The

4th Polish Zebrafish Society Meeting

Abstract Book

Poznań
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Edited by

Emilia Cicha, Agnieszka Karbownik, Mateusz de Mezer, Edyta Szałek, Michał Szulc, Helena Wielgus

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Introduction

Hello,

On the behalf of the Polish Zebrafish Society and the Local Organizing Committee I would like to warmly welcome you on the 4th Polish Zebrafish Society Meeting. This time we will be hosted by the Poznan University of Medical Sciences.

This is a clear sign that the zebrafish makes bigger waves in Poland. With around 140 registered participants and just over 50 posters, it is a clear sign that not only zebrafish gains on the popularity, but also our Society grows in strength. We do hope that our Society will grow even more after this conference. With numerous benefits to the members, we warmly invite you to join us.

We also hope that this conference will meet your expectations of a good scientific forum for the idea exchange, networking and establishing new and fruitful collaborations.

Very kind regards,

Chair

Przemko Tylżanowski

Program of the 4th Polish Zebrafish Society Meeting

DAY I	13.02.2025
10:00 - 11:00	**Science popularization lecture (PL)**: "Jak mała rybka pomaga w badaniach naukowych?" **Magda Dubińska-Magiera**, **Marta Migocka-Patrzałek,** *University of Wrocław* ***Location:*** *Congress and Didactic Centre, Room A*
10:00 - 11:00	***Zebrafish Breeding and Welfare Section Meeting*** **Location:** Medical Biology Centre, Room 2009
11:00 - 12:00	**Welcome: Vice-Rector for Science Professor Michał Nowicki, MD, PhD,** *Poznań University of Medical Sciences*
12:00-12.30	**Open lecture**: "Fishing for a Disease - Zebrafish in Biomedical Research" **Przemko Tylżanowski**, *Medical University of Lublin, University of Leuven* Discussion panel **Location:** Medical Biology Centre, Room 1010
12:00 - 13:00	**Zebrafish Breeding and Welfare Section Meeting** "Inside the Champalimaud Fish Platform: Zebrafish Care & Colony Management" **Joana Monteiro**, *Champalimaud Foundation, Lisbon Portugal, (lecture on-line)* **Location:** Medical Biology Centre, Room 2009
from 12:30-	Registration
13:00 - 14:30	General Assembly of the Polish Zebrafish Society **Location:** Medical Biology Centre, Room 1010
14:30 - 14:45	Opening of the Conference **Przemko Tylżanowski,** *Chairman of Polish Zebrafish Society* **Location**: Medical Biology Centre, Room 1010
14:45 - 15:30	**Keynote Lecture** - "Zebrafish Avatar-test for personalized medicine" **Rita Fior**, *Champalimaud Center, Lisbon, Portugal* **Location**: Medical Biology Centre, Room 1010

15:30 - 16:30	Session I – **Oncology** **Chairman**: **Przemko Tylżanowski** **Location**: Medical Biology Centre, Room 1010 1) Evaluation of the Zebrafish Xenograft Model in Veterinary Oncology. **Małgorzata Chmielewska-Krzesińska**, *University of Warmia and Mazury in Olsztyn* 2) Evaluation of the Anticancer Effects of Cisplatin and EX527 in the Treatment of Head and Neck Squamous Cell Carcinoma (HNSCC). **Marzena Baran**, *Medical University of Lublin* 3) Evaluation of tyrosine kinase inhibitor-associated cardiovascular toxicity. **Emilia Seta**, *Jagiellonian University in Kraków*
16:30 - 17:00	Coffee break
17:00 - 18:45	Session II – **Immunity** **Chairman**: **Piotr Podlasz** **Location:** Medical Biology Centre, Room 1010 1) Zebrafish as a real-time model to investigate phagocyte activity and inflammation in response to amyloid-b1-42. **Magdalena Marcinkowska**, *Jagiellonian University in Kraków* 2) Clock gene cry1 knock-out affects the survival and immune response against viral infection in zebrafish larvae model. **Mikołaj Mazur**, *Jagiellonian University in Kraków* 3) The effect of 25-hydroxycholesterol (25HC) on viral load in zebrafish larvae infected with Tilapia Lake Virus (TiLV), Spring Viraemia of Carp Virus (SVCV) and Nervous Necrosis Virus (NNV). **Justyna Starzyk**, *Jagiellonian University in Kraków* 4) Streptococcus pneumoniae is targeted by two non-canonical autophagy pathways within zebrafish macrophages. **Bartosz Michno,** *Jagiellonian University in Kraków* 5) Enzybiotics: A Cutting-Edge Solution for Tackling Pathogenic Bacteria, Combating Antimicrobial Resistance and Improving Animal Care. **Małgorzata Korzeniowska**, *Mossakowski Medical Research Institute - Polish Academy of Sciences* 6) Balancing Genetic Diversity, Cost Efficiency, and Animal Welfare in Zebrafish Facility Management. **Magdalena Gral**, *International Institute of Molecular and Cell Biology in Warsaw*
18:45 - 21:00	**Poster session and "Get together party"** **Location:** Ground floor corridor

DAY II	**14.02.2025**
9:00 - 10:45	Session III – **Diseases Models** **Chairman**: **Anna Sarosiak** **Location**: Medical Biology Centre, Room 1010 1) Genomics dissection into heart development and disease. **Cecilia Winata**, *International Institute of Molecular and Cell Biology in Warsaw* 2) Generation and analysis of muscle glycogen phosphorylase (Pygm) deficiency zebrafish model. **Magda Migocka-Patrzałek**, *University of Wrocław* 3) Functional studies of hereditary human mutations leading to craniofacial malformations. **Paulina Krzesińska**, *Medical University of Lublin* 4) Vibrational spectroscopy imaging in zebrafish larvae model of obesity. **Grzegorz Kalisz**, *Medical University of Lublin* 5) Using zebrafish larvae to model type 2 diabetes: effects of subchronic glucose exposure. **Jagoda Szponar**, *Medical University of Lublin* 6) Towards an understanding of mitochondrial Ca2+ transport using the zebrafish. **Iga Wasilewska**, *Mossakowski Medical Research Institute - Polish Academy of Sciences*
10:45 - 11:15	Coffee break
11:15 - 13:00	Session IV – **Neurobiology & Behavior** **Chairman: Magdalena Chadzińska** **Location**: Medical Biology Centre, Room 1010 1) Innovative Approach to PTZ-Induced Seizure Analysis in Zebrafish. **Małgorzata Potoczna**, *University of Warmia and Mazury in Olsztyn, Transpharmation Poland Ltd.* 2) Zebrafish as a model to evaluate the efficacy of zirconium-based Metal-Organic Frameworks in amphetamine detoxification. **Anna Boguszewska-Czubara**, *University of Lublin* 3) Study of the involvement of selected bioactive substances from Withania somnifera in the modulation of behavioral responses and gene expression for specific subunits of the GABAA receptor in danio rerio larvae under the influence of ethanol. **Weronika Jarczak**, *Poznań University of Medical Sciences* 4) Short-term exposure to nanoplastic affects nervous system-associated gene expression in the Zebrafish (Danio rerio) brain: a pilot study. **Wojciech Langwiński**, *Poznań University of Medical Sciences* 5) Assessment of indoximod toxicity in a zebrafish model. **Beata Sieklucka**, *Medical University of Białystok*
13:00 - 13:15	Conclusions, award ceremony and closing remarks **Location**: Medical Biology Centre, Room 1010

Abstracts

INITIAL STUDIES ON ZEBRAFISH (*DANIO RERIO*) C-TYPE LECTIN RECEPTORS

Adamek M.[1*], Rakus K.[2], Breitkopf V.[1], Monteiro J.[1], Podlasz P.[3], Mojżesz M.[2], Zawisza M.[2], Krebs T.[1], Pooranachandran N.[2], Widziołek-Pooranachandran M.[2], Falco A.[4], Chadzińska M.[2], Steinhagen D.[1], Lepenies B.[1]

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Myeloid CLRs serve as pattern recognition receptors (PRRs) in innate immunity, often recognising evolutionarily conserved pathogen-associated molecular patterns (PAMPs). They are predominantly expressed by innate immune cell subsets such as monocytes, macrophages, dendritic cells, NK cells and neutrophils. Ligation of CLRs by pathogen-derived ligands acts as a 'danger signal', initiating a pro-inflammatory response. While the effect of CLRs on the immune response has been extensively studied in mice and humans, little is known about the role of CLRs in immunity in other veterinary species, including fish. Consequently, very few studies have been conducted in the context of bacterial and viral infections in fish, even in well-studied model organisms such as zebrafish (*Danio rerio*).

The zebrafish genome was screened for the presence of novel CLRs. C lectin receptor genes were molecularly cloned and analysed for structural transmembrane classification. The mRNA expression of selected CTRs were studied during infections with fish pathogenic viruses including chum salmon reovirus (CSV), carp viremia virus (SVCV) and tilapia lake virus (TiLV). In addition, expression was studied in zebrafish injected with recombinant IL-6, CRP-5 and in rag1 mutants. Moreover, putative CLR-Fc fusion proteins were used to screen for novel CLR-pathogen interactions. The extracellular portion of each CLR was ligated into a pFuse-hIgG1-Fc expression vector and CLR-hFc fusion proteins were produced in CHO-S cells and purified from the supernatant using HiTrap Protein G HP columns. ELISA-based detection of CLR/bacteria interactions was used for initial pre-screening with several fish pathogens, which was further confirmed by flow cytometry-based binding analysis.

After screening the zebrafish genome, two putative CLRs, tentatively named UP463 and UP690, were selected for further study. They are likely to be homologs of mammalian MINCLE and SIGNR3, encoded by the genes named *up463* and *up690*. When the mRNA expression of the two putative CLRs encoding genes *up463* and *up690* was measured, a strong down-regulation was observed in fish under viral infection. IL-6, CRP-5 injection and *rag1* deletion did not regulate expression. The recombinant CLR-hFc fusion proteins showed no binding capacity for fish pathogenic bacteria.

These initial results provide a good basis for further functional studies of CLRs. Viral infection seems to downregulate the expression of CLRs. The expression is not regulated by the acute phase response and the receptors do not bind pathogenic bacteria. Therefore, our current working hypothesis is that UP463 and UP690 might be more involved in viral recognition and that their activation might lead to a better antiviral response. To test this, we will use additional methods such as CRISPants and whole body overexpression in zebrafish larvae to test the activity of UP463 and UP690 during viral infection.

IDIOSYNCRASY OF ANTISENSE OLIGONUCLEOTIDE TARGETING PROTEIN-CODING GENE EMBEDDED WITH NON-CODING RNA IN VIVO

Ali H.[1*], Anbalagan S.[1]

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Antisense oligonucleotides (ASO) are powerful tools to alter gene expression and ASO's are even under clinical use and clinical trials to treat human diseases. Whether ASO's targeting protein-coding genes embedded with non-coding RNA's affect non-coding RNA expression or function is relatively unknown. While studying the development of the posterior pituitary, an important neuroendocrine interface, we observed that an ASO targeting only the splice site but not the translation site of the gene *slit3* disrupts pituitary axonal morphogenesis. In addition to altered *slit3* splicing, we also observed an increase in the expression of *slit3* and *slit3* intron-embedded primary *mir218a-1* transcripts.

The ASO-induced phenotype was not observed when mature *mir218a-1* was blocked by an ASO or in *mir218a-1*$^{-/-}$ knockout zebrafish embryos We believe our results highlight a blind spot in ASO-based research and call for a careful evaluation of results from ASO studies when targeting protein-coding genes embedded with non-coding RNAs.

ANALYSIS OF A NEW, ZEBRAFISH PYGM-/- LINE IN MCARDLE STUDIES

Altinay A.N.[1*], Stefanik E.[1], Daczewska M.[1], Migocka-Patrzałek M.[1]

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McArdle disease (Glycogen Storage Disease Type V) is an autosomal recessive disorder arising from impaired activity of PYGM (muscle glycogen phosphorylase) due to mutations in the gene encoding this protein.

In our project, we aim to develop an animal model for McArdle disease using zebrafish (*Danio rerio*). To create accurate model of the impaired human gene, we obtained a pygm-/- line strain by knocking-out the pygm gene in zebrafish using CRISPR-Cas9 technique. The mutation was confirmed by assays using restriction enzymes interactions with DNA samples. To obtain results, following assessments were made. Birefringence analysis was used to visualize and quantify the changes in structure of muscle fibers in both wild-type and mutant larvae. Comparison between samples showed a decrease in light intensity in images of mutants, indicating damage to muscle tissue. Additionally, morphological measurements of zebrafish at 72 hpf revealed that mutants were shorter in length compared to wild-type and demonstrate physical anomalies.

To summarize, our results allow the conclusion that the new zebrafish mutant line pygm-/-with impaired muscular glycogenolysis due to lack of PYGM, exhibits signs of McArdle symptoms such as changes in muscle structure. Although more detailed analysis is needed, we could conclude that pygm-/- line will optimistically serve as a new tool in studying McArdle disease.

This work was supported by the National Science Centre, Poland (2021/43/D/NZ4/00081).

GASORECEPTORS AND GASOCRINE SIGNALING

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In the scientific literature, gas-sensing biomolecules are primarily proteins (gasoreceptors) such as the heme-based NO (nitric oxide)-sensing gasoreceptors soluble guanylate cyclase, REV-ERBβ transcription factor in mammals and the O_2-sensing gasoreceptors FixL kinase and Dosphosphodiesterase in microorganisms that trigger gasocrine signaling.

[1,2] Whether zebrafish contain similar gasoreceptors that sense gaseous molecules such as N_2, C_2H_4, H_2S etc. per se is still unknown.[1] The experimental identification of gas-sensing proteins and other biomolecules (nucleic acids) in zebrafish can potentially help to verify the following postulates of gasocrine signaling. 1) All living organisms composed of one or more cells require gasocrine signaling to sense, communicate, survive and propagate. 2) Gasocrine signaling mediated via gasoreceptor proteins (or perhaps riboceptors) is the most essential cellular and inter-organism signaling. 3) All cells and acellular entities arising from or replicating in pre-existing cells require gasocrine signaling.

References:
1. Aono S. Gas sensing in cells. Royal Society of Chemistry. Great Britain. 2017.
2. Anbalagan S. Gas-sensing riboceptors. RNA Biol. 2024. DOI: 10.1080/15476286.2024.2379607

EVALUATION OF THE ANTICANCER EFFECTS OF CISPLATIN AND EX527 IN THE TREATMENT OF HEAD AND NECK SQUAMOUS CELL CARCINOMA (HNSCC)

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Head and neck squamous cell carcinoma (HNSCC) is a highly heterogeneous malignancy often characterized by aggressive growth and resistance to standard therapies. Cisplatin, a commonly used chemotherapeutic agent for HNSCC, exerts its effects by inducing DNA damage in cancer cells. However, resistance to cisplatin limits its therapeutic efficacy. The sirtuin inhibitor EX527, targeting SIRT1, has the potential to enhance the effects of cisplatin by influencing epigenetic regulation and DNA repair mechanisms in cancer cells. The objective of this study is to investigate the synergistic effects of cisplatin and EX527 on HNSCC tumor growth in vitro.

To evaluate the potential synergy between cisplatin and EX527 in inhibiting the growth of HNSCC cancer cells and to identify the molecular mechanisms underlying their effects.

Patient-derived HNSCC cells will be cultured in vitro and divided into four groups: control, cisplatin-treated, EX527-treated, and combination treatment with cisplatin and EX527. After 48 hours of treatment, the following analyses will be performed:

1. Cell viability assay: Using the MTT assay to assess the effect of treatments on cell proliferation.
2. Apoptosis analysis: Employing flow cytometry to evaluate apoptosis levels.
3. Assessment of the cell cycle progression in cell lines after treatment with the tested substances using flow cytometry and staining with propidium iodide (PI) in combination with RNase.
4. In vitro imaging: Staining with Vybrant DiD to monitor morphological changes in the cells.

Combination therapy with cisplatin and EX527 is anticipated to exhibit stronger anticancer effects than either agent alone. The inhibition of DNA repair by EX527 may sensitize HNSCC cells to cisplatin, resulting in enhanced apoptosis and reduced proliferation.

The combination of cisplatin and EX527 may represent a novel therapeutic strategy for the treatment of HNSCC. Targeting epigenetic regulatory mechanisms with EX527 could enhance the efficacy of cisplatin by disrupting DNA repair processes and inducing apoptosis in cancer cells.

Cisplatin is a standard treatment for HNSCC, but its effectiveness is limited by tumor resistance. Sirtuins, including SIRT1, play a crucial role in DNA repair and epigenetic regulation, making them attractive therapeutic targets. By inhibiting SIRT1 activity, EX527 may disrupt adaptive mechanisms in cancer cells and enhance their sensitivity to cisplatin, justifying the use of this combination in the study.

KNOCKOUT OF CALCIUM SENSOR – *STIM2* GENE CAUSES MICROGLIAL ACTIVATION AND CELL LOSS IN ZEBRAFISH RETINA

Baranykova S.[1*], Gupta R.K.[1,7], Kajdasz A.[2,3], Wasilewska I.[4], Macias M.[5], Szybinska A.[5], Węgierski T.[5], Nahia K.A.[6], Mondal S.S.[6], Winata C.L.[6], Kuźnicki J.[1], Majewski Ł.[1]

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Calcium plays one of the important roles in regulation of microglial functioning. Variations in intracellular calcium levels regulate migration of microglia, its transition between different functional states, phagocytosis and release of cytokines. Increases of intracellular calcium levels are associated with microglial activation as well.

STIM2 is a calcium sensor and a key player in store operated calcium entry machinery. Our data shows that knock out of *stim2* in zebrafish causes a significant increase of number of microglial cells in retina along with loss of ganglion cells (RGCs), GABAergic cells, cristae in mitochondrial photoreceptors and decrease of glycogen deposition in liver. Some of the phenotypical changes are similar to those in glaucoma, such as thinning of retinal layers and loss of RGCs.

We hypothesize that lack of STIM2 leads to ganglion cells loss because of microglial activation, and if so, *stim2* KO might be a good model to study some features of glaucoma. Our goal is to identify the mechanisms through which microglia may cause the changes that are observed in the retina of knockouts.

Funded by National Science Centre, Poland, PRELUDIUM 22 (no. 2023/49/N/NZ3/02921) to S.B., National Science Centre (no. 2016/23/B/NZ3/03142) to J.K.

ZEBRAFISH AS A MODEL TO EVALUATE THE EFFICACY OF ZIRCONIUM-BASED METAL-ORGANIC FRAMEWORKS IN AMPHETAMINE DETOXIFICATION

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Zebrafish (*Danio rerio*) larvae were employed as an in vivo model to evaluate the efficacy of zirconium-based metal-organic frameworks (Zr-MOFs) in mitigating amphetamine (AMP) toxicity. Three Zr-MOFs: MOF-808, UiO-67 and NU-1000, were tested for their ability to adsorb and neutralize AMP.

Metal-organic frameworks (MOFs) are highly porous materials composed of inorganic metal clusters and organic linkers, offering exceptional surface area, tunable pore sizes and chemical stability. Their unique structure allows precise adsorption and sequestration of target molecules, making them ideal for applications in detoxification, drug delivery and environmental remediation.

According to the World Health Organization (WHO), over 296 million individuals aged 15−64 globally abused drugs in 2021, reflecting a 23% increase compared to the previous decade. Drug addiction, characterized as a chronic relapsing mental disorder, often involves the brain's reward system, including the mesolimbic and mesocortical pathways, which mediate the release of dopamine and other neurotransmitters.

Behavioral and physiological parameters in zebrafish larvae exposed to AMP, both with and without Zr-MOF treatment, were meticulously analyzed. AMP exposure induced notable behavioral hyperactivity and developmental disruptions, which were significantly alleviated upon co-treatment with Zr-MOFs. NU-1000 demonstrated the highest adsorption efficiency, achieving up to 90% AMP removal under simulated physiological conditions. The study further confirmed the low toxicity of Zr-MOFs themselves, as evidenced by unchanged survival rates, normal hatching, and unaffected locomotor activity in larvae treated with MOFs alone.

These findings demonstrate the suitability of zebrafish as a sensitive, high-throughput model for assessing both the efficacy and safety of Zr-MOFs, supporting their application as effective tools for AMP detoxification in acute overdose scenarios. These findings open pathways for integrating MOFs into modern toxicology and environmental health research.

Founding: National Science Centre, Poland, "MOF-antidote: Novel detoxification materials based on metal-organic frameworks for drugs of abuse removal: synthesis, chemical characterization, toxicity, and efficacy in in vivo and in vitro studies" UMO-2021/43/B/NZ7/00827.

ZEBRAFISH AS A MODEL FOR EVALUATING ZIRCONIUM-BASED METAL-ORGANIC FRAMEWORKS IN DETOXIFICATION OF MDMA OVERDOSES

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The rising incidence of acute drug overdoses highlights the urgent need for innovative detoxification strategies for emergency applications. This study explores the application of porous zirconium-based metal-organic frameworks (Zr-MOFs) as a novel approach to mitigate the toxic effects of 3,4-methylenedioxymethamphetamine (MDMA), one of widely abused psychoactive substances. According to the World Health Organization (WHO), over 296 million individuals aged 15−64 globally abused drugs in 2021, reflecting a 23% increase compared to the previous decade. Drug addiction, characterized as a chronic relapsing mental disorder, often involves the brain's reward system, including the mesolimbic and mesocortical pathways, which mediate the release of dopamine and other neurotransmitters.Zebrafish (*Danio rerio*) were employed as an in vivo model to study the acute toxicity and therapeutic effects of Zr-MOFs. Zebrafish embryos and larvae, due to their high permeability and well-established homology to mammalian systems, were exposed to MDMA at varying concentrations. The Fish Embryo Toxicity (FET) test assessed survival, hatching rates, developmental abnormalities, and cardiovascular responses, while locomotor activity assays quantified behavioral changes.

Our results revealed that MDMA increased heart rate at concentrations ≥50 μM and stimulated locomotor activity at 25 μM and 50 μM. Co-incubation with Zr-MOFs, including UiO-67, NU-1000, and MOF-808, significantly mitigated MDMA-induced effects, with UiO-67 notably reducing MDMA-elevated heart rate and swim distance. Similar trends on locomotor activity were observed with NU-1000 and MOF-808, demonstrating their therapeutic potential. These findings suggest that Zr-MOFs may effectively counteract the acute toxic effects of AMP and MDMA by modulating cardiovascular and behavioral outcomes. This research underscores the potential of Zr-MOFs as emergency detoxification agents for amphetamine-class substances, providing a foundation for future studies involving mammalian models and clinical applications.

Founding: National Science Centre,Poland, "MOF-antidote: Noveldetoxification materials based on metal−organic frameworksfor drugs of abuse removal: synthesis, chemical characterizaon,toxicity, and efficacy in in vivo and in vitro studiem" UMO-2021/43/B/NZ7/00827.

THE ROLE OF PROLIDASE IN CHEMOTHERAPY RESISTANCE OF MCF7 BREAST CANCER CELLS

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Tumor resistance to chemotherapy is a significant reason for treatment failure. One of the molecular factors contributing to this resistance is the intracellular level of prolidase (PEPD), a multifunctional enzyme able to bind and inactivate p53, thus preventing chemotherapy-induced apoptosis. However, oxidative stress can cause dissociation of the PEPD-p53 complex. The present study aimed to investigate the effect of oxidative stress on p53-dependent apoptosis in breast cancer cells with different levels of PEPD expression. The study used MCF7 breast cancer cells (wild type), MCF7 cells with prolidase overexpression ($MCF7^{PL}$) and zebrafish model. Apoptosis was induced by doxorubicin, a direct activator of p53, while oxidative stress was induced by tert-butyl hydroperoxide (t-BHP) and counteracted with ascorbic acid, an antioxidant. Apoptosis and DNA biosynthesis were measured using various methods, including fluorescence microscopy and Western blotting.

The results showed that doxorubicin induced apoptosis in MCF7 wild-type cells and zebrafish models in a dose-dependent manner, but the effect was reduced in $MCF7^{PL}$ cells. Oxidative stress induced by t-BHP significantly increased apoptosis in $MCF7^{PL}$ cells, an effect was counteracted by antioxidant vitamin C. The molecular mechanism of apoptosis induction under the conditions and cell models tested was associated with increased expression and translocation to the cell nucleus of p53 and increased expression of active forms of caspases 9 and 7. This was accompanied by decreased in DNA biosynthesis. These findings suggest that prolidase overexpression contributes to chemotherapy resistance by inhibiting p53-dependent apoptosis induced by doxorubicin. However, oxidative stress enhances apoptosis in these cells, which can be reversed by antioxidants. Therefore, a combination therapy involving a p53-activating drug and an oxidative stress inducer could be a promising strategy for treating tumors with prolidase overexpression.

EVALUATION OF THE ZEBRAFISH XENOGRAFT MODEL IN VETERINARY ONCOLOGY

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The aim of this study was to verify the feasibility of using the zebrafish (*Danio rerio*) xenograft model to assess the survivability and metastatic potential of canine mammary tumor (CMT) cells, and to develop a platform enabling high-throughput testing of anticancer drugs in veterinary oncology. The research focused on three cell lines—CMT-U27, CMT-U229, and CMT-U113—to determine their ability to form tumors and metastases in zebrafish embryos.

Cells from the CMT-U27, CMT-U229, and CMT-U113 lines were injected into zebrafish embryos at 2 days post-fertilization (2 dpf) using microinjection techniques. Subsequently, fish survival and tumor progression were monitored, including local tumor growth and potential metastasis from the primary injection site. Analyses were conducted primarily through fluorescence microscopy and quantitative methods assessing cancer cell growth dynamics and spread.

Results
1. All tested CMT lines (CMT-U27, CMT-U229, and CMT-U113) demonstrated the ability to survive in the zebrafish xenograft model, confirming their compatibility with this model organism.
2. The CMT-U27 and CMT-U229 lines showed the ability to metastasise from the primary injection site to other areas of the embryo, suggesting that their aggressive and invasive characteristics are preserved in this model.
3. Zebrafish embryo survival was generally high, indicating the utility of the zebrafish model for further optimisation of experimental procedures and studies on oncology therapies.

The findings confirm that the zebrafish xenograft model can effectively be used to study the invasiveness and metastatic properties of canine mammary tumor cell lines and can serve as a valuable platform for high-throughput testing of potential anticancer drugs. The ability of CMT-U27 and CMT-U229 to form metastases in zebrafish embryos like that observed in mammals points to a high degree of phenotypic and functional consistency. Meanwhile, the sustained viability of the CMT-U113 line opens opportunities for further research on various types of canine mammary tumors. The use of zebrafish embryos as a system for rapid evaluation of antitumor activity offers a chance to speed up screening procedures and optimize therapies in veterinary oncology. The zebrafish model allows for shorter study times and reduced costs compared to traditional models, which provides an additional advantage in implementing innovative cancer treatment strategies in animals—and potentially also in humans.

A LIGAND-RECEPTOR INTERACTOME ATLAS OF THE ZEBRAFISH

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Studies in zebrafish can unravel the functions of cellular communication and thus identify novel bench-to-bedside drugs targeting cellular communication signaling molecules. Due to the incomplete annotation of zebrafish proteome, the knowledge of zebrafish receptors, ligands, and tools to explore their interactome is limited[1]. To address this gap, we *de novo* predicted the cellular localization of zebrafish reference proteome using deep learning algorithm. We combined the predicted and existing annotations on cellular localization of zebrafish proteins and created repositories of zebrafish ligands, membrane receptome, and interactome as well as associated diseases and targeting drugs.

Unlike other tools, our interactome atlas is based on both the physical interaction data of zebrafish proteins, interologous proteins and existing human ligand-receptor pair databases. The resources are available as R and Python scripts (https://github.com/DanioTalk). Our atlas provides a novel resource for researchers interested in exploring cellular communication in zebrafish, as we demonstrate in applications studying synapse and axo-glial interactome[2]. Our methodology can be applied to build and explore the ligand-receptor atlas of other model organisms that lacks ligand-receptor interaction mapping tools.

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HOW INACTIVATION OF MITOCHONDRIAL CALCIUM UNIPORTER PROTECTS DOPAMINERGIC NEURONS

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The susceptibility of dopaminergic neurons (DNs) towards degeneration in diseases like Parkinson's remains a mystery. The condition can be familial, spontaneous, and environmentally induced with harmful herbicides such as MPTP. While therapy often relies on the supplementation of L-DOPA to replace the loss of dopamine, the long-term effects include dyskinesia and neurotoxicity. Although prior studies highlighted the importance of calcium perturbations and mitochondrial dysfunction in DNs, the therapies involving the drug Isradipine did not provide satisfactory results. Moreover, recent findings have highlighted that iron ions regulate the calcium levels in mitochondria by monitoring mitochondrial calcium uniporter (MCU) oligomerization. Interestingly, our previous data underscored the importance of mcu inhibition in the protection of dopaminergic neurons in MPTP treated and *pink1* zebrafish mutants.

Findings of other authors with *lrrk2* mutants in fibroblasts and neuronal cultures highlighted MCU inhibitions in protecting DNs. Based on these, we hypothesize ferroptosis is a key player in DN cell death by regulating MCU. With our initial findings, we found that erastin significantly impacts the DN population in wild type zebrafish leading to their reduction. We also found that MCU inhibition by Ru360 in MPTP-treated wild type zebrafish rescues DNs. While a similar rescue could not be obtained in *pink1* mutants of zebrafish lines using Ru360 we plan to test the effect of Ru360 in *lrrk2* mutant line, which we now generate. Further, we aim to characterize ferroptosis in zebrafish models of *pink1* and *lrrk2* mutants and identify MCU-sensitive genes.

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MECHANISTIC UNDERSTANDING OF TSC-ASSOCIATED NEUROPSYCHIATRIC DISORDERS IN TSC2-DEFICIENT ZEBRAFISH MODEL

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Tuberous Sclerosis Complex (TSC) is an autosomal dominant disease caused by mutations in genes encoding TSC1/TSC2 proteins. These proteins form a complex that negatively regulates mechanistic/mammalian target of rapamycin complex 1 (mTORC1), which integrates signals to control cell growth, metabolism, and development. Without proper regulation, mTORC1 becomes hyperactive, leading to a wide range of symptoms, including seizures, cortical malformations, and the neurological and behavioral symptoms collectively referred to as TANDs (TSC-Associated Neuropsychiatric Disorders) like autism spectrum disorder, intellectual disability, or anxiety. Interestingly, in TSC patients, TANDs do not always correlate with the mTORC1 expression level, suggesting that other molecular pathways interact with mTORC1 to produce these phenotypes.

Rac1 is required for commissural axon development specifically in the cortex and controls axon crossing through the anterior commissure and corpus callosum. Hyperactive Rac1 also caused excitatory-inhibitory imbalance and increased brain activity with impaired synchronization. Our previous results showed that inhibition of Rac1 rescued anxiety-related hypervelocity of $tsc2^{vu242/vu242}$ fish, but did not affect epilepsy-related decreased locomotion, suggesting that Rac1 involvement in anxiety-related behavior is separate from epilepsy. Moreover, $tsc2^{vu242/vu242}$ mutant exhibited thinner anterior commissure and presented problems with axons crossing the brain midline. Therefore, we hypothesize that the disruption of commissural axons may result in anxiety through Rac1.

We pharmacologically validated the TSC model by demonstrating the rescue effect of exhibition to NSC, a Rac1 inhibitor on selected behavioral and molecular levels. Behavioral analyses of Tsc2-deficient zebrafish in the open field indicated that movements of $tsc2^{vu242/vu242}$ fish treated with NSC were less confined to the peripheral area of the well compared with wild-type controls suggesting that Rac1 inhibition by NSC decreased anxiety-like behavior. Results from immunofluorescence analysis of the fish brains have shown that NSC decrease the fluorescence intensity of phosphorylated ribosomal protein s6 in $tsc2^{vu242/vu242}$ compared to sibling controls, suggesting that Rac1 and the mTORC1 pathways might be correlated.

Transcriptome profiling provided deeper insights into the neuropathology of TSC and the effects of NSC treatment. The gene expression profiles of untreated WT and $tsc2^{vu242/vu242}$ larvae revealed 1260 differentially expressed genes (DEGs). Gene Ontology (GO) enrichment analysis of biological processes based on these DEGs identified GO terms associated with axon development and neuron projection guidance, confirming disrupted brain connectivity in our model. Notably, NSC treatment restored the expression of nearly 1 000 of these DEGs in the control mutants ($tsc2^{vu242/vu242}$), including upstream and downstream targets of Rac1. Some of these genes encode binding proteins involved in actin cytoskeleton dynamics, specifically axon growth and dendritic spine development in the brain.

The presented results point Rac1 pathway as an important pathway influencing anxiety in TSC, expanding our understanding of the complex mechanisms that underlie TSC-associated neuropsychiatric disorders and revealing a potential therapeutic target.

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EFFECT OF MELATONIN ON THE GROWTH OF HUMAN MELANOMA XENOGRAFTS IN DANIO RERIO

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Melatonin (N-acetyl-5-methoxytryptamine) is a hormone produced by the pineal gland, known for regulating circadian rhythms. It has pleiotropic antioxidant, anti-inflammatory, immunomodulatory and oncosuppressive effects in cancer cells, including melanoma, as shown in vitro. As its suppressive role in in vivo systems is less understood, we investigated the effect of melatonin on the growth of human melanoma A375 and WM266-4 tumors injected in zebrafish (Danio rerio) embryos. The aim of the study was to verify the oncosuppressive properties of melatonin in a melanoma xenograft model.

To achieve the goal we used two approaches: (1) cells were pretreated with 1mM melatonin for 48 hours and then injected into 48-hour-old embryos for 96 hours; (2) cells were injected in 48-hour-old zebrafish embryos and then fish were treated for 96 hours with melatonin at 1 or 25 μM, as higher concentrations affect the development of Danio rerio. At the end of treatment, pictures of larvae were taken, and tumor size was measured using a fluorescent microscope and ImageJ software. Tumor growth was expressed as fold change over the size of cell mass at 3 hours post injection.

In contrast to melatonin added to the fish medium, pre-treatment with melatonin inhibits tumor growth in the zebrafish model, as observed for both A375 and WM266.4 melanoma cell lines. Our data confirms the oncosuppressive effects of melatonin on melanoma cells, although highlights that zebrafish embryos are not suitable to test systemic administration.

BALANCING GENETIC DIVERSITY, COST EFFICIENCY, AND ANIMAL WELFARE IN ZEBRAFISH FACILITY MANAGEMENT

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The Zebrafish Core Facility (ZCF) at the International Institute of Molecular and Cell Biology in Warsaw (IIMCB) is the largest zebrafish breeding facility in Poland, serving both internal and external users. The services provided by ZCF include the maintenance and breeding of wild-type, transgenic, and mutant strains; development of breeding programs; space and husbandry systems management; collection of material for DNA isolation; conducting practical training for working with animals; as well as sperm freezing (SF) and *in vitro* (IVF) procedures. Managing this diverse portfolio of services required the implementation of robust systems to streamline laboratory operations and resource management, including oversight of the animal population, equipment, and human resources. Over the years, we have established a framework that ensures efficient facility operations, balancing animal welfare with their use in research. All animal-related data are cataloged and maintained in a digitized database which tracks both current and historical data on all the maintained strains, recording newly born or introduced fish and assigning them unique cohort numbers. This system allows us to track the genealogical tree of strains, monitor generations, and manage the number of fish. Thanks to detailed record-keeping, we can maintain an appropriately low level of inbreeding in our breeding programs.

We routinely conduct health assessments of the fish, including routine examinations and emergency interventions. These are carried out externally at certified external centers and internally by a veterinary doctor permanently based at the Institute. The laboratory space is organized with a dedicated room for fry breeding (“the nursery”), a quarantine area, and husbandry rooms where breeding racks are assigned to each laboratory according to their needs. This arrangement facilitates efficient management of available breeding space, ensures visual organization, and simplifies tracking of the number and types of strains maintained by each user. To effectively monitor the number and types of lines maintained at the facility, we have implemented a fry queue system, which requires each user to inform us in advance of their intention to breed a new strain.

The implementation of SF and IVF has significantly enhanced the management of rare fish strains. These methods enable us to conserve unique genetic material and preserve genetic diversity, safeguarding against genetic drift or the loss of specific strains. Additionally, they have improved the long-term preservation of wild-type lines, mitigating the risks associated with inbreeding. Finally, SF and IVF improve cost-effectiveness and space optimization by minimizing expenses related to housing, feeding, and maintaining large zebrafish populations by making more efficient use of the facility’s limited space.

Through its comprehensive services and innovative approaches, ZCF has positioned itself as a vital resource for zebrafish-based research, ensuring continued support for diverse research needs while advancing the understanding of zebrafish as a model organism.

STUDY OF THE INVOLVEMENT OF SELECTED BIOACTIVE SUBSTANCES FROM *WITHANIA SOMNIFERA* IN THE MODULATION OF BEHAVIORAL RESPONSES AND GENE EXPRESSION FOR SPECIFIC SUBUNITS OF THE GABAA RECEPTOR IN *DANIO RERIO* LARVAE UNDER THE INFLUENCE OF ETHANOL

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Therapy of alcoholism should be based on reducing the desire to continue taking alcohol and alleviating persistent withdrawal symptoms. Registered drugs for the treatment of alcoholism have limited safety of use and many side effects, so substances of plant origin, such as Withania somnifera, may be helpful as an adjunct to therapy. With this in mind, an experiment was performed using *Danio rerio* larvae as model organisms to test the effect of selected compounds contained in *Withania somnifera* extract (vitanolid A and vitanone) on the behavioral activity of test individuals after administration of different concentrations of ethanol.

Using RT-q-PCR, the gene expression profile for selected GABA-A receptor subunits (Gabra1, Gabra2, Gabrd and Gabrg2) was assessed from ethanol and administered vitanoids. Behavioral activity was assessed by measuring two parameters (distance traveled and locomotion) during changes in lighting conditions (light-dark). It was shown that ethanol at a concentration of 2% had an excitatory effect, while there was an inhibitory effect when a concentration of 4% was administered. Prior exposure to vitanon exacerbated the anxiolytic effect of alcohol, as evidenced by an increase in distance traveled and a decrease in locomotion during the bright phase of the test.

In contrast, exposure to vitanolide A had a different effect - an increase in motility of ethanol-influenced larvae was observed under light conditions. After performing RT-q-PCR reactions for the genes mentioned above, a concentration-dependent decrease in expression levels under ethanol was observed for Gabrg1, Gabrd and Gabrg2. There was no significant effect on Gabrg2 gene expression. Administration of vitanone alone resulted in a decrease in the expression of Gabra1, Gabra2 and Gabrd genes. The decrease was also observed after exposure to this compound for Gabra1 and Gabrg2 genes for single concentrations of EtOH, so it cannot be concluded that vitanone modified the effect of ethanol. The impact of vitanolide A was observed when the expression levels for Gabra1, Gabra2 and Gabrg2 genes were measured. There was a decrease in expression for the first two genes, and an increase for the Gabrg2 gene. Prior exposure to the compound increased Gabrg2 gene expression after ethanol administration at all concentrations tested.

Based on the results, both ethanol and selected W. somnifera compounds may indirectly affect GABA-ergic transmission by modifying the expression of genes for GABA-A receptor subunits, which most likely translates into changes in its structure and function.

VIBRATIONAL SPECTROSCOPY IMAGING IN ZEBRAFISH LARVAE MODEL OF OBESITY

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Zebrafish larvae, as a model of diseases such as cardiovascular disorders, cancer, neurodegenerative diseases, etc., aid significantly in the testing of potential therapeutics. Most of the research uses imaging with fluorescence microscopy based on added labels rather than intact larvae. Vibrational spectroscopy, including Raman and Fourier Transform Infrared (FTIR) spectroscopy, is a powerful analytical technique that probes the molecular vibrations of chemical bonds within a sample. It provides a "molecular fingerprint" revealing detailed chemical composition and structure information. It is a label-free, non-destructive, high-resolution technique requiring very little sample preparation. Hyperspectral imaging with vibrational techniques like Raman or Fourier transform infrared (FT-IR) spectroscopy is well established and interest in imaging is steadily growing. This study explored the sample preparation and investigation ofobesity-induced changes revealed with hyperspectral imaging.

Five dpf wild-type zebrafish larvae were used. The obesity was induced with a daily feed of 20 mg of hard- boiled egg yolk for 5 days. After sacrificing in ice water, larvae were either fixed with paraformaldehyde (PFA), glutaraldehyde (GA) or embedded in gelatin and agarose, sliced to 10 □m and subjected to Raman (Witec alpha300R, Germany) and FTIR (Nicolet Continuum, Thermo, USA) imaging.

The combined FT-IR, Raman and mass spectrometry imaging of control and obesity groups were performed on consecutive slices of 10 dpf larvae. The study involving mass spectrometry required optimization of sample handling and selecting gelatin as an embedding compound. FTIR and Raman mapping revealed that frozen samples exhibited better-preserved tissue structure than chemical fixation methods. PFA-treated samples showed uniform amide distribution, while GA resulted in tissue disruptions and denser protein networks. Embedding provided a more informative distribution of amides, lipids, and phosphates, although sample handling was challenging due to stiffness at low temperatures. Spectral analysis and hierarchical cluster analysis demonstrated differences between fixation methods, with agarose-embedded showing the most unique features.
The processing of zebrafish larvae for vibrational spectroscopic imaging coupled with e.g. ass spectrometry is challenging due to required minimal mass spectrum background to avoid nterference in detection. Frozen samples offer better tissue preservation for vibrational spectroscopy imaging, each method has its advantages and limitations. Coupled evaluation by Raman and FT-IR imaging in the zebrafish obesity model provides information on potentially obesity-related degeneration in amides of the brain, indicating pathways for consideration of disease management.

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MM-129 AS A POTENTIAL SENOTHERAPEUTIC CANDIDATE AGAINST COLON CANCER

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Cellular senescence is a state of stable, terminal cell cycle arrest associated with various macromolecular changes and a hypersecretory phenotype. In cancer it could be a double-edged sword. While it serves as a crucial tumor-suppressive mechanism by halting the proliferation of potentially malignant cells, in certain conditions and contexts, malignant and non-malignant cells with lastingly persistent senescence can acquire pro-tumorigenic properties. The purpose of the present study was to examine the senotherapeutic properties of MM-129, a novel promising drug candidate against colon cancer.

1,2,4-triazine derivative was assessed for serotherapeutic potential through *in vitro* (DLD-1, HT-29 cells) and on zebrafish xenografts. The mechanistic studies investigated the cellular affinity of new 1,2,4-triazine derivative by measuring levels of crucial biomarkers of cellular senescence.

The results indicated that 1,2,4-triazine derivative significantly reduced cell viability of the colon cancer cell lines and tumor growth in zebrafish challenged with DLD-1 and HT-29 cells. It also significantly decreased the level of the senescence-associated β-galactosidase major senescence marker as well as the transcription factor FOXO4 (forkhead box protein O4) that plays a key role in maintaining the senescent cell viability. Moreover, MM-129 attenuated the pathological SASP (senescence-associated secretory phenotype) which can substantially support tumorigenesis.

These preclinical results suggest that MM-129 may modulate the cellular senescence and may be considered a novel therapeutic strategy against colon cancer It has the potential as a safe and well-tolerated anticancer formulation for future treatment of patients with colon cancer.

THE INVOLVEMENT OF CXCR4 AND MYD88 SIGNALING PATHWAYS IN THE REDISTRIBUTION OF NEUTROPHILIC GRANULOCYTES IN ZEBRAFISH

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In all vertebrates, infection and stress induce the mobilization of neutrophils into the blood circulation, however, the molecular mechanisms governing their redistribution remain poorly understood. CXCR4 signaling is known to regulate the retention of immature neutrophils within hematopoietic tissues and the trafficking of old granulocytes from circulation for clearance, while Myd88-dependent pathways, often activated by Toll-like receptors (TLRs), are essential for neutrophil activation and recruitment during inflammation. Both pathways are also involved in neutrophil maturation, with Myd88 influencing the activation state and CXCR4 contributing to the balance between immature and aged neutrophil populations. Here, we use zebrafish larvae as a model to study the impact of Cxcr4 signaling and Myd88-dependent pathways on the neutrophil mobilization, maturation, and recruitment to inflammatory site. Moreover, we tested if neutrophil redistribution to the site of inflammation can be modulated by the stress hormone - cortisol.

We employed two experimental approaches: (1) neutrophil-specific Cxcr4a/b double knockout line and (2) Myd88 crispants in a double transgenic zebrafish line, Tg(lysC:CFP-NTR)^{vi002}/Tg(BACmmp9:Citrine-CAAX)^{vi003}. Larvae (2 dpf) were subjected to vehicle or cortisol pretreatment (24 h), followed by tail fin amputation (6 h). Confocal imaging was used to quantify neutrophil recruitment to the damage site and their retention in caudal hematopoietic tissue (CHT).

Our results reveal that Cxcr4 knockout significantly enhances neutrophil recruitment to the fin amputation area compared to Non-Targeting Control (NTC), while this phenomenon was not observed in cortisol-treated Cxcr4 knockout larvae with amputated fin. Furthermore, in control and cortisol-treated CXCR4 knockout larvae, fin amputation reduced the number of neutrophils in CHT compared to Cxcr4 knockout larvae with intact fin. Using the Tg(lysC:CFP-NTR)^{vi002}/Tg(BACmmp9:Citrine-CAAX)^{vi003} line, we analyzed the response of immature (Lys^+MMP9^-) and mature (Lys^+MMP9^+) neutrophils to the amputation. In this experimental approach, we also used Myd88 crispants. Both in the vehicle and cortisol-treated larvae in the fin amputation area, an increased number of matured neutrophils was observed. Interestingly, in Myd88 crispants with intact fins, a higher number of matured neutrophils in the analyzed area in vehicle- and cortisol- treated larvae was observed, however such a phenomenon was not found upon fin amputation. Moreover, cortisol-treated control larvae had a significantly higher number of Lys^+MMP9^- neutrophils in the analyzed area. The number of immature and matured neutrophils did not differ in the CHT of control larvae and Myd88 crispants.

Our results suggest that Cxcr4 and Myd88 pathways play distinct roles in neutrophil redistribution during inflammatory response.

ENZYBIOTICS: A CUTTING-EDGE SOLUTION FOR TACKLING PATHOGENIC BACTERIA, COMBATING ANTIMICROBIAL RESISTANCE AND IMPROVING ANIMAL CARE

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The growing threat of antimicrobial resistance is a critical global health challenge that requires urgent and coordinated action. Therefore, the development of effective strategies to prevent, control, and treat bacterial diseases while minimizing the use of antibiotics is essential. Enzybiotics (enzyme + antibiotic), naturally occurring or engineered bacteriolytic enzymes, offer a promising alternative to antibiotics due to their specificity, efficacy and reduced likelihood of resistance development. We explore peptidoglycan hydrolases, enzymes that modify bacterial cell walls, determine their structure, specificity, and stability. By leveraging these unique properties, we aim to transform these enzymes into highly effective, precisely-targeted weapons against pathogenic bacteria, including multidrug-resistant strains. With this approach, we also can minimize collateral effects on beneficial microbiota and reduce the emergence of resistance.

In vitro testing of generated enzybiotics across multiple bacterial species demonstrated their remarkable ability to selectively and efficiently eradicate 99.99% of target pathogens, including staphylococci, streptococci, enterococci, *Listeria* spp, and *Yersinia* spp., even those resistant to antibiotics. Comprehensive toxicity evaluations of 36 enzybiotics were conducted through *in vitro* assays using mammalian cell lines (mice fibroblasts 3T3 NIH and human keratinocytes HaCAT) and *in vivo* tests in two model organisms: the great wax moth (*Galleria mellonella*) larvae and zebrafish (*Danio rerio*) embryos. Most enzymes demonstrated non-toxic and non-teratogenic properties. Field trials in a salmon challenge model and on dairy cows further validated the safety and efficacy of selected enzybiotics in relevant environment.

This non-antibiotic approach is anticipated to improve animal welfare, reduce economic losses in agriculture and limit the use of antibiotics, thereby mitigating the development of antibiotic resistance in pathogenic bacteria. Furthermore, this study highlights the utility of non-mammalian models in advancing both basic and applied research.

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EFFECT OF PROBIOTIC BACILLUS SP. ON CYPRINID MEAT

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Sustainable and adequate fish nutrition is a crucial aspect of aquaculture, directly influencing farming efficiency, fish health, and the quality of final products (Jones et al., 2020).The present study aims to focus on essential components in fish nutrition and their significance, based on current scientific research. As highlighted in studies conducted by Gatlin et al. (2007), nutrients such as proteins, fats, carbohydrates, vitamins, and minerals play a key role in the proper development and functioning of fish. The balanced provision of these nutrients is imperative for effective fish nutrition in aquaculture, and current scientific research serves as the foundation for formulating optimal diets, thereby enhancing farming efficiency and promoting fish health.

The role of components influencing fish immunity, such as probiotics, is increasingly recognized as a significant aspect of nutrition. Numerous studies have been conducted on certain strains of *Bacillus* bacteria, which exhibit probiotic properties, and their potential benefits for the health of host organisms, including fish, have been extensively discussed in scientific literature. Among the probiotic bacterial strains most frequently studied, *Bacillus subtilis* stands out. Research conducted on various animal species, including fish, suggests beneficial effects on gut health, growth performance, and immunity (Song, W., et al. 2014).It is important to note that the significance of a probiotic bacterial strain may be related to the specific animal species, farming conditions, and environment.The mechanism of action of probiotic *Bacillus* strains is complex and involves various mechanisms that affect the health and functioning of the host organism. The following mechanisms have been identified in the scientific literature as being central to the action of probiotic *Bacillus*. *Bacillus* can compete with pathogenic bacteria for resources and space, thereby limiting the growth of the latter *Bacillus* can produce antibacterial substances, such as antibiotics, that inhibit the growth of pathogens (Cutting, 2011). Probiotic *Bacillus* can activate immune system cells, leading to enhanced host defense. This includes the activation of macrophages, increased antibody production, and other immune responses (Huang, J. M., et al. 2019). *Bacillus* also exhibits the ability to produce digestive enzymes, which can support the digestion and absorption processes of nutrients in the host's gastrointestinal tract (Latorre, et al. 2018).

In the course of the present studies, the microbiome of carp (*Cyprinus carpio* L.) was isolated and multiplied, and its spore form was used to replace probiotic *Bacillus* used in feeds. Four distinct types of feeds were produced: feed devoid of probiotic addition – group 0, with the addition of commercially available *Bacillus subtilis* – group 1, feed with the addition of *Bacillus subtilis* isolated from the carp environment – group 2, and feed with the addition of
commercially available *Bacillus subtilis* but using easily digestible ingredients – group 3. Studies conducted on carp fry demonstrated the impact of probiotic *Bacillus subtilis* isolated from carp intestines on meat palatability and texture, with higher concentrations of Na and Fe observed in fish muscles, and the profile index and quality index were significantly higher in this group. These findings provide a compelling rationale for the continuation of research in this area.

FUNCTIONAL STUDIES OF HEREDITARY HUMAN MUTATIONS LEADING TO CRANIOFACIAL MALFORMATIONS

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Non-Syndromic Cleft Lip and Palate (NCL/P) has a complex genetic background and several mutations have been linked already to this defect . We have identified a novel family with autosomal dominant NCL/P, and linked it to mutations in *EHHADH* and *MASP1* genes. The genes are associated with peroxisomal β-oxidation pathway and the complement activation pathway pathways respectively. To investigate the genotypephenotype link, we chose the strategy based on zebrafish gain and loss of function studies.

The wild-type (wt) or mutant (mt) mRNA was injected into a single cell stage zebrafish embryo and the larvae were analyzed during the next 5 days of development. The morphological analysis included morphology and *whole mount in situ hybridization*. Loss-offunction experiments will use site directed mutagenesis using CRISPR/Cas9 strategy by introducing the human mutation into the zebrafish genome and carry out the phenotypic analysis as described above.

Injections of mRNAs encoding wt or mt variants of both genes resulted in a dosedependent phenotypes. Gain of function experiment using wt mRNA overexpression, resulted in defects including shortened mandible and dorsal bowing of the embryo. Importantly, mt mRNA caused additional and striking defects including cyclopic eyes due to the absence of the forebrain, cleft lips and/or palates, and severe pericardial edema. Double injections produced more severe phenotypes as compared to individual overexpression.

By reproducing the patient craniofacial phenotype in the zebrafish model, our research demonstrates that it is an effective model to explore the molecular basis of this phenotype. It suggests also that both genes are involved in evolutionarily conserved processes regulating the craniofacial development. We are finalizing CRISPR-driven genomic site-directed mutagenesis to identify genetic markers associated with these malformations, enhancing our understanding of craniofacial development. Future research will investigate the molecular signature of the phenotype, analyze fatty acid levels, and study the impact of mutations on C3 protein expression to gain insights into the underlying molecular mechanisms.

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SHORT-TERM EXPOSURE TO NANOPLASTIC AFFECTS NERVOUS SYSTEM-ASSOCIATED GENE EXPRESSION IN THE ZEBRAFISH (DANIO RERIO) BRAIN: A PILOT STUDY

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The increasing accumulation of plastic particles in the environment has become a crucial global health concern. Microscopic plastic particles, that do not biodegrade, accumulate over time in living organisms, food, and drinking water. Most studies have focused on microplastic (particle size 1–1000 μm) that progressively accumulate in critical internal organs leading to persistent inflammation, oxidative stress, fibrosis, cell damage, and organ failure. Particular attention has been given to the impact of microplastics on the nervous system, both through their deposition in other organs causing systemic inflammation and from direct deposition in brain tissue. However, the implications of nanoplastics (particle size 1–1000 nm) on nervous system function remain largely unexplored. Due to their smaller size, nanoplastics can more easily cross biological barriers, such as the intestinal epithelium and blood-brain barrier, and be taken up by cells via endocytosis. This characteristic raises potential concerns for human health. In this study, we aim to investigate the short-term effects of nanoplastic exposure on gene expression involved in the modulation of the antioxidant system (*Gr*), neural cell proliferation (*Pcna*), inflammation (*Hmgb1*), apoptosis (*Casp3*, *Casp8*), cholinergic (*AChE*) and dopaminergic (*Th1*, *Th2*) systems in the brain of *Danio rerio*.

Wild-type zebrafish (*Danio rerio*) were used at 2 days post-hatching. They were exposed to nanoplastics (particle size 30 nm) at a concentration of 100 mg/L for 72 hours. As a control, zebrafish were treated with phosphate-buffered saline (PBS) at the same volume as the nanoplastic treatment. He brains were dissected under a binocular microscope and immediately placed in QIAzol Lysis Reagent. RNA was isolated from pooled samples (5 brains per sample). cDNA was synthesized using a Reverse Transcription Kit (Promega), and gene expression was measured using SYBR Green MasterMix (Promega) and the comparative (ΔΔCt) method. Data were acquired using the ABI Prism 7900HT (Applied Biosystems) with SDS 2.4 software, and analysis was performed using DataAssist v3.01.

Short-term exposure to nanoplastic resulted in a significant upregulation of gene expression for tyrosine hydroxylase 1 (*Th1*) and 2 (*Th2*), and acetylcholinesterase (*AChE*), suggesting dysregulation of the dopaminergic and cholinergic systems, respectivelly. This dysregulation was associated with increased neural inflammation, cytotoxicity, and apoptosis, as indicated by elevated expression of *Hmgb1*, *Casp3*, and *Casp8*. Additionally, we observed increased expression of the neural cell proliferation marker (*Pcna*) and antioxidant system-associated genes (*GR*), suggesting a potential link to abnormal cell cycle regulation and carcinogenesis.

This pilot study demonstrates that short-term exposure to nanoplastics induces significant changes in gene expression associated with neural system function in zebrafish brain. These findings suggest that nanoplastics may have a detrimental impact on the nervous system and could potentially be associated with the pathogenesis of human neurological diseases.

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ZEBRAFISH AS A MODEL IN NOVEL EMBRYONAL RHBDOMYOSARCOMA THERAPIES DEVELOPMENT

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Rhabdomyosarcomas (RMSs) are a diverse group of malignant tumours originating from myogenic tissue. These tumours arise from undifferentiated primary mesenchymal cells capable of differentiating into skeletal muscle. Among RMSs, embryonal rhabdomyosarcoma (ERMS) is one of the most common types affecting children. Standard treatment for ERMS typically involves a combination of surgery, chemotherapy, and radiation therapy (RT), adjusted to the stage of the disease. Chemotherapy and RT are considered as fundamental approaches in ERMS management. However, chemotherapy is significantly challenging: it induces multidrug resistance (MDR) proteins expression and severe toxic side effects. The majority of antitumour agents kill not only cancer cells but also healthy tissues. Additionally, many cytostatic drugs are approved only for adult, limiting their use in paediatric patients. Similarly, RT rises considerable risks for infants and young children due to its high toxicity.

This project aims to evaluate the therapeutic and anticancer potential of selected natural substances in the treatment of ERMS using zebrafish xenograft models. Our research will focus on biochanin A (BioA), caffeic acid phenethyl ester (CAPE), and cucurbitacin E (CurE) as innovative, natural, and relatively unexplored agents in ERMS therapy. Vinorelbine (VIN) and daunorubicin (DAU) will serve as positive controls. The findings from this study will enhance our understanding of the mechanisms of action and cellular responses to these novel, natural chemotherapeutics. The resulting data could pave the way for less toxic, more effective anticancer strategies, particularly for paediatric ERMS patients.

Here we present the results of zebrafish larvae survivability tests where potential antiERMS agents (BioA, CAPE, CurE) were used. Obtained data are preliminary studies for further research of zebrafish ERMS xenograft model.

BENCHTOP X-BAND EPR FOR IN VIVO STUDIES OF ZEBRAFISH MELANOMA

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Melanoma, one of the most aggressive forms of skin cancer accounting for 75% of skin cancer-related deaths arises from the malignant transformation of pigment cells (melanocytes). While ultraviolet light (UVA and UVB) exposure is strongly associated with melanoma formation, the underlying biological mechanisms remain poorly understood. A key area of investigation involves the roles of different melanin types and their radicals in melanoma progression as understanding of these mechanisms is vital for advancing diagnostic capabilities and therapeutic strategies. This study focuses on two types of melanin which have significant role in melanoma development and progression.

Eumelanin is a heterogeneous macromolecule with antioxidant properties, free-radical scavenging activity, and UV protection capabilities. Its primary components, 5,6-dihydroxyindole (DHI) and 5,6-dihydroxyindole-2-carboxylic acid (DHICA), exist in various redox states, including hydroxyquinone, semiquinone, and indolequinone. Conversely, pheomelanin offers weak UV protection and can amplify UV-induced reactive oxygen species production. Its main structural components are 1,4-benzothiazines and 1,3-benzothiazole. Both eumelanin and pheomelanin possess paramagnetic properties due to stable semiquinone radicals in their structures. These radicals can be studied using electron paramagnetic resonance (EPR) spectroscopy, which has provided significant insights into melanin biochemistry and melanoma progression. EPR signals differ between eumelanin and pheomelanin and correlate with their antioxidant properties, radical stability, and UV absorption. For instance, eumelanin's antioxidant activity increases with higher DHICA content, while pheomelanin induces reactive oxygen species via its benzothiazine components. The ratio of eumelanin to pheomelanin radicals is a critical parameter in melanoma research. Additionally, EPR signal intensity has been shown to correlate with tumor growth stages, and L-band EPR (1–1.4 GHz) has proven effective in in vivo melanoma studies on mouse and rat models, with potential application in humans.

Our pilot study on wild-type, albino, wild-type with chemically blocked melanin production, and wild-type with melanoma xenograft zebrafish embryos demonstrated that EPR is suitable to detect various levels of melanin and distinguish eumelanin from pheumelanin radicals . These findings underscore the potential of EPR of zebrafish embryos as a valuable tool for preclinical melanoma research and the development of novel therapeutic approaches.

The “A new capillary and a method for determining the melanin radical for use in preclinical studies of potential drugs for melanoma in the Danio rerio embryo model” FENG.02.07-IP.05-0059/23 project is carried out within the FENG POC program of the program of the Foundation for PolishScience co-financed by the European Union under the European Funds for Smart Economy2021-2027 (FENG).

ZEBRAFISH AS A REAL-TIME MODEL TO INVESTIGATE PHAGOCYTE ACTIVITY AND INFLAMMATION IN RESPONSE TO AMYLOID-B1-42

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Alzheimer's disease (AD) is a chronic neurodegenerative disease and one of the leading causes of dementia worldwide. Although AD is associated with genetic, environmental, and inflammatory factors, its exact mechanism still remains unclear. The disease is characterized by hallmark features, such as the accumulation of extracellular amyloid-beta (Aβ) plaques and the formation of intracellular tau protein tangles. At the cellular level, AD leads to synaptic loss, neuronal degeneration, microglial activation, neuroinflammation, and impairment in learning and memory.

In this study, we employed a zebrafish larvae hindbrain ventricle injection model to investigate the effects of the most toxic form of Aβ - oligomeric Aβ1-42, on the response of microglia/macrophages and neutrophils, as well as on neuroinflammation. We used a transgenic zebrafish line with fluorescently labeled microglia/macrophages and neutrophils, along with reporter lines expressing fluorescently labeled pro-inflammatory cytokines (Il-1β and Tnfα). Our results demonstrate a significant increase in the number of microglia/macrophages in the brain following the administration of Aβ1-42, with a slight increase in neutrophil numbers. Additionally, we observed that microglia/macrophages interacted with and rapidly phagocytosed Aβ1-42, indicating their protective response against Aβ. Furthermore, we detected Aβ1-42-induced upregulation of the expression of pro-inflammatory cytokines.

We believe that zebrafish larvae are a reliable model for studying neurodegenerative diseases. This model is particularly valuable for investigating the early cellular and molecular mechanisms involved in AD and could aid in the discovery of new therapeutic targets.

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CLOCK GENE *CRY1* KNOCK-OUT AFFECTS THE SURVIVAL AND IMMUNE RESPONSE AGAINST VIRAL INFECTION IN ZEBRAFISH LARVAE MODEL

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Circadian clock is a crucial mechanism for survival and adaptation to the environment. Present in virtually all organisms, it regulates many physiological processes and make them follow a rhythm. Rhythmicity of those processes is generated by the transcription-translational negative feedback loop of the clock genes: *per*, *cry*, *bmal* and *clock* that regulate their own expression and the expression of the clock-controlled genes.

It is known in mammals, that the immune system and circadian clock interact in order to maintain a homeostasis and activation of the immune system can affect the circadian clock mechanism, but also, the interruption of the clock can affect the immune response.

In this study, we aimed to study the effects of clock gene knock-out on the immune response upon Tilapia Lake Virus (TiLV) infection in the zebrafish larvae.

Experiments were conducted on 2.5 dpf zebrafish larvae mutants with knock-out of either *cry1a* (*cry1a*$^{-/-}$) or *cry1b* (*cry1b*$^{-/-}$) gene and their respective wild type control (*cry1a*$^{+/+}$ and *cry1b*$^{+/+}$) that were injected with medium containing Tilapia Lake Virus (TiLV, 1 × 107 TCID50/ml) or with the control medium. We studied zebrafish survival, morphology, including monitoring of virus-induced pathological changes as well as viral load and expression of genes involved in anti-viral immune response.

We observed that the survival of TiLV-infected larvae was affected by the *cry1a* and *cry1b* gene knock-out leading to *cry1a*$^{-/-}$ and *cry1b*$^{-/-}$ fish dying earlier than the wild type controls. Moreover, the RT-qPCR study revealed differences in the expression of anti-viral response related genes between the wild type and mutant larvae. In *cry1a*$^{-/-}$ larvae, expression of genes encoding pattern recognition receptors (*rig-I* and *tlr3*) and interferon regulatory factors (*irf3* and *irf7*), was significantly lower compared to the wild type. In turn, *cry1b*$^{-/-}$ mutants showed significantly higher expression of *irf7* and *il-1β* (encoding a proinflammatory cytokine interleukin-1 beta), but lower expression of *mxa* (encoding an anti-viral protein mxa).

Our results show that the *cry1a* as well as the *cry1b* knock-out leads to the impaired survival and immune response during TiLV infection in zebrafish larvae and that zebrafish model allows to study the mechanisms of bidirectional interaction between biological clock and innate immune response.

STREPTOCOCCUS PNEUMONIAE IS TARGETED BY TWO NON-CANONICAL AUTOPHAGY PATHWAYS WITHIN ZEBRAFISH MACROPHAGES

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Streptococcus pneumoniae is an opportunistic pathogen responsible for several life-threatening diseases such as pneumonia and meningitis, causing over 1 million deaths worldwide each year. In mammals, the pulmonary defense against *S. pneumoniae* is mostly mediated by alveolar macrophages, which can effectively internalize and degrade bacteria. Recent studies have implicated canonical and non-canonical autophagy-related processes, such as xenophagy and LC3-associated phagocytosis (LAP), in bacterial clearance.

In our study, we utilize a well-established *in vivo* zebrafish larval infection model to investigate the role of autophagy in host defense against pneumococcal infection. Using a transgenic autophagy reporter CMV:GFP-Lc3 line, we tracked the autophagic response to internalized bacteria within zebrafish macrophages. The amenability to specific gene silencing of zebrafish facilitated the identification of different autophagy-related pathways possibly evoked during infection.

Our findings reveal that the autophagy marker Lc3 is rapidly and abundantly recruited to bacteria-containing vesicles within macrophages in an atg5- and atg16l1-dependent manner, indicating an active autophagic response. The genetic inhibition of *atg5*- and *atg16l1* led to impaired acidification of phagosomes containing internalized pneumococci, significantly delaying bacterial clearance. Furthermore, our data demonstrate that Lc3 recruitment is partially mediated by LAP, as knockdown of LAP-regulating genes, *cyba* and *rubcn*, reduced Lc3 association with phagosomes and diminished pneumococcal degradation, confirming the host protective function of this process.

Interestingly, we observed no involvement of xenophagy within *S. pneumoniae*-infected macrophages, suggesting the activation of an other non-canonical autophagy pathway, distinct from LAP, that targets pneumococci-containing phagosomes. In pursuit of identifying the alternative autophagic route, we found that production of pneumococcal pore-forming toxin - pneumolysin induces LAP-independent Lc3 lipidation. This Lc3 decoration of pneumolysin-positive pneumococci can be abolished by knockdown of *tecpr1a* (tectonin beta-propeller repeat-containing 1a) which has been recently identified to induce another non-canonical autophagy pathway called STIL (sphingomyelin–TECPR1-induced LC3 lipidation).

Collectively, our observations shed new light on the host immune response against *S. pneumoniae*, demonstrating that non-canonical autophagy pathways play a supportive role in bacterial degradation by macrophages in zebrafish larvae. Additionally, to our knowledge, this is the first report demonstrating TECPR1-mediated LC3 lipidation of bacteria-containing phagosomes in an in vivo system.

IMPACT OF *PORPHYROMONAS GINGIVALIS* INFECTION ON THE BLOOD-BRAIN BARRIER FUNCTIONALITY

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A growing body of evidence highlights the role of oral bacterial pathogens, including *Porphyromonas gingivalis* (Pg), in the development of neuroinflammatory and neurodegenerative diseases. The detection of *Pg* DNA and its main virulence factors, gingipains, in the brains of Alzheimer's disease (AD) patients strongly supports this hypothesis. However, the mechanism by which Pg crosses the blood-brain barrier (BBB) and disseminates into the central nervous system remains poorly understood.

In this study, we investigated the impact of Pg on BBB functionality using a zebrafish systemic infection larvae model. Additionally, we assessed the effect of gingipains on BBB permeability. To evaluate changes in BBB functionality/permeability, zebrafish larvae infected with either the wild-type *Pg* strain or its gingipain-deficient mutant (*ΔK/R-ab*) were intravenously injected with two different fluorescent tracers: fluorophore-conjugated dextran or DAPI. Furthermore, whole-mount immunohistochemical staining with anti-claudin-5 and anti-ZO-1 antibodies was conducted to assess the degradation of tight junction proteins.

We found that systemic infection with wild-type *Pg* resulted in excessive leakage of fluorescent tracers into the brain parenchyma, indicating increased permeability of brain vessels. Moreover, *Pg*-induced changes in brain vasculature, such as narrowing or blockage of the vessel lumen followed by degradation of tight junction proteins, were observed. Notably, immunohistochemistry analysis showed high degradation of claudin-5, a tight junction protein required for BBB structure and function, in response to *Pg* infection. BBB disruption occurred in a gingipain-dependent manner.

Our results provides new insights into how *Pg* contributes to BBB damage, showing the role of gingipains in increasing brain vessel permeability. These findings suggest that *Pg*-induced alterations in the brain vasculature could be important in the bacteria dissemination in the rain and in development of neurodegenerative diseases, such as AD.

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GENERATION AND ANALYSIS OF MUSCLE GLYCOGEN PHOSPHORYLASE (PYGM) DEFICIENCY ZEBRAFISH MODEL

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The muscle glycogen phosphorylase (PYGM) deficiency in humans leads to metabolic disorder, glycogen storage disease type V (McArdle disease). It may be caused by several mutations in the pygm gene leading to a lack of functional protein. The lack of this key enzyme impairs glycogenosis, the process allowing muscles to obtain the main source of energy, glucose, from glycogen. The energy flow necessary for proper muscle work is distorted. The symptoms observed in human patients include muscle damage and pain leading to the inability to undertake physical effort.

Our goal is to develop a zebrafish (Danio rerio) model of McArdle disease and study the metabolic aspects of this glycogenosis, offering insights into its pathophysiology. We have shown that the Pygm level varies at both the mRNA and protein levels in distinct stages of zebrafish development, which is correlated with glycogen level. The morpholino pygm knockdown resulted in altered, disintegrated muscle structure and accumulation of glycogen granules in the subsarcolemmal region. Furthermore, lower Pygm in larvae elevates glycogen levels and leads to morphological muscle changes mimicking the symptoms of human McArdle disease. Subsequently, we used the CRISPR-Cas9 technique to generate a stable pygm-/- zebrafish line by knocking out the pygm gene. Our preliminary results show that indeed the mutants exhibit alternations in both morphological and behavioral aspects, mimicking the symptoms of human patients. Additionally, we postulate that PYGM role should be considered from a wider perspective.

PYGM differs from other PG isoforms in expression pattern and biochemical properties. It is not only necessary for providing energy for muscle contraction but also plays an important role in tissues other than muscle, such as the brain, lymphoid tissues, and blood. PYGM is involved not only in glycogen metabolism but also in diverse physiological and pathological processes.

In conclusion, the zebrafish pygm-/- line could be a potentially useful model of human metabolic McArdle disease and an applicable tool to study PYGM functions in muscle and other tissues. Moreover, we may be able to get to know more about metabolic strategies used by cells to overcome glucose deficiencies such as the utilization of fatty acids in β-oxidation.

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OVERCOMING TAMOXIFEN RESISTANCE IN BREAST CANCER THERAPY USING HISTONE DEACETYLASE INHIBITORS IN THE ZEBRAFISH MODEL

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Breast cancer (BC) is one of the most commonly diagnosed malignancies in women worldwide, with tamoxifen (TAMOX) resistance posing a significant clinical challenge, particularly in cases of ESR1 mutations. This project utilized a zebrafish larval model to evaluate the combined therapy of TAMOX with histone deacetylase inhibitors (HDIs), assessing its effectiveness in wild-type (WT) and ESR1-mutated breast cancer cells.

An in vivo zebrafish model was employed to study tumor growth dynamics following the injection of breast cancer cells into the yolk sac. Larvae were treated with TAMOX in combination with HDIs (Vorinostat or Valproic Acid), and changes in tumor volume were analyzed using advanced imaging techniques.

The zebrafish model demonstrated high utility in assessing the effectiveness of anticancer therapies, enabling rapid and efficient testing of drug combinations. Preliminary results suggest synergistic effects of TAMOX and HDIs, leading to tumor growth inhibition in both WT and ESR1-mutated cell lines.

The zebrafish model is a versatile research tool for preclinical evaluation of novel therapeutic strategies. Its unique characteristics, such as larval transparency, the ability to rapidly assess drug responses, and low costs, make it particularly appealing in oncology. The findings of this study confirm the potential of zebrafish in investigating mechanisms of drug resistance and in developing new anticancer treatments.

ROLE OF GLIAL PITUICYTES IN NEUROHYPOPHYSEAL SYNAPTIC MORPHOGENESIS

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Neurohypophysis (posterior pituitary) is a major neuroendocrine interface in the brain through which water homeostasis is maintained. Neurohypophysis majorly consists of glial pituicytes, neuropeptides oxytocin- and vasopressin-loaded loaded synapses and permeable capillaries. We recently identified that pituicyte-derived secreted factor can regulate neurohypophyseal neurovascular morphogenesis.

However, the role of other secreted factors expressed in neurohypophysis in neurovascular morphogenesis is unknown. Towards this goal, we have been employing pharmacological and genetic perturbations to explore the roles of candidate molecules that could regulate neurohypophyseal synapse morphogenesis. Our studies of the glial pituicytes are expected to reveal novel players in the development of a key neuroendocrine interface conserved in vertebrates.

THE ROLE OF GLIAL PITUICYTES IN ADENOHYPOPHYSIS DEVELOPMENT

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The neurohypophysis, or posterior pituitary, is a vital neuroendocrine interface regulating water balance and reproduction. Neurohypophysis is closely associated with the adenohypophysis, or anterior pituitary, that contain various neurosecretory cells with diverse functions ranging from growth, metabolism and stress regulation. Even though the role of neurohypophyseal glial pituicytes in adenohypophysis function has been addressed in the past, whether glial pituicytes can also regulate adenohypophysis development is unclear.

We hypothesize that inter-cellular signaling between pituicytes and adenohypophyseal cells can regulate adenohypophyseal cell development and our preliminary data in larval zebrafish supports this hypothesis. Studying the role of glial pituicytes in zebrafish larvae may potentially reveal conserved mechanisms and therapeutic targets for disorders associated with pituitary developmental defects.

INNOVATIVE APPROACH TO PTZ-INDUCED SEIZURE ANALYSIS IN ZEBRAFISH

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Zebrafish are increasingly being used as a model for epilepsy research due to their rapid development and genetic similarity to humans. The pentylenetetrazol (PTZ)-induced seizure model in zebrafish is a well-established platform for the evaluation of anticonvulsant drugs. However, traditional behavioural analysis methods, particularly those based on movement, often lack sensitivity and fail to fully capture the complexity of seizure-like behaviours. In this study, we present a novel algorithm designed to improve the detection and analysis of epileptic-like activity in zebrafish, providing more accurate insights into drug effects.

This research evaluates the effects of several anticonvulsant drugs, including diazepam, stiripentol, sodium valproate, gabapentin, carbamazepine, retigabine and fenfluramine, using the PTZ-induced seizure model. Our algorithm incorporates a new behavioural parameter that outperforms the conventional 'distance moved' unit. This improved parameter allows for more accurate identification of seizure-like behaviour and provides finer resolution of drug effects.
In addition to enhanced sensitivity, the new algorithm enables the visualization of individual fish activity, offering detailed insights into how each drug modulates seizure dynamics. Our results indicate that relying solely on 'distance moved' is insufficient to fully comprehend the spectrum of drug effects. However, when combined with our new parameter, it provides a more comprehensive and reliable interpretation of drug action. This is particularly useful for distinguishing between anticonvulsant and sedative effects, which are often confounded in traditional movement-based analyses. The pharmacological effects observed in zebrafish were consistent with results from PTZ-induced seizure models in mice and rats, reinforcing the translational value of the zebrafish model for preclinical drug testing. These results suggest that the zebrafish PTZ model, coupled with our advanced behavioural analysis tool, provides a highly sensitive and reliable system for screening anticonvulsant drugs, with implications for drug discovery in epilepsy.

In conclusion, the combination of the PTZ-induced zebrafish model and the novel analysis algorithm provides a powerful and efficient platform for studying epilepsy and evaluating new anticonvulsant compounds. By improving behavioural sensitivity and providing more detailed insights into drug mechanisms, this approach holds great promise for accelerating the development of effective therapies for epilepsy.

PROTECTIVE EFFECT OF CANNABIDIOL ON SALINOMYCIN NEUROTOXICITY- AN IN VITRO STUDY

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Salinomycin is used in veterinary medicine for the treatment and prevention of coccidiosis in farm animals. Its pharmacological and toxicological mechanism of action involves the formation of lipid-soluble complexes with monovalent cations. The acute toxicity of the drug is strongly species-dependent, with turkeys and horses being the most sensitive species tested: LD50 0.6 mg/kg bw compared to LD50- 50 mg/kg bw and 21 mg/kg bw in rats and rabbits, respectively. The narrow safety margin of veterinary drug and the susceptibility of some species to its toxic properties can lead to poisoning of both target and non-target farm animals. Clinical cases of such severe, often fatal poisoning have been reported. Symptoms of poisoning included cardiovascular effects, striated muscle necrosis, neuropathy and gastrointestinal disturbances. Therefore, they emphasize the need for studies assessing its safety, in particular regarding human exposure through the consumption of animal products. Identifying substances with neuroprotective properties that counteract the toxic effects of a drug on nerve cells may have significant public health implications.

The aim of this study was to assess the potential protective effects of cannabidiol (CBD) against salinomycin-induced neurotoxicity. Cannabidiol is one of over 80 chemical compounds classified as cannabinoids, which are naturally occurring components of Cannabis. Notably, unlike THC, another cannabinoid in the same group, CBD lacks psychoactive effects and addictive potential, enhancing its safety as a therapeutic agent. Currently, extensive research is being conducted globally to explore the medical applications of cannabinoids. Due to its documented anticonvulsant properties, CBD's primary clinical use is in the treatment of seizure disorders. Furthermore, cannabidiol exhibits anti-inflammatory, analgesic, anxiolytic, antidepressant, antipsychotic, anticoagulant, and, importantly for the present study, neuroprotective properties.

The study was conducted on cells derived from the human neuroblastoma cell line SH-SY5Y. The cells were exposed for 72 hours to salinomycin and mixtures of salinomycin and CBD at two different concentrations. The antibiotic was applied in a concentration range of 0.38 µg/ml to 50 µg/ml. Mixtures of salinomycin and cannabidiol were used at two non-toxic concentrations: 1.56 µg/ml (S+C1) and 3.125 µg/ml (S+C2). The neurotoxicity of the antibiotic and the protective effect of cannabidiol were evaluated using the MTT, NRU, TPC, LDH, and BrdU assays. Based on the results obtained, IC50 values were calculated. The interaction between salinomycin and CBD was assessed using the combination index (CI). Oxidative stress was evaluated with the DCFH-DA assay, while cell death was analyzed through Hoechst 33342+PI staining. Statistical analysis of the data was performed using GraphPad software.

The salinomycin significantly ($p \leq 0.05$) inhibited lysosomal activity, disintegration of the cellular membrane cells and decreased total protein contentof SH-SY5Y cells from low the concentration of 0.38 µg/ml. At the concentration of 1,56 µg/ml, inhibited mitochondrial activity and synthesis DNA. Exposure of cells to the drug mixture containing CBD caused an inhibitory effect at high concentrations of both mixtures. Analysis of the IC_{50} values for salinomycin and its two mixtures with CBD (S+C1 and S+C2) on SH-SY5Y nerve cells showed that the IC_{50} values were lowest in the NRU and TPC assays compared to the MTT, LDH and BrdU assays. However, in both tests, a proportional increase in the IC_{50} values of the drug was observed as the CBD concentration in the mixture increased. In the MTT and TPC assays, a significant increase in the IC_{50} values was observed in the $S+C_2$ mixture compared to the IC_{50} of the drug alone and the $S+C_1$ mixture. The increase in the IC_{50} value for the mixtures S+C1 and S+C2 compared to the effect of the drug alone indicated an antagonistic or agonistic nature of the interaction specially in the MTT and TPC assays for SH-SY5Y cells. The effect of the veterinary drug alone induced stronger ROS production compared to its mixture with CBD. The increase in ROS production was proportional to the concentration of salinomycin. When the cells were exposed to S+C1 or S+C2, there was an approximate 10% or 20% decrease in ROS levels, respectively, compared to the lowest concentration of drug alone. A slight increase in apoptotic and necrotic cells was observed in SH-SY5Y neuronal cells after exposure to salinomycin. Mixtures of salinomycin and CBD caused a decrease in both apoptotic and necrotic cell death.

High concentrations of salinomycin present in food may lead to disorders in the human nervous system. CBD has shown neuroprotective effects on the nervous system exposed to veterinary drugs through the consumption of food products of animal origin. Further studies using the *Danio rerio* model are necessary to investigate the interaction between veterinary pharmaceuticals and cannabidiol in the context of protecting the health of both consumers and animals.

FUNCTIONAL STUDIES OF IDENTIFIED NOVEL MUTATION RESPONSIBLE FOR THE HEREDITARY FORM OF CLUBFOOT DISEASE

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We study the molecular basis of Clubfoot, a hereditary limb disorder. Recently, we identified a novel mutation associated with that condition and the objective of the study is to establish the genotype-phenotype correlation.

For the studies, we chose zebrafish model. The *wt* or *mt* mRNA were injected at a single cell stage and the embryos were analyzed during the next 5 days of development. The morphological analysis included various morphometric analyses and will be followed by molecular description of the phenotypes using *whole mount in situ hybridization*. Loss-of-function experiments will focus on the site directed mutagenesis using CRISPR/Cas9 strategy. Specifically, we will introduce the corresponding human mutation into the zebrafish genome and carry out the phenotypic analysis as described for the gain of function studies.

We have identified an autosomal dominant mutation in Clubfoot patients within one of the tropomyosin genes. The gain of function experiments indicate that the mutation has a severe effect on the zebrafish development and specifically the musculoskeletal system. The specific phenotypes include tail bent ventrally suggesting a disturbance within the myofibers. Additionally, the somites are misshapen reflecting the muscular malformation. Larvae were also shorter along the anterior-posterior axis, with empty swim bladders, malformed mandible and a small pericardial edema, as compared to *GFP* injected zebrafish.

Our data indicate that overexpressing wild-type (wt) *tpm2* in zebrafish results in phenotypes such as pericardial edema, shortened overall body morphology, and mild mandible protrusions. These phenotypes are less severe compared to those caused by the *tpm2* mutation, which disrupts key developmental processes, including dorsal-ventral axis formation, somite integrity, skeletal structure development, and overall body morphology. These findings establish a strong genotype-phenotype correlation, underscoring the mutation's role in musculoskeletal abnormalities. Concurrently, we are conducting *in-vitro* experiments using mouse myoblasts (C2C12) to investigate how the *tpm2* mutant disrupts myotube morphogenesis.

Our next step involves employing CRISPR/Cas9-mediated gene editing to further explore the impact of the identified *tpm2* mutation (mt) in zebrafish. This approach will substantiate our current findings, providing a comprehensive understanding of the molecular mechanisms underlying clubfoot, and elucidate the role of the mutation in gene function and expression.

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ROLE OF GLIAL PITUICYTES IN REGULATING NEUROHYPOPHYSEAL VASCULAR PERMEABILITY

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The blood-brain barrier (BBB) serves as a critical safeguard for the central nervous system (CNS), but it also presents a formidable obstacle to effective therapeutic drug delivery. Interestingly, the neurohypophysis (NH), one of the circumventricular organs (CVOs), circumvents the BBB due to its specialized fenestrated vasculature, which allows selective molecular permeability. Neurohypophysis majorly consists of glial pituicytes, neuropeptides oxytocin- and vasopressin-loaded loaded synapses and permeable capillaries. Despite its unique physiological role, the molecular mechanisms that regulate vascular permeability in the NH need to be explored further.

Our research investigates the genetic and molecular pathways underlying NH vascular permeability, with the aim of identifying key regulators that could be leveraged to modulate BBB permeability. Through transcriptomic datamining, we have identified candidate genes and signaling pathways that may govern NH-specific vascular fenestrations and permeability dynamics. Towards this goal, we have been employing pharmacological and genetic perturbations to explore the roles of candidate molecules that could regulate neurohypophyseal vascular permeability. Our studies are expected to reveal novel players in the vascular development of a key neuroendocrine interface conserved in vertebrates. These findings shed light on the distinct vascular architecture of the NH and provide a foundation for developing new innovative strategies to selectively deliver therapeutics to the CNS.

ZEBRAFISH INFECTION MODEL TO STUDY SYSTEMIC EFFECT OF ORAL BIOFILM INFECTIONS

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A growing body of evidence indicate that oral bacterial infections associated with periodontitis, can contribute to the development of systemic diseases such as cardiovascular and neurodegenerative conditions. Periodontitis is caused by bacteria residing in dental plaque, a complex biofilm structure that protects the bacteria from host immune response and drug penetration. Despite advancements in this research field, there is a still lack of suitable models to decipher the complex dynamics of infection and the mechanisms of interaction between oral bacteria and the host.

In this study, we used the transparent zebrafish larval model to investigate (i) the pathogenic potential of selected oral bacteria (commensal, opportunistic, or pathogenic); (ii) kinetics of systemic infections, and (iii) the bacterial potential to induce a inflammatory response in the host. Moreover, we studied (iv) the role of phagocytes during infection utilizing transgenic lines with fluorescently labelled macrophages and neutrophils. Zebrafish larvae were infected intravenously with bacterial species representing the core oral microbiome (i.e., *Streptococcus mitis*, *Fusobacterium nucleatum*, *Porphyromonas gingivalis*, *Aggregatibacter actinomycetemcomitans*). To go beyond classical reductionist approach of single-bacteria planktonic infection, we also developed an infection model with biofilm-residing bacteria. This biofilm mimics the structural and functional complexity of natural oral biofilms, providing a more relevant conditions for studying host-pathogen interactions.

We observed a dose-dependent pathogenic potential for all studied bacteria, with *Fusobacterium nucleatum* being especially immunogenic at a low dose, and despite its very fast eradication by the host. Furthermore, infection with biofilm-residing bacteria suggests distinct infection kinetics, emphasizing the importance of mimicking the natural disease environment more closely.

Zebrafish larval infection model is a powerful tool for investigating the oral pathogens and host interactions in a context of systemic diseases, offering novel opportunities for the development of targeted therapeutic strategies.

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EVALUATION OF TYROSINE KINASE INHIBITOR-ASSOCIATED CARDIOVASCULAR TOXICITY

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Tyrosine kinase inhibitors (TKIs) of BCR-ABL, a class of chemotherapeutic agents, contributed to a major breakthrough in therapy against chronic myeloid leukaemia (CML) and other malignancies. Unfortunately, it has been shown treatment with TKIs could contribute to life-threatening adverse cardiovascular events in oncological patients, however the underlying mechanisms are largely unknown.

In this study, we employed the zebrafish model to determine the effect of TKI from every generation (imatinib, nilotinib, ponatinib and asciminib) on cardiac and vascular dysfunction, evaluated by such parameters as pericardial oedema or hyperpermeability and vasoconstriction, respectively.

Firstly, we explored the cardiotoxicity of TKIs by treating wild-type zebrafish larvae (ABTL) via immersion at 2 days post fertilization (dpf). Subsequently, the area of pericardium was measured in three timepoints (24, 48 and 72 hours post treatment). To assess vascular toxicity, the transgenic zebrafish larvae with labelled endothelium were injected with FITC-dextran at 2 dpf stage. The injected larvae were treated with TKIs for 12 or 40 hours and then imaged using a confocal microscope. To quantify the extravasation of dextran, mean fluorescence intensity was measured inside and outside dorsal aorta and finally the signal ratio (outside/inside) was calculated. Moreover, measurements of blood vessel diameters were taken. Finally, the uptake of TKIs into zebrafish larvae were measured with ultra pressure liquid chromatography coupled with tandem mass spectrometry (UPLC-MS/MS).

In order to investigate the cellular mechanisms driving cardiovascular toxicity associated with ponatinib treatment, we generated a morpholino-mediated genetic knockdown of neutrophils and macrophages. Furthermore, zebrafish thrombocytes were genetically ablated with the use of CRISPR-Cas9 method. We observed a pericardial oedema in nilotinib- and ponatinib-treated larvae after 48 or 24 hours, respectively. Among all TKIs, only ponatinib induced vascular hyperpermeability and vasoconstriction after 12 hours. Nevertheless, 40 hours long treatment with nilotinib or ponatinib resulted in similar vasculotoxicity in both groups. We also found the absorption of imatinib and asciminib by zebrafish larvae was very low, contrary to nilotinib and ponatinib.

Additionally, neither leukocyte nor thrombocyte depletion contributed to alleviation of ponatinib- related toxic effects. Overall, we believe zebrafish is a valuable model for studying TKI cardiovascular toxicity *in vivo*.

ASSESSMENT OF INDOXIMOD TOXICITY IN A ZEBRAFISH MODEL

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Colorectal cancer (CRC) is the third leading cause of cancer-related deaths worldwide, but CRC effective treatment of CRC is still lacking [1]. Recently, it was postulated that tumor cells acquire the ability to avoid immune mechanisms, which leads to their increased growth. One of these mechanisms is modifying tryptophan (TRP) metabolism via the kynurenine pathway. It has been reported that cancer cells secrete TRP catabolizing enzymes, such as indoleamine 2,3-dioxygenase (IDO1, IDO2) and tryptophan 2,3- dioxygenase (TDO2), which drive the formation of an immunosuppressive microenvironment [2]. Due to that fact, inhibitors of this pathway – e.g., indoximod become a new agent that can be used in an antitumor treatment. Indoximod acts as an IDO1/TDO2 inhibitor and restores immune defense response [3].

The study aimed to evaluate the toxicity of indoximod using the zebrafish model and cell viability assay. The FET (Fish Embryo Toxicity) was conducted with modifications [4], whereas the cytotoxicity of indoximod was estimated by MTT assay. Newly fertilized zebrafish embryos (0-2) Hpf or 72 hpf larvae were transferred to 24-well plates filled with standard medium and a series of concentrations of fingolimod (100, 300, 1000 μM). Every 24 hours, indicators of lethality, early spontaneous movement, hatching rate, additional development alteration, and embryo malformations were observed. In the case of embryos and larvae 24, 48,72, and 96 h after treatment, the survival rate, heart rate, total body length, and morphological deformities were examined. Survival rate was significantly lowered at a concentration of 1000 μM in zebrafish embryos and larvae. Additionally, at a concentration of 1000 μM, the most prominent effects on the embryo and larvae phenotypic features were observed (spinal scoliosis, pericardial and yolk sack edema, and tail curvature) (***$p<0.001$).

Exposure to indoximod did not affect the embryo hatching rate, cardiac function, and total body length. Moreover, the effect of indoximod on the viability of colon adenocarcinoma cell line DLD-1 assessed by MTT assay showed no cytotoxic potency on cells. Our study suggests that indoximod has the potential to be considered a safe and well-tolerated anticancer formulation and seems to be a promising candidate for future treatment of patients with colon cancer.

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THE EFFECT OF 25-HYDROXYCHOLESTEROL (25HC) ON VIRAL LOAD IN ZEBRAFISH LARVAE INFECTED WITH TILAPIA LAKE VIRUS (TILV), SPRING VIRAEMIA OF CARP VIRUS (SVCV) AND NERVOUS NECROSIS VIRUS (NNV)

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Cholesterol 25-hydroxylase (CH25H) is an enzyme involved in cholesterol metabolism. CH25H catalyses production of 25-hydroxycholesterol (25HC), which is a member of the family of cholesterol oxidation derivatives known as oxysterols. This compound can also be formed via non-enzymatic pathway mediated by ROS (reactive oxygen species). In mammals, 25HC has been shown to influence viral entry into cells and may play a role in the antiviral response. However, its role in the antiviral response of fish remains largely unexplored. The aim of our project is to study the role of 25HC in the antiviral mechanisms of fish. We hypothesize that 25HC can suppress viral replication during infection, thereby mitigating disease progression.

To test this, we studied the effect of 25HC stimulation of zebrafish larvae prior infection with three fish viruses: Tilapia Lake Virus (TiLV), Spring Viremia of Carp Virus (SVCV), and Nervous Necrosis Virus (NNV). Zebrafish larvae at 2 days post-fertilization (dpf) were injected with 25HC into the pericardium, followed by viral infection 2 hours later. Control fish were injected with solvent and also infected 2 hour later. Viral load was studied using RT-qPCR method in selected sampling points post infection. Viral load was significantly lower in zebrafish larvae treated with 25HC as compared to control fish. These findings suggest that 25HC treatment effectively lowers viral load in zebrafish larvae during infection with 3 different viruses.

Additionally, using CRISPR/Cas9 technology we established a novel zebrafish knockout line, *ch25hb*$^{-/-}$, characterized by a 5 base-pair deletion in the *ch25hb* gene encoding CH25H. We didn't see any differences in the development or viability of the mutant larvae compared to the wild-type larvae. Zebrafish mutant (*ch25hb*$^{-/-}$) larvae and control (*ch25hb*$^{+/+}$) larvae were infected with TiLV *via* pericardial injection at 2 dpf. The results of this experiment show that there are no differences in the viral load between control fish and *ch25hb* mutants. In summary, our research expands the knowledge on the role of 25HC during viral infection in zebrafish larvae. Stimulation of zebrafish larvae with 25HC prior infection has an effect on the course of the disease and effectively reduces the viral load of the viruses tested, but we didn’t observe any effect of the *ch25hb* gene knockout during TiLV infection.

BEHAVIORAL ANALYSIS OF ZEBRAFISH (*DANIO RERIO*) WITH GLYCOGEN PHOSPHORYLASE KNOCK-OUT

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Glycogen phosphorylase (PYGM) is a key enzyme in the first step of glycogenolysis. It is responsible for converting glycogen into glucose, the primary energy source for skeletal muscle activity. Mutations in this gene lead to McArdle's disease. Patients affected by this condition are unable to engage in excessive physical exertion due to the lack of energy.

The aim of our study is to develop a new animal model of human McArdle's disease using zebrafish (*Danio rerio*). We hypothesize that employing CRISPR-Cas9 technology will efficiently knock out the *pygm* gene in Zebrafish, resulting in a strain (*pygm-/-*) that closely mimics human McArdle disease. Zebrafish possess two genes, *pygma* and *pygmb*, which correspond to the human PYGM gene, displaying significant similarity with humans (85.0% amino acid sequence identity and 76.1% nucleotide sequence identity).

One of the key aspects to investigate during the evaluation of the new line is the assessment of the behaviour of the obtained zebrafish line. The larvae and adult pygm-/-zebrafish way of behaving reflect some symptoms of McArdle's disease observed in affected patient, such as, a reduced ability to engage in physical activity.

In summary, the goal of our project is to create a new zebrafish line with silenced expression of the muscle isoform of glycogen phosphorylase. This line will serve as an animal model of human McArdle's disease, enabling us to better understand the disease's underlying mechanisms and explore potential new therapies.

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FUNCTIONAL CHARACTERIZATION OF CHD ASSOCIATED PUTATIVE ENHANCERS IN ZEBRAFISH MODEL

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Enhancers are non-coding cis-regulatory elements in the genome that harbor transcription factor binding sites and drive gene expression in a spatiotemporal manner. Many enhancers are known to share functional homology across species despite sequence dissimilarity, being able to drive similar expression patterns in different organisms. This enables the functional characterization of human enhancers in model organisms including the zebrafish.

Through computational analysis of publicly available human genomic resources including GWAS and ClinVar databases, we identified putative enhancers associated with congenital heart diseases. The objective of this study is to perform functional characterization of selected candidate human enhancers identified from the aforementioned analysis in the zebrafish model system. To check the enhancer activity, we performed *in vivo* enhancer reporter assay utilizing the Tol2-mediated transposition system. We cloned ~500 bp genomic regions of candidate enhancers into either pGG4 or E1b transgenic constructs which carries fluorescent reporter genes under the control of minimal promoters *hsp70* and E1b, respectively. These constructs were injected into zebrafish embryo at 1-cell stage and fluorescent expression was assayed throughout development up to 3 days post fertilization (dpf). I will present the results from our ongoing analysis of these enhancers and discuss the potential insights into their mechanisms in heart development and disease.

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USING ZEBRAFISH LARVAE TO MODEL TYPE 2 DIABETES: EFFECTS OF SUBCHRONIC GLUCOSE EXPOSURE

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Acquired metabolic disorders are on the rise due to the obesity pandemic caused by a low-nutrition yet calorie- dense diet and sedentary lifestyle. According to the Polish Society of Obesity Treatment, the prevalence of obesity is reported to lead to around 200 secondary diseases, most notably type 2 diabetes. It is estimated that approximately 537 million adults have diabetes, while 90% of those numbers are type 2 diabetes cases. If left untreated, diabetes can cause cardiovascular failure, retinopathy, poor wound healing, or impaired cognitive function all leading to permanent disability. Expanding the range of diabetes therapeutics requires continuousevolution of validated animal models parallel with the latest discoveries and advancements.

Zebrafish are a reliable model for studying metabolic disorders due to their pancreas structure, glucose homeostasis, and lipid metabolism, which are similar to that of mammals. Our initial research involved establishing the toxicity profile of glucose as a hyperglycaemic agent, metformin as a first-line therapy drug for diabetes treatment, and mannitol as an osmotic control. The substances were tested by incubating 3-4 hpf zebrafish eggs in solutions of glucose, mannitol, and metformin of various concentrations for 96h. No significant toxicity was registered in the tested concentrations. The model for type 2 diabetes was established by exposing 7 dpf zebrafish larvae to 80 mM glucose, which induced hyperglycaemia without increasing larval mortality. A metformin concentration of 10 μM successfully reduced hyperglycaemia to the physiological level.

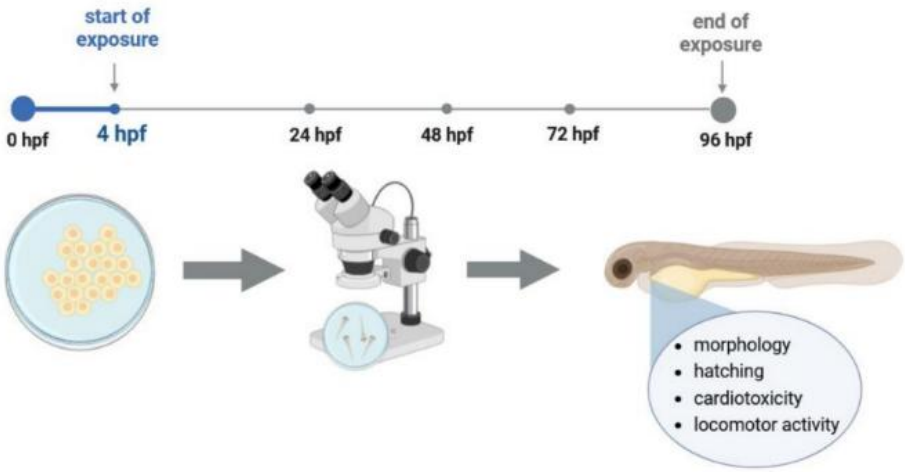

Fig. 1 The Fish Embryo Toxicity test protocol for glucose, mannitol, and metformin including the selection of 4 hpf proliferating zebrafish embryos, substance exposure, and specimen observation.

SAFETY STUDY OF A NEW COMPOUND WITH TUBULIN INHIBITORY PROPERTIES USING A FISH EMBRYO ACUTE TOXICITY (FET) TEST

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The subject of the study was a new compound with the acronym MS-PK27b which shows a potent tubulin assembly inhibition properties. Tubulin exists as a heterodimer consisting of α and β subunits which polymerize to form microtubules. Since microtubules have a number of functions in eukaryotic organisms including chromosome segregation, motility and the maintenance of cellular morphology, therefore tubulins are the proposed target for drugs against cancer, helminths and kinetoplastid parasites [1]. MS-PK27b screened using *in vitro* assays displays nanomolar cytotoxicity against multiple cancer cell lines including T-lymphoblastic leukemia CCRF-CEM cells, leukemia K562 cells and their multi-resistant counterparts (cellosaurus CEM/DNR and K562 lymphoblast cells), lung carcinoma epithelial cell line (A549) and human colon cancer cell line (HCT116 and HCT116p53-/-). Cytotoxic studies showed that MS-PK27b is not toxic to normal human fibroblast cell line (BJ) [2].

To determine acute toxicity of MS-PK27b on embryonic stages of fish we use a Fish Embryo Acute Toxicity (FET) test [3] with the zebrafish (*Danio rerio*) according to OECD guidelines for the testing of chemicals using zebrafish (ABTL-strain) larvae. For this purpose newly fertilized zebrafish eggs were exposed to MS-PK27b for a period of 96 hrs. Every 24 hrs four apical observations were recorded as indicators of lethality: coagulation of fertilized eggs, lack of somite formation, lack of detachment of the tail-bud from the yolk sac and lack of heartbeat. For testing 3,4- dichloroaniline was used as a positive control at a fixed concentration of 4 mg/L. At the end of the exposure period, acute toxicity was determined based on a positive outcome in any of the four apical observations recorded, and the LC50 values at 96 hrs for mortality was calculated.

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TOWARDS AN UNDERSTANDING OF MITOCHONDRIAL CA^{2+} TRANSPORT USING THE ZEBRAFISH

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Mitochondrial Ca^{2+} homeostasis is critical for the proper function of these organelles. Ca^{2+} in mitochondria can affect cristae shape and control oxidative phosphorylation. In addition, mitochondria serve as cellular Ca^{2+} sinks, making mitochondrial Ca^{2+} uptake important for cellular Ca^{2+} signaling in general and involved in the regulation of important processes such as gene expression and apoptosis. The mechanisms that control mitochondrial Ca^{2+} transport are not fully understood. Ca^{2+} enters the mitochondrial matrix via the mitochondrial Ca^{2+} uniporter (MCU) and is mainly extruded by the Na^{+}-Ca^{2+} exchanger (NCLX). However, Mcu deficiency in various species does not result in obvious phenotypes, suggesting the existence of additional calcium transport systems. Tmbim5 has recently emerged as a novel player in mitochondrial Ca^{2+} homeostasis, but its function in Ca^{2+} transport remains to be elucidated.

In this study, we investigated the phenotype induced by *tmbim5* knockout in zebrafish. We observed that loss of Tmbim5 results in impaired growth, muscle atrophy, and increased brain cell death. We took advantage of the translucency of zebrafish larvae to monitor changes in mitochondrial Ca^{2+} levels and mitochondrial membrane potential *in vivo*. In living larvae, Tmbim5 depletion did not affect mitochondrial Ca^{2+} levels, but reduced mitochondrial membrane potential. To investigate potential interactions with known Ca^{2+} transport systems, we generated *tmbim5/mcu* and *tmbim5/slc8b1* double knockouts. Both lines were viable without major phenotypes. The simultaneous deletion of Tmbim5 actually reduced and did not exacerbate the mortality observed in *slc8b1* knockout larvae. The NCLX inhibitor CGP-37157 lost its behavioral effects in Tmbim5-deficient but not wild-type or Slc8b1-deficient zebrafish, suggesting common, possibly antagonistic, pathways. The mild phenotypes of double knockouts indicate either strong compensatory mechanisms or that additional Ca^{2+} transport pathways maintain mitochondrial Ca^{2+} homeostasis in the absence of these proteins.

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GENOMICS DISSECTION INTO HEART DEVELOPMENT AND DISEASE

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The heart performs a vital function in circulating oxygen-rich blood and nutrients throughout the body. The core genetic program underlying heart development is largely conserved across metazoans, and aberrations to this process can result in congenital heart disease. Despite our knowledge of key factors regulating various steps of heart morphogenesis, little is known about their downstream gene regulatory networks.

Our previous transcriptomic and epigenomic analyses of the developing zebrafish heart revealed significant gene expression changes and chromatin rearrangements throughout different stages of heart morphogenesis, likely representing genetic regulatory hubs driving key events of heart development. Furthermore, the loss of function of cardiac transcription factors Gata5, Tbx5a, and Hand2 affected these regulatory networks, resulting in global changes in chromatin accessibility profiles. Among genomic regions with dynamic chromatin accessibility were highly conserved non- coding regulatory elements that represent putative enhancers implicated in heart development. To elucidate the role of these enhancers in heart development and disease, we combine both experimental and computational approaches for discovery and biological validation in the zebrafish model system. Ultimately, we aim to characterize the contribution of the dynamic transcriptional regulatory landscape to heart development and identify novel elements (both genic and non-genic) associated with congenital heart disease.

SAFETY AND EFFECTIVENESS OF QUINONE METHIDES IN ANTICANCER THERAPY

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Bladder cancer (BC) is a significant oncological challenge, and invasive treatments such as catheterization are associated with an increased risk of infections, particularly *Candida albicans*. To address this, this study focuses on developing an innovative, multifunctional therapeutic system based on quinone methides (QM) conjugated with receptor tyrosine kinase (RTK) inhibitors. QMs demonstrate promising antifungal activity, and their conjugation with RTK inhibitors, used in BC therapy, aims to simultaneously combat the tumor and prevent infections.

A panel of QM-based compounds was synthesized and their antimicrobial activity was evaluated. Arylcyanomethylenequinone oxime and its acylated derivative (acetylated arylcyanomethylenequinone oxime) exhibited the strongest activity against *C. albicans*, with minimum inhibitory concentrations (MICs) of 4 and 2 µg/mL, respectively. Importantly, these compounds did not significantly impact the cytotoxicity of erdafitinib and sunitinib in bladder cancer cell lines (T24 and UMUC3). To assess the toxicity profile, a zebrafish fish embryo toxicity (FET) assay was performed. The results indicated lower toxicity for acetylated arylcyanomethylenequinone oxime compared to its parent compound. However, only arylcyanomethylenequinone oxime demonstrated antitumorigenic activity against xenograft model in zebrafish, where the drug decreased the size of UMUC3 tumors by about 50% in comparison to vehicle control. In subsequent studies, acetylated arylcyanomethylenequinone oxime will be conjugated with erdafitinib and sunitinib, and the resulting conjugates will be evaluated in a zebrafish xenograft model for a comprehensive assessment of their therapeutic potential.

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BEET WASTE TOXICITY IN ZEBRAFISH EMBRYO MODEL

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Beetroot and sugar beet, two of the cultivated forms of *Beta vulgaris* plant, are grown globally for various purposes, mainly sugar production and food production. After the extraction of sugar from sugar beets or juice from beetroots, the residual byproducts, such as beet tops, beet pulp, and peels, are commonly regarded as agricultural waste.

The global production in 2020 was over 250 mln ton for sugar beet and over 300 mln for beetroot. That also means large amount of agricultural waste associated with the processing.

Studying the toxicity of agri-food by-products is crucial for ensuring consumer safety, environmental protection, and promoting sustainable practices in the food industry. The by-products of beets often contain bioactive compounds, such as vitamins, minerals, phenolics, carotenoids, nitrates, and betalains, that can have both beneficial and harmful effects, especially on aquatic organisms when disposed on landfield. It was shown that compounds present in apple pomace, which is also an important agrifood waste, both show some own toxicity towards model aquatic organisms and can increase the toxicity of other compounds such as pesticides.

In this work we have studied the effect of the water and ethanol-water extracts of beetroot and sugar beet pulp as well as of beetroot juice on zebrafish embryos. In the concentration of 0.1 mg/L for all the extracts obtained from the pulp no toxicity towards zebrafish embryos was observed, moreover they showed some ability to protect embryos from a model pesticide (endosulfan β) exposure when incubated in the presence of both pesticide and an beet pulp extract. However, extracts from juice showed high toxicity towards zebrafish embryos, probably due to high sugar content.

INTRANASAL INJECTION OF TILAPIA LAKE VIRUS (TILV) LEADS TO ANTIVIRAL AND INFLAMMATORY RESPONSE IN THE BRAIN OF ZEBRAFISH

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Tilapia lake virus (TiLV) is an enveloped virus with a genome of linear negative-sense single-stranded RNA. TiLV is responsible for mass mortality of farmed and wild Nile tilapia (*Oreochromis niloticus*) and has wreaked havoc on the global tilapia aquaculture.

Recently, we established zebrafish-TiLV infection model using adult fish (intraperitoneal injection) and larvae (injection into duct of Cuvier). In case of adult fish, virus replicated in multiple organs of zebrafish with the brain being the organ with the highest viral load. Moreover, both in zebrafish adult fish and larvae, strong antiviral and inflammatory response, as well as activation of microglia/macrophages was observed in the brain of TiLV-infected fish. Given the neurotropic nature of the virus, in order to further evaluate the TiLV-zebrafish interaction, we developed a new route of infection via intranasal injection.

Adult zebrafish (line AB-TL) were infected by intranasal injection of 2 µl of medium with TiLV per nasal cavity or were mock-infected using Hamilton pipettes. We demonstrated that intranasal infection of adult zebrafish resulted in TiLV replication in the brain and to the smaller extend in the other organs (spleen, kidney and liver). Infected fish displayed typical sickness behavior. Using RT-qPCR we analyzed expression of genes involved in antiviral response, inflammatory response and genes encoding markers of macrophages/microglia and astrocytes activation in the brain of TiLV-infected zebrafish. At 14th dpi, we observed an up- regulation of the expression of genes encoding pathogen recognition receptors *tlr3*, *rig1*, transcription factor *irf7*, antiviral protein mxa and pro-inflammatory cytokine *il-1β*. Moreover, at the same time point, up-regulation of macrophage/microglia markers (*csf1r*, *apoeb*, *cd68*) and astrocyte marker (*gfap*) was demonstrated in the brain of TiLV-infected zebrafish. Up-regulation of studied genes was correlated with increased viral load in the brain. This shows that the brain is a significant target for TiLV and that intranasal injection can be the route of infection to study brain- virus interactions.

PTZ webpage

www.zebrafish.org.pl

UMP webpage

https://www.ump.edu.pl/

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