitch Event*, urging them to present projects to potential investors. Objectives were set for each session: i) create an original company name and logo; ii) choose a disorder or pathology; iii) choose a signalling pathway and a therapeutic target; iv) design a new drug or innovative methodology for disease treatment; v) show potential results and prospects; vi) present orally at the Investor Pitch Event to convince investors to back their proposals. Among the disorders, freely chosen by the teams, were endometriosis, cytokine storm, addictions, and hepatitis B-derived liver carcinoma. In addition to integrating contents of the master's degree, this trained soft skills such as collaboration, creativity, critical thinking, problem solving, time management, initiative and communication. Instructors used a rubric for evaluation and students assessed their experience. Results: i) 85% of the students considered the experience as motivating for performing the tasks; ii) all of them found free arrangement in teams to be adequate; iii) 30% had some conflict that they managed conveniently; iv) 95% found the team positive for learning and, v) all of them committed to present their project at a student congress. In conclusion, the role-playing game proved to be effective for developing quality research projects and for training soft skills fundamental for professional insertion of postgraduates. InDoBio 5.0 Teaching Innovation Group; Funding: UAHEV/1517.

P-E-01-06
Blocking Biochemistry – evaluation of an immersive course design

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Biochemistry at Oxford is a 4-year integrated Master's course with a yearly cohort of 100-115. We have recently completed a major course redesign of years 2 and 3. This redesign, the first in 25 years, was motivated by the need to respond to significant theoretical and technological transformations in biochemistry, coupled with the recognition that inspiring and training the next generation of biochemists required a significant change in content and delivery. A guiding pedagogical principle was using an immersive course design in which teaching is delivered as a series of week-long blocks allowing students to take a deep dive into a biochemical topic, with time and support built in for integration and synopsis. The immersive structure was designed to best meet the needs of our students who increasingly reported challenges in exploring multiple topics simultaneously. We also diversified the assessment of the course away from end of course essay-based assessment to include elements of continuous assessment and to make the assessment more authentic. This was designed with the dual aim of supporting diverse student learning and reducing student stress. The first cohort completed the new Part I course in July 2023. Evaluation data were gathered through weekly questionnaires and termly in-person sessions. Students are overwhelmingly positive about the new course structure and particularly value the opportunities for focused learning, the integrated practical teaching and the interactive use of the VLE. We have also initiated a quantitative evaluation where students from both old and new courses sat identical assessments. Initial analysis suggests that students on the new design have better data handling and interpretation skills compared to the old design. In this poster we explore the design of the course along with evaluating its impact on student learning and outcomes, as well as the challenges of this immersive block teaching in biochemistry for staff and students.

P-E-01-07
Proposals of bioinformatics and chemiformatics application for education in food technology and related fields of study at University of Warmia and Mazury in Olsztyn, Poland

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The first bioinformatics exercise has been introduced at the Faculty of Food Science at UWM in Olsztyn, as a part of biochemistry course for food technology students in 2004, year after launching of the BIOPEP-UWM database [1]. Exercise involves matching bioactive peptides to protein sequences and proteolysis simulation using BIOPEP-UWM database https://biochemia.uwm.edu.pl/en/biopep-uwm-2/ [1]. Bioinformatics tools serve also fort exercises concerning seraching in databases of allergenic proteins and prediction of allergenicity (courses of biochemistry and alergen management for students of food technology as well as food safety and certification). Description of tools serving for these purposes is included in program of lectures. Cheminformatics includes description of databases (e.g, PubChem, ChemSpider, FooDB, KEGG and BRENDA) as well as exercise concerning prediction of toxicity and absorption of low molecular compounds by human organism. Links to chemical databases and programs are available via the MetaComBio website: https://biochemia.uwm.edu.pl/metachemibio/about-metacombio/ [2]. Speciality „Food engineering" includes course named „Enzymology and bioinformatics" providing integrated information from the areas of cheminformatics and bioinformatics (e.g. searching for information about low-molecular compounds, their enzymatic reaction and metabolic pathways). Application of the above tools follows contemporary trend to use Internet as a major source of knowledge. References: 1. Iwaniak A et al. (2024) Curr Opin Food Sci 55, 101108. 2. Minkiewicz P et al. (2015) J Chem Educ 92, 874-876. Funded by the Minister of Science under „the Regional Initiative of Excellence Program."

P-E-01-08
Network research projects: organization of educational programs in life sciences for schoolchildren and teachers

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Network research projects are a universal tool for involving schoolchildren and their teachers in educational research projects in life sciences. The project "Atlas of Soil Microorganisms" was implemented in 75 regions in 2021–2023. As a result of its implementation, more than 6,000 schoolchildren and teachers took part in it and collected over 20,000 samples of soil and soil microorganisms. An essential feature of the work of "citizen scientists" was that they not only collected soil samples and recorded a geotag but also carried out initial screening of samples, analyzed the presence of plant growth-promoting bacteria (PGPB) in the samples, which are relevant for sustainable agriculture. Based on the results of the project, the researchers of the ICBFM SB RAS and partner organizations published more than 40 papers in peer-reviewed scientific journals. Several new bacteriophages enzymes relevant for the genetic technologies, were identified in samples collected by citizen scientists. In some projects, a decisive contribution was made by schoolchildren of 7–10 grades. It has been shown that the application of a consortium of soil PGPB together with the application of 50% of the recommended volume of a complex of mineral fertilizers allows one to obtain the same increase in shoots and roots, as well as seedling biomass, as with the application of the full volume of fertilizers. Moreover, qPCR analysis showed that the introduction of a microbial consortium with fertilizers into the soil leads to an increase in the number of copies of siderophore biosynthesis genes by four orders; the number of copies of nitrogen metabolism genes was increased by 10–100 times, phytase genes were increased in 10 times. The experience of network research project implementation is easy to scale to other regions, so this practice is an excellent example of easy entry into project activities in high school. The work was supported by the Atlas of Soil Microorganisms project (075-15-2021-1085).

P-E-01-09
Transdisciplinary approaches for education innovation in biological, chemical and biomedical sciences: fostering creativity, critical thinking and collaborative learning

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The coauthors of this communication are involved in undergraduate and postgraduate teaching in the fields of Biology, Chemistry and Biomedicine. We are implementing an Education Innovation Project aiming to foster creativity, critical thinking and collaborative learning in our students. This project promotes meaningful and relevant learning through the use of collaborative learning strategies based on cases (real or simulated, CBL), problems (PBL), projects (PrBL), and challenge-based learning (ChBL). We will emphasize that the cases, problems, projects and challenges designed and proposed simulate authentic real-world tasks or simulate professional situations. We will also promote that our students acquire skills and practice the search and selection of relevant scientific information, the identification of pseudo-scientific, manipulated or technically false information, the comprehensive reading of scientific articles, the presentation and discussion of results. These skills are key to foster their critical thinking and their capacities in science communication. Our project also includes the involvement of professor and students in several annual workshops, namely, a Vocational Guidance and Entrepreneurship Promotion Workshop, a Metabolism Workshop, and a Advances in Molecular Oncology Workshop. This work is supported by an Educative Innovation Project (PIE22-118, funded by University of Málaga). [Grants: PID2023-148504OB-I00 (Spanish Government). Funds from BIO 267 (Andalusian Government, University of Málaga, EU FEDER)].

P-E-01-10
The effects of introducing new teaching methods and student-oriented teaching in the short course "Biochemistry with elements of chemistry"

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An attractive learning environment increases the number of satisfied learners. Techniques increasing engagement and student-centered education should be a standard of modern teaching. Short courses with small student numbers pose challenges. Students start with a different initial knowledge. Implementation of active teaching methods creates an opportunity to engage all students. The study was designed to report changes implemented in the biochemistry course for the paramedics. As part of the course reorganization, techniques that aimed to create conditions for improving and equalizing the students' learning experience were applied. The main change in the course concept was a shift from knowledge transfer to active student participation in the learning process. Remote teaching on an e-learning platform was introduced. Participants could self-regulate the amount of time spent mastering this part of the course as needed. Applied case-based learning (CBL), worksheet tools, and laboratory reports enhanced the ability to critically analyze results and structured the knowledge. The original survey was used. It was anonymous, voluntary, and conducted after the completion of the course and before the exam. 62 first-year students took part in the survey (34 the year before the and 28 the year after the changes). Before the changes, the majority of individuals evaluating the course (78%) gave a rating of 4 on a scale of 0-5, and only 3% gave a rating of 5. The course change increased by fully satisfied with 32% rating 5 (while 53% a rating of 4). Additionally, course material quality evaluations were conducted. The percentage of those who considered it sufficient and well-delivered was 37% (before the changes) and 48% (after the changes). Those considering the material sufficient but not interestingly delivered decreased from 31% to 15%. However, those considering the material too broad and difficult increased from 28% to 37%.

This motivates further changes within the course. *The authors marked with an asterisk equally contributed to the work.

P-E-01-11
Nursing-Escape Room, an integrative tool in the biochemical laboratory for the study of diabetes mellitus

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Inclusion of game in the classroom aims to motivate students in order to promote meaningful learning. All students in 1st year of the Nursing Degree participated in this experience after the practical sessions of the Biochemistry course. Students assessed: i) concentration of glucose (serum); ii) percentage of glycated haemoglobin (haemolysate); and iii) concentration of ketone bodies (serum), to monitor diabetes mellitus. The activity was aimed at reinforcing concepts, knowledge of the bases of the techniques and integration of the metabolic pathways involved. In addition, the experience intended to train skills specific to laboratory work, including determining concentrations, mastering the use of micropipettes, using spectrophotometers, deducing the suitability of different samples, etc. Four Nursing-Escape Rooms were held with students organised in groups of 7-8 members outfitted with a team colour. One instructor per team checked that tests were carried out correctly and that group members understood the concepts being reviewed. At the end of the game, students rated the impact of the activity on their study and learning. They considered very positive to have participated in the Nursing-Escape, as a "different" way of learning and solving doubts. Likewise, instructors evaluated the effect the game had on understanding of the contents during the experience, perceiving deductive reasoning in the tests, and once the game was over, considering the grade in assessment of the contents. The results obtained support that the introduction of challenge-based games in the laboratory helps foster motivation, an essential condition for achieving meaningful learning. InDoBio 5.0 Biochemistry Teaching Innovation Group (UAHGI21171) Funding: Proyectos para el Fomento de la Innovación en el proceso de Enseñanza¬Aprendizaje. Vicerrectorado de Estrategia y Planificación de la UAH. "UAHEV/1517 Escape Rooms UAH: motivación para aprender en el laboratorio de Bioquímica, 2023."

P-E-01-12
Synthesizing proteins: a virtual learning experience

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Virtual games have gained prominence in science teaching because of their role in students' cognitive development. In biology teaching, games are suggested by the Brazilian national curriculum as a strategy for approaching science topics. Our research group, based at EIC/CIBFar, has been developing interactive educational resources containing 3-D images, which is a popular and widely disseminated form of communication and learning, contributing to the construction of knowledge in different areas of science. One of these resources is a board game aimed at high school and undergraduate courses called Synthesizing Proteins, with an investigative and applied approach. This topic is involved in practically all biological phenomena, so understanding this phenomenon is essential to understanding how cells work and how life is established. Recently EIC/CIBFar team developed an electronic version of the game, with a programming language that can be accessed in different browsers and installed on Windows or Linux systems. It simulates the protein synthesis process within a eukaryotic cell from the gene, following the steps of transcription, translation and protein synthetized addressing. The computer resources used were Inkscape, Unity, Gimp and the C# language. The game begins with the user having to choose one of four proteins after which an animation starts showing a physiological situation in which the protein is involved. Next, the user will complete five missions: 1) identifying the components of eukaryotic cells and finding their nucleus to continue; 2) getting to know the nucleus components and transcribing the gene for mRNA synthesis; 3) carrying the mRNA outreach the nucleus to the cytoplasm for decoding at ribosomes on the RER, starting protein synthesis; 4) processing, folding and forming complexes of the synthesized protein, so that it becomes functionally active; 5) to give destination of the synthesized protein to into or out of the cell after answer a quiz. *The authors marked with an asterisk equally contributed to the work.

P-E-01-13
BIOqui-EScape as an active and participative methodology for undergraduates in Biology Degree

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Active and participatory methodologies in university teaching are essential since they promote student engagement, motivation, and meaningful learning. By encouraging interaction, collaboration and critical thinking, they allow students to construct their

own knowledge, apply what they learn to real situations and develop problem-solving skills. Another advantage is that they foster intrinsic motivation and interest in learning, increasing information retention and the transferability of knowledge to different contexts. By focusing on students as protagonists of their learning, these methodologies prepare more effectively future professionals to face the challenges of today's world. Our experience was carried out with students of the Biochemistry course in 2nd year of the Biology Degree. A virtual escape room entitled "Searching the inhibitor" was developed on the *Genially* application. It served as a final review of all the knowledge acquired in seminars over four months. Two virtual rooms were created, with clues hidden in the laboratory equipment, which students had to find in groups of three. The objective was to deduce the sequence of a pentapeptide that blocks an enzyme essential for the survival of a deadly virus. In order to achieve this, the students had to solve five challenges. The sequence obtained was entered in the final screen of the Escape, which communicated whether the result was correct. At the end of the session, the students filled in a form to evaluate the activity. They perceived the methodology as highly motivating, as well as helpful for them to consolidate the knowledge imparted and to solve any doubts arisen during study of the seminars. InDoBio 5.0 Biochemistry Teaching Innovation Group (UAHGI21171) Funding: Proyectos para el Fomento de la Innovación en el proceso de Enseñanza–Aprendizaje. Vicerrectorado de Estrategia y Planificación de la UAH. "UAHEV/1517 Escape Rooms UAH: motivación para aprender en el laboratorio de Bioquímica, 2023."

P-E-01-14
Learning protein synthesis via a tabletop card game: "The ribosome game" put to the test from primary school to university courses

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We developed an interactive team card game to increase knowledge about protein synthesis and the role of amino acids in protein structure. Protein synthesis is one of the fundamental mechanisms for life, and involves an organelle of the cell, the ribosome, which gives this game its name. The ribosome reads and decodes DNA information that reaches it via messenger RNA and translates the genetic code to achieve the correct assembly of proteins by combining the individual bricks that make it up, the amino acids. In the proposed "Ribosome Game," two teams of players are rivals in "playing the ribosome" and producing the best protein (longer and with extra bonus points) using cards that "speak the language of instructions" (the genetic code) and which allow, taken in groups of three cards, to 'buy' an amino acid, according to the decoding rules of the ribosome. The 'Ribosome Game' tests were accompanied by data collection to assess impact on more than 1800 players in the age range between 7 and 34. Data are reported on the learning outcome on the specific process and amino acids function as well as on the perception of the complexity of biochemistry and on engaging students in STEMs. Significant improvements were observed comparing questionnaires, collected before and after the test matches, on learning and interest selected indicators. As a further approach to innovation in teaching protein synthesis, the visualisation of created proteins was achieved via Alphafold2 at https://colab.research.google.com/github/sokrypton/ColabFold/blob/main/AlphaFold2.ipynb#scrollTo=kOblAo-xetgx and virtual reality on Metaquest2 devices using the software Nanome. The project was supported by the Proof-of-Value (POV) funding scheme 2022 sponsored by Compagnia di Sanpaolo and University of Torino. Endorsement and support in-kind was also provided by: Il Salotto di Giano, Fondazione Paideia, Quercetti, ANISN, Xké: Il laboratorio della curiosità, IIS Leardi, Liceo Porporato.

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Biochemistry case studies: a new online course for medical students

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Biochemistry is a quite difficult subject to study for medical students. According to teaching evaluation surveys, students feel a lack of connection between a boring theory and a real clinical medicine. We were thinking for a long time about revitalising the teaching of medical biochemistry and showing students how biochemistry relates to clinical medicine. We have therefore developed an online course using biochemical case reports. In the course, there are twelve case studies, i.e. twelve patients with twelve different medical problems (diseases). Each case is presented as a first-line physician (a general practitioner, a paediatrician, a doctor at the central reception desk of a hospital) would see it. The text provides only information that help students to find their way and solve the problem. It is actually a kind of puzzle with a clue. The task is to get through the mysterious problem (disease) and solve the puzzle. Students are given a glossary of terms, then a supplementary examination and a differential diagnosis. From these, the students choose the correct solution, which unlocks the last part, the clinical pearls for the case and the deep biochemical explanation of the problem. In the academic year 2023/24, a total of 219 general medicine students participated in this course. Students who completed the course had to fill out a survey. 90% of the students evaluated the obtained information very useful for their final biochemistry exam. The most popular case studies were anorexia nervosa (20%), gout, diabetes mellitus and phenylketonuria (11% each). The number of case studies was sufficient for 97% of the students and 91% of them found the case studies easy to understand. Only 9% of the students stated that the knowledge acquired after one and a half years of medical studies was not sufficient to assess the specific pathology in the body and that some diseases were unknown to these students.

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Developing education and training in undergraduate level biochemistry laboratories in Türkiye and Northern Cyprus

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Biochemistry is a vast and multidisciplinary field of research that encompasses the study of chemical processes in living organisms. Undergraduate biochemistry laboratory courses include practicals that require students to use laboratory math skills, such as pH, absorbance, concentration, dilution, and solution calculations. This study investigated the impact of incorporating practical tests into biochemistry laboratory practice on student success and aimed to explore the correlation between student learning styles and academic achievements. The study population consisted of 235 Year I students, 43.4% male (n = 102) and 56.6% female (n = 133) from the Medical Faculty at Gazi University and Kyrenia University, with an average age of 19.87 ± 1.81. The study was conducted as a descriptive and cross-sectional study, and the quantitative data was collected from pre-test and post-test measures. There was a significant difference between pre-test and post-test scores ($p < 0.001$), indicating that incorporating tests into biochemistry laboratory practice was beneficial for medical students. These practicals helped the students learn more effectively, allowing them to apply theoretical concepts to real-world situations and understand the relevance of the ideas they learned in class. Moreover, the practical tests of biochemical calculations encouraged students to work collaboratively, communicate effectively, and develop problem-solving skills. Consequently, practical tests are a valuable tool to enhance student success in biochemistry laboratory courses. The findings of this study have significant implications for the design and implementation of laboratory courses in biochemistry and related fields. Incorporating practical tests into laboratory practice may help students develop the skills and knowledge necessary for success in their future careers. Keywords: Medical students, biochemistry, undergraduate education *The authors marked with an asterisk equally contributed to the work.

Postgraduate Teaching/Learning

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Application of active learning strategies to improve student engagement

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Engagement and active participation are essential in postgraduate education, fostering critical thinking and deeper understanding. This study investigates the effectiveness of incorporating active learning strategies into master courses to enhance student engagement. The approach was tested with graduate students in various Applied Microbiology, Medical Biochemistry, and Microbe Biotechnology courses. Through a mixed-methods approach, various active learning techniques, such as flipped classrooms, problem-based learning, case studies, and collaborative projects were implemented to teach different aspects of microbial biochemistry. Significant improvement in student participation rates and knowledge retention compared to the traditional lecture-based approach was recorded. Qualitative data from student surveys and focus group discussions highlighted increased motivation, deeper comprehension of complex concepts, and enhanced peer interaction. These findings highlight the importance of integrating active learning strategies into postgraduate teaching practices to cultivate a dynamic learning environment and contribute to the academic success and professional development of the students. *The authors marked with an asterisk equally contributed to the work.

P-E-02-03
Gamification in marine biology: a strategy to improve perspectives of basic research

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Gamification has emerged as a valuable pedagogical tool motivating students to participate and engage more intensively through game mechanics and it has successfully been applied in Higher Education. In our study, we explore the application of gamification to basic research discoveries in marine biology, aiming to improve learning efficiency, motivation, and participation. We developed the "*SEA game: Search, Explore and Apply*" featuring two sets of cards: one picturing marine models such as sea star larvae, jellyfish, sea urchin eggs or the giant squid; and the other showcasing research discoveries such as phagocytosis, the green fluorescent protein, cyclins, or the neural action potential. We included notable cases from Nobel Prize-winning research or commercially available drugs when possible. We implemented the *SEA game* in the course UE SBME19B of the *Master Sciences de la Mer* of *Université de Caen Normandie* for the academic year 2023–2024 and conducted an evaluation to validate our hypothesis. In this pilot study, ten students were invited to experience and provide their feedback. Results indicated that the *SEA* game improves student motivation, participation and learning efficiency. Quantitatively, their ability to provide successful examples of marine research increased from 0 to 4–9 before and after the game, respectively. Qualitatively, participant feedback highlighted positive aspects such as engaging in discussions during gameplay or interest in learning more about some cases, while concerns focused on technical limitations such as the number of cases and time constraints. Despite the additional workload for teaching staff, integrating the *SEA* game into the classroom proves to be a valuable pedagogical tool. Future plans involve expanding the *SEA* game's repertoire of cases and inviting more students to participate.