

Lublin
23-25.09.2026

8th Polish Zebrafish Society Workshop



Abstract book



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Edited by Marta Migocka-Patrzałek

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Welcome from the Chair

From the Chair's chair

How is the time passing by....

We welcome you to already “**8th Polish Zebrafish Society Workshop**” organized by the Polish Zebrafish Society. We are pleased to be gathering in Lublin this year, thanks to the outstanding efforts of **the team at the Experimental Medicine Centre of the Medical University of Lublin**.

The training is intended for all researchers interested in using zebrafish (*Danio rerio*) as a model organism or extending already existing skills.

On the first day of the Workshop, **we invite everyone for open lectures (no registration required)**, regarding investigating the use of zebrafish as a model organism in a number of biomedically relevant settings (1:00 p.m. - 5:30 p.m., 23rd September 2026). Stay with us after lectures to enjoy **get together at the Poster Session!**

During the next two days (24-25th September 2026) we will offer a **practical training** to familiarize participants with zebrafish as a model organism demonstrating basic techniques, imaging, xenografts tools and behavioral studies, to mention some of them. Additionally, much sought after CRISPR-Cas9 gene editing technology will be presented.

I must say I am not only humbled by the number of applications but also excited by several new centers beginning to grow in Poland. We, the Board, feel that the **zebrafish as a model organism has gained traction in Poland** and we are very happy we can catalyze that change.

Przemko Tylżanowski

Chair of the Polish Zebrafish Society

Localisation & Venues

Open Lectures & Poster Sessions: Main Lecture Hall, Collegium Universum, Medical University of Lublin, ul. W. Chodźki 1, 20-093 Lublin, Poland.

Practical Workshop: Experimental Medicine Center (Ośrodek Medycyny Doświadczalnej - OMD), Medical University of Lublin, ul. Dr K. Jaczewskiego 8d, 20-090 Lublin, Poland.



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Organizers

Polish Zebrafish Society (Polskie Towarzystwo Zebrafish - PTZ)

PTZ Management Committee:

- Przemko Tylżanowski (Medical University of Lublin, Poland / KU Leuven, Belgium) - Chair
- Piotr Podlasz (University of Warmia and Mazury in Olsztyn, Poland)
- Marta Migocka-Patrzałek (University of Wrocław, Poland)
- Tomasz Prajsnar (Jagiellonian University, Kraków, Poland)
- Anna Sarosiak (University Center for Women's and Newborn Health, Medical University of Warsaw, Poland)

PTZ Management Committee

**Przemko
Tylżanowski**

Medical University of Lublin, Poland
University of Leuven, Belgium



**Piotr
Podlasz**

University of Warmia and
Mazury Olsztyn



**Marta
Migocka-Patrzałek**

University of Wrocław



**Tomasz
Prajsnar**

Jagiellonian University
Kraków



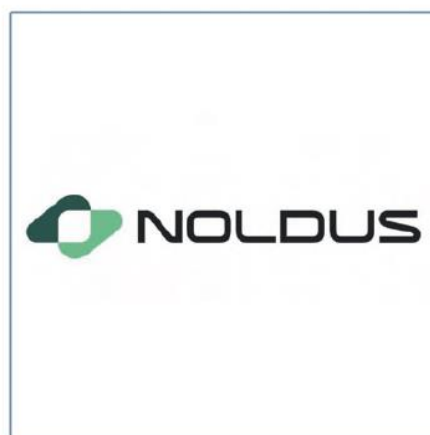
**Anna
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Sponsors & Partners

We would like to thank our generous sponsors!



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Workshop Program

Program

DAY I

23.09.26

13.00-17.30 LECTURES and POSTER SESSION - open to public

(including coffee break)

13:00 -13:10 - Welcome and Introduction - Przemko Tylżanowski - Chairman of Polish Zebrafish Society,

13:10 -13:40 - "Many Stripes of Zebrafish" - Przemko Tylżanowski -Medical University of Lublin, KU Leuven, Belgium

13:40 -14:10 - "The Zebrafish Brain Under Challenge: Neurobiology, Behavior and Toxicology" - Piotr Podlasz - University of Warmia and Mazury in Olsztyn

14:10 -14:40 - "Seeing Immunity and Host-Pathogen Interactions in Action: Insights from the Zebrafish Model"-Tomasz Prajsnar - Jagiellonian University, Krakow

14:40 -15:10 - "Decoding heart development and disease through zebrafish genomics". - Cecilia Winata -International Institute of Molecular and Cell Biology in Warsaw

~ 20 min break

15:30 -16:00 - "From Model Organism to Microscopy Innovation: Zebrafish Imaging and iCAT"" - Ondrej Svoboda - Institute of Molecular Genetics of the Czech Academy of Sciences, Prague, Czech Republic

16:00 -16:30 - "From Humans to Fish and Back: Modeling Monogenic Diseases in Zebrafish" - Marta Migocka-Patrzałek - University of Wrocław

16:30 -17:00 - "How to keep your zebrafish happy and healthy: A practical guide to good husbandry" - Magdalena Gral - Zebrafish Core Facility, International Institute of Molecular and Cell Biology in Warsaw

17:00 -17:30 - "Casting a wider net: the versatility of Zebrafish models in cancer research": Anna Boguszevska-Czubara, Medical University of Lublin

17.30 - 20.00 **Poster session/ get together**

DAY II 24.09.26

8.00-8.15 Registration and Welcome

8.15-10.15 Practicals - part I

10:15-10:30 Coffee break

10.30-12.30 Practicals -part II

12.30-14.00 Lunch & Discussion

14:00-16:00 Practicals -part III

16:00-16:15 Coffee break

16.15-18.15 Practicals -part IV

19.00- Social event ?

DAY III 25.09.26

9.00- 10.15 Practicals - part V

10.15-11.30 Practicals - part VI

11:30-11:45 Coffee break

11.45-13.00 Practicals - part VII

13:00-14.15 Practicals - part VIII

14.15-15.30 Lunch & Summary

Course Learning Outcomes

The 8th Polish Zebrafish Society Workshop is an intensive professional training course designed for biomedical scientists, PhD candidates, and researchers. The course provides specialized theoretical knowledge and hands-on laboratory experience in utilizing zebrafish as a model organism in toxicology, oncology, neurobiology, and genomic research.

The 8th Polish Zebrafish Society Workshop provides an intensive 3-day curriculum (27.5 total workload hours: 17.5 contact hours + 10 self-study hours, the estimated workload of the course is equivalent to approximately 1 ECTS credit). Upon successful completion of the course, participants demonstrate the following verified competencies:

Knowledge (Participant knows & understands):

- Biology, anatomy, husbandry standards, and developmental staging of zebrafish in research.
- Principles of toxicological testing (FET test), compound dilutions, and developmental toxicity scoring.
- Theoretical workflow and bioinformatic target selection for CRISPR/Cas9 genome editing.
- Advanced 3D fluorescence imaging techniques, light-sheet microscopy, and THUNDER computational clearing.

Skills (Participant is able to):

- Execute single-cell stage microinjections and tumor cell injections for human xenograft models.
- Perform adult organ microdissections (brain, heart, gills, gut, gonads, kidneys).
- Conduct automated larval behavioral tracking (PTZ seizure models).
- Acquire 3D microscopy datasets.

Social Competences (Participant is prepared to):

- Adhere strictly to European legal regulations and ethical standards regarding laboratory animal welfare (3Rs principles).
- Independently apply zebrafish disease models and collaborate effectively within multidisciplinary research teams.

Polish Zebrafish Society Recommendations

Strengthen Your Research Applications with Official Polish Zebrafish Society Recommendations!

When submitting grant proposals to funding agencies (e.g., NCN, NCBR, FNP, Horizon Europe) or applying for local ethics committee approvals, referencing official standards established by the Polish Zebrafish Society (PTZ) significantly strengthens the methodological rigor and ethical justification of your research.

Key Regulatory & Ethical Arguments:

- **High Genetic Orthology:** Zebrafish share ~70% of human genes and ~84% of human disease-causing genes, making them an ideal vertebrate model for human disease modeling and drug discovery.
- **3Rs Compliance (Replacement & Reduction):** Under EU Directive 2010/63/EU, non-feeding larvae up to 5 days post-fertilization (5 dpf) are legally defined as non-protected early life stages, enabling high-throughput screening prior to mammalian testing.
- **Cost-Effectiveness & Scale:** Large clutch sizes (100–300 eggs per pair) and rapid ex vivo development allow statistical power that is unfeasible in rodent models.
- **Optical Transparency:** Enables non-invasive real-time in vivo visualization of cellular processes, organogenesis, and physiological responses at single-cell resolution.

Official Publication Citation:

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Polish version available at: <https://zebrafish.org.pl/> > About > Recommendations

1. Lecture Abstracts

[L1] Decoding Heart Development and Disease Through Zebrafish Genomics — Presenter: Cecilia Winata

Cecilia Winata^{*1}, Shikha Vashist¹, Costantino Parisi¹, Aman Suryan¹

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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. While key transcription factors such as Gata5, Tbx5a, Hand2 are known to regulate cardiogenesis, their downstream gene networks and chromatin-level mechanisms remain unresolved. Using the zebrafish model organism, we integrate transcriptomics and epigenomics (ATAC-seq) to characterize the transcriptome and chromatin landscape during heart development. Our analyses revealed dynamic gene expression and chromatin rearrangements throughout different stages of heart morphogenesis, likely representing genetic regulatory hubs driving key events of heart development. Furthermore, the loss of cardiac transcription factors perturbs global chromatin accessibility, particularly at evolutionarily conserved non-coding regions, suggesting these as candidate enhancers involved in heart development. To elucidate the role of these enhancers in heart development and disease, we employ both experimental and computational approaches for discovery and functional validation in the zebrafish. Ultimately, we aim to define 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.

Key words: *heart development, genomics, single cell transcriptomics, gene regulation, zebrafish*

[L2] From Model Organism to Microscopy Innovation: Zebrafish Imaging and iCAT — Presenter: Ondrej Svoboda

Ondrej Svoboda*¹

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Zebrafish owe much of their value as a vertebrate model to optical access. Progress in the zebrafish field has therefore tracked progress in microscopy, from stereomicroscopes to confocal, spinning-disk and light-sheet systems, each trading resolution, speed and photodamage differently. One degree of freedom has remained difficult to obtain: controlled rotation of the specimen on a conventional stand. To address this, we developed iCAT, an open-source axial rotation platform. iCAT adds 360° specimen rotation, heating and camera preview to microscopes already in use. Applied to primitive erythropoiesis, it showed erythroid progenitors separating from the endothelial lineage at 50% epiboly. The iCAT's design is 3D printed and solder-free, and the hardware, firmware, and build instructions are publicly available.

Key words: *zebrafish, microscopy, imaging, iCAT*

[L3] From Humans to Fish and Back: Modelling Monogenic Diseases in Zebrafish — Presenter: Marta Migocka-Patrzałek

Marta Migocka-Patrzałek*¹

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Monogenic diseases affect millions of people worldwide, with approximately 80% having identified genetic origins. The zebrafish (*Danio rerio*) has emerged as a prominent vertebrate model organism to bridge human genetics and clinical research, supported by ~70% gene orthology and ~80% conservation of human disease-associated genes. A structured translational pipeline—from identifying pathogenic human variants and selecting zebrafish orthologs to genome engineering, phenotypic characterization, and high-throughput therapeutic screening—enables findings to be translated back to

patient.

Functional modeling utilizes genome engineering techniques ranging from transient Morpholino knockdowns to stable CRISPR-Cas9 gene editing. These tools have established zebrafish models for diverse human monogenic disorders, including Cystic Fibrosis (CFTR), Duchenne Muscular Dystrophy (DMD), Tuberous Sclerosis Complex (TSC1/TSC2), Li-Fraumeni syndrome (TP53), Noonan Syndrome (PTPN11, KRAS, RAF1), and Glycogen Storage Disease (PYGM). Over years research priorities have evolved from developmental biology in the 2000s and disease modeling in the 2010s toward precision medicine, variant validation, and drug discovery in the 2020s. While zebrafish offer key advantages in scale, cost reduction, and integrated imaging, challenges such as partial teleost genome duplication and incomplete physiological conservation mean mammalian validation is often required before clinical translation. Overall, zebrafish serve as a powerful platform for understanding disease mechanisms and accelerating drug discovery for human monogenic conditions.

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

Key words: *zebrafish (Danio rerio), monogenic diseases, CRISPR-Cas9, precision medicine, drug discovery*

2. Poster Abstracts

[P1] Liver Regeneration Following Aflatoxin B1-Induced Injury in Zebrafish Larvae — Presenter: Patrycja Nikiel

Author Names: **Patrycja Nikiel***, Dominika Czekalska

Affiliations: Medical University of Łódź, Łódź, Poland

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Introduction: Aflatoxin B1 (AFB1) is a hepatotoxic mycotoxin capable of inducing oxidative stress, hepatocyte damage and apoptosis. Zebrafish (*Danio rerio*) larvae provide a useful model for studying liver injury and regeneration due to their rapid development, optical transparency and suitability for fluorescence imaging.

Aim: The aim of this theoretical study is to assess how the severity of AFB1-induced liver injury influences the regenerative response of the zebrafish liver.

Materials and methods: Tg(fabp10a:DsRed) zebrafish larvae will be divided into a control group and three groups exposed to 0.1, 0.5 or 1.0 ppm AFB1 in feed. The proposed AFB1 concentrations should be validated in a pilot study before the experiment. Exposure will be conducted from 6 to 8 days post-fertilization (dpf), followed by a 72-hour recovery period on toxin-free feed. Liver recovery will be assessed at 0, 24, 48 and 72 hours after AFB1 withdrawal. DsRed fluorescence will be used to evaluate liver area and fluorescence intensity. Hepatocyte proliferation will be assessed by EdU incorporation, while apoptosis will be evaluated by active caspase-3 immunofluorescence. Separate larvae will be collected for EdU and caspase-3 analyses at each sampling point.

Expected results: Increasing AFB1 exposure is expected to reduce liver area and increase apoptosis. During recovery, hepatocyte proliferation and liver area are expected to increase, while higher AFB1 exposure may result in persistent apoptosis and slower recovery.

Expected conclusions: The regenerative response of the zebrafish liver is expected to depend on the severity of AFB1-induced injury, with stronger exposure associated with less effective recovery.

Key words: *Zebrafish larvae, aflatoxin B1, hepatotoxicity, liver recovery, In vivo fluorescence imaging*

[P2] Evaluating the safety profile and anti-obesity potential of innovative plant-derived food formulations in diet-induced obese zebrafish. — Presenter: Natalia Lepkowska

Natalia Lepkowska*1, Justyna Magdalena Hermanowicz1, Sandra Budziak1, Renata Markiewicz-Żukowska2, Anna Puścion-Jakubik2, Monika Grabia-Lis2, Justyna Moskwa2, Sylwia Naliwajko2, Katarzyna Socha2, Michał Tomczyk3

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Obesity is a complex chronic disease closely linked to metabolic dysregulation. Because metabolic disturbances both cause and worsen obesity, targeted dietary interventions like functional foods offer promising supportive therapy. In this study, an active complex (AS) containing quercetin, rutin, betaine, zinc, and mulberry extract was incorporated into three food matrices (F1, F2, and F3). Our objective was to evaluate the safety and metabolic effects of novel food formulations using a zebrafish model of diet-induced obesity.

Methods: A complex of active substances (AS) was incorporated into three nutritional matrices (formulation F1, F2, F3). The safety of the complex was assessed using toxicity evaluation and zebrafish microinjection. To evaluate the effects of AS in the diet-induced obesity (DIO) model, standard food was enriched with 10% of the F1, F2 or F3 formulation. After 3 weeks, body weight, glycemia and lipid profile were measured.

Results: Exposure to the AS complex (100µg/mL) showed safety profiles comparable to controls in zebrafish embryos and larvae. In DIO zebrafish, formulations F1, F2, and F3 reduced body weight, fasting glucose, total cholesterol, triglycerides, abdominal fluorescence, and adipocyte surface area in subcutaneous and visceral fat, with F1 and F2 restoring glucose levels near control values.

Conclusions: In adult DIO *Danio rerio*, formulations F1–F3 effectively counteracted diet-induced metabolic dysregulation, reducing body weight and tissue-level lipid deposition. This efficacy likely stems from the synergistic effects of key bioactives. Overall, these formulations show strong potential

as functional foods for obesity management, though clinical translation requires accounting for interspecies differences.

Key words: *zebrafish, obesity, functional food*

[P3] New reports on iron related proteins:

Molecular characterization of two ferroportin genes in common carp (*Cyprinus carpio* L.) and its expression pattern — Presenter: Teresa Kamińska-Gibas

Teresa Kamińska-Gibas* 1, Joanna Szczygiał 1, Annemiek Blasweiler 2, Łukasz Gajda 3, Ebru Yilmaz 4, Patrycja Jurecka 1, Ludmiła Kolek 1, Marek Ples 5, Ilgiz Irnazarow 1

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Iron is a crucial element for all living organisms, serving essential roles in biochemical processes. Although animals obtain iron primarily from food, they have not developed a pathway for excretion, necessitating a feedback mechanism to regulate intestinal iron absorption. The balance between iron uptake, transport, and storage is regulated by a complex network of interacting proteins. Ferroportin (fpn, also known as SLC40A1) plays a crucial role as the sole known iron efflux transporter, moving iron from within cells to the bloodstream. Detailed data on fish ferroportin is lacking, as it has only been characterized in a few species such as zebrafish, turbot, sea bass, and some others. Compared to other Cyprinidae species, common carp is an allotetraploid, which undergone a whole-genome duplication event, resulting in the retention of two copies of its genome, known as subgenome

A and subgenome B. The two subgenomes are highly similar, with an estimated nucleotide identity of 95–98%, still having some differences in gene content and expression patterns. In the present study we characterized coding sequence, gene and protein organization of the ferroportin in common carp (*Cyprinus carpio* L.). We also performed RNA-seq analysis to evaluate its constitutive transcription levels across different tissues. Our results indicate that *C. carpio* possesses two functional fpns with distinct expression patterns, highlighting the potential for functional divergence and expression differentiation among fpns in this species.

Key words: *common carp, ferroportin, iron metabolism*

**[P4] Zebrafish (*Danio rerio*) as a model for studying the toxicity of chemical warfare agents —
Presenter: Wojciech Wilczyński**

Wojciech Wilczyński*^{1,2,3}

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More than a million tons of chemical weapons were disposed of in aquatic environments during the 20th century. Due to corrosion, chemical warfare agents (CWAs) are being released from submerged munitions into surrounding waters and sediments at an increasing rate, resulting in environmental contamination. Despite this, there was a lack of research on the risk assessment and remediation of sea-dumped CWAs, particularly regarding their health effects on fish – experimental data on both acute and chronic CWA toxicity to fish were missing.

To address these gaps, standardized studies were conducted employing the zebrafish, *Danio rerio*, as a model species: (1) to assess the toxicity of CWAs to embryos during acute exposure (OECD Test No.

236), and (2) to assess the toxicity of CWAs to juveniles during chronic exposure (OECD Test No. 215). The presented research utilized multiple endpoints, including standard toxicity thresholds (NOEC, EC50, LC50), oxidative stress biomarkers, bioaccumulation, and changes in intestinal microbiota.

Several CWAs were found to exhibit far greater toxicity than predicted by modelling approaches, with even CWA degradation products inducing oxidative stress and teratogenic effects. CWAs were also found to effectively bioaccumulate in zebrafish tissues, and microbial markers suggested their biotransformation in the intestines.

These studies contribute essential data on the ecological risks of CWA release, enhancing the understanding of their long-term effects on aquatic ecosystems and informing strategies for environmental management and remediation.

The research was financed by grant no. 2020/37/N/NZ8/04099 (National Science Centre, Poland).

Key words: *Aquatic Toxicology; Chemical weapons; Zebrafish*

[P5] Opioids Beyond Pain Control: Remodeling of the Kynurenine Pathway in MCF-7 Breast Cancer Cells — Presenter: Marianna Chodowiec

Marianna Chodowiec*, Anna Tankiewicz-Kwedlo, Dariusz Pawlak, Department of Pharmacodynamics, Medical University of Białystok, Białystok, Poland

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Background: Opioids may affect cancer cells through mechanisms extending beyond analgesia, including alterations in cellular metabolism. The kynurenine pathway (KP), the major route of tryptophan metabolism, generates bioactive metabolites involved in immune regulation, redox homeostasis, and tumor biology. This study investigated whether morphine and naltrexone, including low-concentration naltrexone, alter KP metabolism in estrogen-dependent MCF-7 breast cancer cells.

Methods: MCF-7 cells were exposed for 24 h to low-concentration naltrexone (LDN), naltrexone (NTX), morphine (MOR), LDN+MOR, or NTX+MOR. Tryptophan (TRP) and its selected KP metabolites- kynurenine (KYN), kynurenic acid (KYNA), anthranilic acid (AA), and 3-hydroxyanthranilic acid (3-HAA)- were quantified by HPLC. KYN/TRP, KYNA/KYN, and AA/KYN ratios were also calculated.

Results: LDN decreased KYN concentration, whereas NTX showed an opposite pattern. MOR alone did not significantly alter KYN concentration, while LDN+MOR further reduced it. Lower concentrations of KYNA and AA were observed following LDN and LDN+MOR, whereas AA concentration was higher after MOR and NTX+MOR. 3-HAA concentration remained relatively stable, with a difference between LDN+MOR and NTX+MOR. Changes in KYN/TRP, KYNA/KYN, and AA/KYN ratios indicated treatment-dependent alterations in TRP-to-KYN metabolism and the relative distribution of KYN between downstream branches.

Conclusions: LDN and NTX produced distinct metabolic profiles and modified the effects of morphine differently. The observed shifts in KP metabolite concentrations and their ratios identify tryptophan-kynurenine metabolism as a metabolically responsive axis to opioid-related compounds in estrogen-dependent breast cancer cells.

Key words: *Breast cancer; Kynurenine pathway; Opioids; Morphine; Naltrexone;*

**[P6] Exploring the Danio rerio Model for the Study of Alcohol-Induced Neurotoxicity —
Presenter: Ewa Gibula-Tarłowska**

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Alcohol exposure during critical periods of brain development may result in persistent alterations in neuronal function and behavior. The mechanisms underlying alcohol-induced neurological dysfunction are complex and involve oxidative stress, neuroinflammation, impaired neuronal development and

alterations in neurotransmitter systems. Better understanding of these mechanisms may contribute to the identification of potential therapeutic strategies.

Our research interests focus on the neurobiological consequences of alcohol exposure, particularly neurodevelopmental abnormalities, behavioral disturbances and molecular mechanisms underlying alcohol-induced neuronal dysfunction. We are interested in extending our current research using the zebrafish (*Danio rerio*) model as a complementary experimental system for studying the effects of early-life alcohol exposure.

The planned research will investigate the effects of ethanol exposure during early zebrafish development, with particular emphasis on neurobehavioral alterations and associated molecular changes. Behavioral phenotypes, including locomotor activity and responses to environmental stimuli, will be evaluated together with selected markers of oxidative stress and neuroinflammation. The model will subsequently provide an opportunity to investigate potential pharmacological approaches aimed at attenuating alcohol-induced neuronal dysfunction.

The *Danio rerio* model offers several advantages for studying developmental neurotoxicity, including conserved neurobiological pathways, well-characterized embryonic development and the possibility of combining behavioral and molecular endpoints. Establishing an experimental zebrafish model of alcohol-induced neurotoxicity may provide a useful platform for investigating mechanisms linking early-life ethanol exposure with persistent neuronal and behavioral alterations.

Key words: *Danio rerio*; zebrafish model; neurodevelopment; ethanol; oxidative stress

[P7] EFFECT OF GALANIN ON THE EFFICIENCY OF PANCREATIC β -CELL REGENERATION IN ZEBRAFISH LARVAE MODEL — Presenter: Dominika Czortek

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Diabetes mellitus is projected to affect over 1.31 billion people by 2050. Progressive loss of pancreatic β -cell mass is a central feature of diabetes pathogenesis, yet no established therapeutic strategies exist to promote β -cell regeneration. Galanin, a neuropeptide densely innervating both β - and δ -cells of the endocrine pancreas, attenuates tissue degeneration and has been implicated in regenerative processes in zebrafish. Given the dense galaninergic innervation of somatostatin-producing δ -cells, previously shown to exhibit plasticity toward an insulin-producing phenotype following β -cell ablation, we hypothesized that galanin modulates β -cell regeneration.

β -cells were selectively ablated via the nitroreductase/metronidazole (NTR/MTZ) system, with larvae immersed in 10mM MTZ for 24 h from 2 dpf. Larvae were then allowed to regenerate for 48 h. All larvae, including controls, underwent an identical heat-shock protocol at 2, 3 and 4 dpf, inducing galanin overexpression in Tg(hsp70l:galn)a135 larvae. β -cell surface area and volume were quantified by confocal microscopy at 3 dpf (immediately post-ablation) and 5 dpf (after regeneration). Immersion in 10 mM MTZ induced effective β -cell ablation, reducing β -cell volume by 79.6%. Compared to regeneration control, larvae from the Tg(hsp70l:galn)a135 line displayed a significantly greater extent of β -cell regeneration in both volume (increasing from 29.5% to 52.8%, $p=0.0498$) and surface area (increasing from 22.8% to 65.9%, $p=0.0262$). However, galanin overexpression did not reduce the extent of ablation-induced damage ($p>0.05$). These findings represent the first report of a positive effect of galanin on pancreatic β -cell regeneration, warranting further mechanistic investigation as a potential regenerative strategy in diabetes.

Key words: *Galanin; Neuropeptide; Zebrafish; β -Cell Regeneration; Diabetes Mellitus*

**[P8] Osteosarcoma Actin Dynamics In Vitro: TPM2 Downregulation Facilitates Cofilin-Mediated Dissociation of Fascin Bundles and Its Potential Investigation in Zebrafish —
Presenter: Małgorzata Siatkowska**

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In osteosarcoma, metastatic progression is associated with actin cytoskeletal remodeling, including upregulation of fascin and downregulation of TPM2-encoded tropomyosins (Tpms) [Siatkowska et al., 2025]. Tropomyosins regulate actin filament organization, while fascin promotes bundling and cofilin-1 drives filament turnover. However, how these activities are coordinated remains unclear. Here, we investigated the interplay between Tpm, cofilin-1 and fascin in actin bundle organization. Using isothermal titration calorimetry, we identified a direct interaction between cofilin-1 and fascin. High-speed co-sedimentation assays showed that Tpm isoforms did not substantially affect cofilin binding to actin, but differed in their resistance to cofilin-induced Tpm displacement. Fascin did not enhance cofilin binding to actin but increased Tpm retention on actin filaments in an isoform-specific manner. In low-speed co-sedimentation assays, cofilin-1 reduced fascin-mediated bundling efficiency to approximately 50%. At lower fascin concentrations, Tpm isoforms further enhanced this inhibition, whereas at higher fascin concentrations bundling stabilized at approximately 40–50%. These findings support a model in which reduced Tpm2 together with increased fascin may alter the balance between actin bundling and cofilin-dependent bundle turnover. An important next step is to determine whether this phenotype translates into altered osteosarcoma cell behavior in vivo. Zebrafish larvae could provide a potential xenograft model for comparing parental and metastatic osteosarcoma cells and testing whether the Tpm2↓/fascin↑ phenotype is associated with altered migration and dissemination. Live imaging could provide an in vivo context for our in vitro findings.

Key words: *Actin cytoskeleton; Tropomyosin; Cofilin; Osteosarcoma; Zebrafish xenograft*

[P9] Evaluation of the effect of atorvastatin on the antitumor response to regorafenib in Dabio rerio larvae — Presenter: Konrad Sarnowski

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Introduction: Regorafenib (REG) is a targeted therapy used in colorectal cancer, but treatment efficacy may be limited by resistance. Atorvastatin (ATO) has demonstrated anticancer activity and may modify the response to REG.

Aim: This study aims to evaluate the impact of ATO on the anticancer response to REG.

Methods: Cytotoxicity and combination effects were assessed in Caco-2 cells. Fish Embryo Toxicity (FET) and Spontaneous Tail Coiling (STC) tests were used to select well tolerated concentrations for subsequent xenograft experiments. A xenograft model of Caco-2 cancer cells was then established in the perivital space of *Danio rerio* to evaluate the antiangiogenic effects of ATO, REG and their combinations.

Results: In vitro, both drugs reduced Caco-2 cell viability in a dose dependent manner. In vivo, initial ATO+REG concentrations caused up to 65% mortality, whereas reduced concentrations were well tolerated with minimal STC changes. In the in vitro model, a dose dependent decrease in cell viability was observed for both drugs. In the xenograft model, active tumour cell migration accompanied by directed angiogenesis was observed in the control group within the subintestinal venous plexus (SIV). REG significantly reduced vascular density and the number of vascular outgrowths, as well as reducing tumour cell survival. Combination therapy led to the inhibition of angiogenesis and deformation of the SIV's basket like structure.

Conclusion: ATO may modify the antitumour and antiangiogenic effects of REG in colorectal cancer models, supporting further investigation of this combination.

Key words: *Regorafenib, atorvastatin, xenograft, colorectal cancer, zebrafish*

[P10] The role of galectins in the pathogenesis of rheumatoid arthritis — Presenter: Paulina Plewa

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Background: Rheumatoid arthritis (RA) is a chronic autoimmune disease characterized by persistent inflammation and progressive joint damage. Galectins are glycan-binding proteins involved in the regulation of immune responses, cell migration, apoptosis, and inflammatory signaling. Their role in RA appears to be complex and context-dependent.

Methods: Synovial tissue and plasma samples were collected from patients with RA and control individuals. The expression of galectin-1 (Gal-1), galectin-3 (Gal-3), galectin-4 (Gal-4), and galectin-7 (Gal-7) was analyzed using qRT-PCR, and ELISA. Associations between galectin expression and selected inflammatory parameters were also evaluated.

Results: A total of 33 patients with RA and 34 controls were included in the analysis of synovial tissue, while plasma galectin levels were assessed in 42 patients with RA and 34 controls. Gal-1 and Gal-3 expression in synovial tissue was significantly higher in the control group compared with patients with RA. No significant differences were observed between the groups in plasma galectin levels. Significant positive correlations were identified between Gal-1 and Gal-3, Gal-4, and Gal-7 expression, suggesting coordinated regulation of galectins within the inflammatory environment. Moreover, Gal-3 expression was negatively associated with S100A8 and S100A9, indicating a potential relationship between galectin signaling and inflammatory activity.

Conclusions: The observed alterations in synovial galectin expression and their associations with inflammatory mediators support a potential role of galectins in RA pathogenesis. Further studies using experimental models, including *Danio rerio*, may help elucidate the molecular mechanisms underlying galectin-mediated regulation of inflammation and immune-cell migration in RA.

Key words: *rheumatoid arthritis, galectin, biomarker, immunomodulation*

[P11] The Effects of Co-exposure to Polyethylene Microplastics and Paracetamol on the Embryonic Development of *Danio rerio* — Presenter: Magdalena Piotrowicz

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The increasing presence of microplastics and pharmaceutical residues in aquatic environment results in organisms being exposed not only to individual contaminants but also to complex contaminant mixtures. Microplastic particles may interact with chemical compounds, through processes such as surface sorption, thereby potentially altering their bioavailability and toxicity. Therefore, a comprehensive assessment of toxicity should account for interactions within contaminants mixtures. The aim of the study was to assess the co-exposure of *Danio rerio* embryos to polyethylene (PE) microparticles and paracetamol. Embryos were exposed to PE microparticles measuring 34-50 µm, paracetamol, and their mixtures containing 5 mg/ml PE and 2 mM paracetamol. To determine whether pre-exposure contact time influenced mixed toxicity, the exposure mixtures were prepared either 1 or 24 hours before exposure. The assessed developmental endpoints included hatching success, heart rate, body length, morphological abnormalities, and the ability of larvae to maintain normal body orientation.

The study comprised two experiments. In the first experiment, hatching dynamics differed depending on the interval between mixture preparation and exposure. Morphological alterations and abnormal larval body orientation were also observed. However, most of these findings were not reproduced in the repeat experiment, in which no statistically significant between group differences in heart rate or

body length were detected. The results do not provide conclusive evidence of a synergistic interaction between polyethylene and paracetamol. Nevertheless, they suggest that the observed effects may depend on mixture preparation conditions, including the contact time between PE particles and paracetamol. Further research and detailed analyses are required to clarify this relationship.

Key words: *microplastics, paracetamol, Danio rerio, environmental contaminants*

[P12] Does a microRNA gate SSRI anxiolysis? dre-miR-135b, SERT paralogues and withanolide co-exposure in Danio rerio larvae — Presenter: Weronika Kieliszkowska

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Selective serotonin reuptake inhibitors remain first-line in anxiety disorders, yet fluoxetine (FLU) acts with delayed onset, early anxiogenesis and adverse effects; withanolides from *Withania somnifera*, acting, among others, as positive modulators of GABA-A signalling, are plausible adjuvants. We asked whether dre-miR-135b, predicted in silico to bind the slc6a4a 3'UTR, tracks FLU anxiolysis in *Danio rerio* larvae and whether withanolides shift it. *D. rerio* larvae were exposed from 3 to 5 dpf (48 h, static renewal, E3) to FLU (0.1 to 100 µg/L behaviourally; 0.1 and 1.0 µg/L molecularly), withanolide A (WTA) or withaferin A (WTF), both 100 µg/L, and their combinations. Locomotion and thigmotaxis were recorded in a 40-min light-dark test (EthoVision XT); dre-miR-135b (reference dre-miR-92b) and slc6a4a, slc6a4b transcripts (reference ones: efla, rpl13a) were quantified by RT-qPCR in pools of ten larvae using the 2^{-ΔΔCt} method. FLU reduced dark-phase hyperactivity at every concentration except

0.5 µg/L, a non-monotonic profile, and at 0.1 µg/L alone paradoxically raised thigmotaxis ($p = 0.013$); FLU 0.1 + WTA lowered dark-phase distance by 74% while leaving light-phase activity unchanged ($p = 0.24$). Only this combination elevated dre-miR-135b about tenfold (Tukey-Kramer $p < 0.001$) and lowered both SERT paralogues (slc6a4b 0.14-fold, slc6a4a 0.27-fold; $p = 0.005$ and $p = 0.028$ versus E3); the rise was lost at FLU 1.0 + WTA ($p < 0.01$) and absent with WTF; FLU alone left both transcripts unchanged. The reciprocal direction of microRNA and transporter transcripts fits withanolide-A-gated, FLU-dose-dependent repression of SERT by dre-miR-135b, and calls for functional verification.

Key words: *fluoxetine; withanolides; dre-miR-135b; slc6a4a/slc6a4b; Danio rerio larvae*

**[P13] Tpm2–Gelsolin Regulation of Actin Dynamics: Prospects for Zebrafish Studies —
Presenter: Małgorzata Siatkowska**

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Actin filaments are dynamic cytoskeletal polymers regulated by actin-binding proteins (ABPs), including gelsolin (GSN) and tropomyosin (Tpm). Tpm binds along actin filaments and modulates the access of other ABPs. GSN is a conserved, Ca²⁺-regulated protein involved in filament severing, capping and remodeling. In humans, alternative transcription produces cytoplasmic isoforms GSN-b and GSN-c and secreted plasma gelsolin (GSN-a). While GSN-a is well characterized for its role in extracellular actin clearance, the cytoplasmic isoforms remain less studied. Tpm2 isoforms are downregulated in osteosarcoma cells and may influence GSN-mediated actin remodeling.

This study characterized the Ca^{2+} dependence of actin binding and depolymerization by human GSN isoforms and examined HMM- and GSN-mediated filament severing in vitro. GSN-a showed lower Ca^{2+} sensitivity, more cooperative actin binding and the highest depolymerizing activity. At isoform-specific pCa_{50} values, GSN-b showed the highest actin affinity, whereas GSN-a showed the lowest. Cycling HMM promoted filament shortening, an effect enhanced by GSN. Low [HMM] promoted fragmentation in the presence of GSN, whereas high [HMM] had little additional effect. Tpm2 isoforms modulated GSN-dependent actin dynamics.

Zebrafish (*Danio rerio*) possess two gelsolin paralogs, *gsna* and *gsnb*, providing an opportunity to investigate their functional conservation and specialization. A larval zebrafish xenograft model will be used to assess human osteosarcoma cells with reduced or restored Tpm2.3 expression. Fluorescence imaging will measure cell survival, proliferation, invasion and dissemination. Manipulation of human GSN and zebrafish *gsna* and *gsnb* will determine whether gelsolin-dependent actin remodeling mediates the effects of Tpm2.3 and whether the paralogs differentially influence tumor-cell behavior.

Key words: *gelsolin, tropomyosin, actin filaments, osteosarcoma*

[P14] SMALL FISH, BIG INSIGHT: DECODING TOXICITY AND THERAPEUTIC DISCOVERY — Presenter: Lucja Saczuk

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Zebrafish are rapidly transforming modern medicine as a transparent, high-fecundity vertebrate model with conserved inflammatory and developmental pathways. This poster presents two perspectives for future work. The first proposal is a toxicological profiling of common supermarket additives, including Sodium Nitrate, Sodium Benzoate, Xanthan Gum, Sulphites and Lecithin. Exposing embryos to increasing concentrations is hypothesised to cause dose-dependent structural malformations. Another proposal is using zebrafish larvae for anti-inflammatory drug discovery. Current gold-standards like NSAIDs and glucocorticoids cause gastrointestinal and renal toxicity with long term use. The aim is to find novel candidates that will reduce fluorescently labelled immune cell recruitment and cytokine secretion after inflammatory stimulus, with minimal systemic toxicity.

Key words: *Toxicology, Food Additives, Anti-inflammatories,*

[P15] Could a Cyclic Peptide Be a Promising Neuroprotective Candidate for Parkinson's Disease? Evaluation in a Zebrafish Model — Presenter: Marcin Fidura

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Peptides have emerged as promising neuroprotective agents due to their ability to modulate cellular pathways involved in neuronal survival and stress responses [1]. The present study investigated the neuroprotective potential of the cyclic opioid peptide Tyr-c(D-Lys-Phe-Phe-Asp)NH₂, previously characterized by high μ -opioid receptor affinity and potent antinociceptive activity [2], in a 6-hydroxydopamine (6-OHDA)-induced in vitro model of Parkinson's disease (PD).

The peptide was synthesized using standard Fmoc solid-phase peptide synthesis and evaluated in differentiated SH-SY5Y (D-SH-SY5Y) neuroblastoma cells and human umbilical vein endothelial cells (HUVECs) [3]. Cells were treated with the peptide (0.1-10 μ M) in the presence or absence of 6-OHDA (100 μ M). Cell viability was assessed using the MTT assay, while reactive oxygen species (ROS) production, mitochondrial membrane potential ($\Delta\Psi_m$), and apoptosis were analyzed using Carboxy-H₂DCFDA, JC-10 dye, and Annexin V/propidium iodide staining, respectively.

Tyr-c(D-Lys-Phe-Phe-Asp)NH₂ exhibited no cytotoxicity in either D-SH-SY5Y or HUVEC cells, confirming a favorable safety profile. Importantly, the peptide significantly protected neuronal cells against 6-OHDA-induced toxicity, improving cell viability, reducing apoptosis, decreasing intracellular ROS levels, and preserving mitochondrial function. These findings indicate that the peptide effectively counteracts key mechanisms involved in dopaminergic neurodegeneration.

Overall, Tyr-c(D-Lys-Phe-Phe-Asp)NH₂ demonstrates promising neuroprotective activity in a cellular model of PD and represents a potential lead compound for the development of novel therapies for neurodegenerative disorders. Future studies will focus on evaluating its safety, toxicity, and neurotoxicity in zebrafish (*Danio rerio*) embryo-based assays and validating its efficacy in more advanced in vitro and in vivo models.

Key words: *Neuroprotection, Parkinson's disease, Cyclic opioid peptide, 6-OHDA, SH-SY5Y cells*

[P16] Cognitive Function in Zebrafish After Gentamicin Exposure and a Cellular Model of Duchenne Muscular Dystrophy. Two Research Experiences. — Presenter: Julia Gil

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The poster presents two research experiences bridging behavioral neurotoxicology in zebrafish and cellular modeling of Duchenne muscular dystrophy (DMD).

In the first project (bachelor's thesis), the effect of gentamicin (GM) exposure on cognitive function was tested in zebrafish larvae using the Free-movement Pattern Y-maze test recorded with the DanioVision system. Larvae were exposed to GM (125–500 µg/ml) for 1h or 24h under four lighting conditions, with scopolamine (4 µg/ml) as a positive control. Under blue light, GM significantly reduced alternation at all tested concentrations and increased repetition at 125 µg/ml, showing working memory impairment; no significant effects were seen under white light, red light, or darkness. The second, ongoing project looks at the therapeutic potential of dystrophin isoforms - Dp71, Dp140, Dp116, and microdystrophin - in a dystrophin-deficient HeLa cellular model (HeLa DMD-KO). Stable cell lines expressing individual dystrophins were made via PiggyBac system and confirmed by Western blot. At the same time, AlphaFold2 is being used to model different Dp71 splicing variants

and their ligand-binding regions. Planned next steps include functional viability assays; from a personal research interest, I am also curious whether the zebrafish model could complement this cellular approach for in vivo validation.

Key words: *neurotoxicity, behavioral tests, cognitive functions, dystrophin, gene therapy*

**[P17] PRELIMINARY SAFETY ASSESSMENT OF TWO NOVEL INDOLE-BASED 2-METHOXYPHENYLPYPERAZINE DERIVATIVES IN THE ZEBRAFISH MODEL —
Presenter: Jolanta Orzelska-Górka**

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The aim of this study was to assess the acute toxicity of two newly synthesized, structurally related indole-based 2-methoxyphenylpiperazine derivatives developed by researchers at the Masaryk University (Brno, Czech Republic). The compounds shared a structural scaffold related to β -adrenergic blocking agents, suggesting potential similarities in biological activity. In vivo toxicity was evaluated in zebrafish (*Danio rerio*) embryos using the Fish Embryo Acute Toxicity (FET) test. Lethal developmental abnormalities were monitored microscopically to determine LC₅₀ values for two compounds. Developmental abnormalities, including yolk sac and pericardial edema and head and tail

deformities, as well as larval heart rate at 96 h post-fertilization, were also assessed. Compound TP 52c showed substantially lower developmental toxicity than compound TP 52b, with an approximately 70-fold higher LC_{50} and developmental abnormalities occurring predominantly at the highest tested concentration. The LC_{50} value was 2.08 μ M (1.03 mg/mL) for TP 52b and TP 52c LC_{50} value was 142,8 μ M (80,72 mg/mL).

Overall, compound TP 52c showed a more favorable safety profile than compound TP 52b in the zebrafish model, with substantially lower developmental toxicity.

Key words: *zebrafish; developmental toxicity; LC_{50} ; phenylpiperazine derivatives; safety assessment*

[P18] From in vitro activity to in vivo validation: evaluating staphylococcal bacteriocins using *Galleria mellonella* and *Danio rerio* larvae models — Presenter: Katarzyna Alicja Dębowska

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According to WHO, antimicrobial resistance (AMR) poses a major threat to public health, and is associated with a substantial and increasing global health burden. Infections caused by multidrug-resistant (MDR) bacteria remain a major challenge as conventional antibiotic therapy may become ineffective. As a result, the direction of current research aims to find alternative treatments. Bacteriocins – ribosomally synthesized antibacterial peptides of bacterial origin – exhibit promising antibacterial properties. Our previous studies demonstrated that cell-free supernatants of bacteriocin-

producing *Staphylococcus felis* inhibit methicillin-resistant *Staphylococcus aureus* (MRSA) and *S. pseudintermedius* (MRSP) in vitro, providing a basis for further evaluation of their antibacterial potential in vivo. The next step involves *Galleria mellonella* larvae and *Danio rerio* embryos. While both in vivo models have overlaps and limitations, their comparative studies may lead to obtaining more credible results, laying the groundwork for future drug development effective in therapy against resistant bacteria. This study is supported by the project LIDER13/0100/2022 (Agreement dated 29.03.2023) titled "StaphiX – an innovative solution based on bacteriocins to control the microbiome of the skin and mucous membranes in veterinary medicine (StaphiX)," funded by the National Centre for Research and Development (NCBiR).

Key words: *antimicrobial r*

esistance, bacteriocins, veterinary microbiology, Galleria mellonella, Danio rerio

**[P19] TPM2 in muscle and craniofacial development: from zebrafish to chick embryos —
Presenter: Sylwia Drzewiecka**

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TPM2 encodes β -tropomyosin, a coiled-coil actin-binding protein that regulates muscle contraction through interactions with actin and troponin. The gene is conserved between zebrafish and humans, with a shared role in thin filament regulation and sarcomere stability. In a clubfoot-affected family, we identified a heterozygous splice-site variant causing an in-frame deletion (r.462_737del), predicted to remove 92 residues (p.Glu72_Ala166) and truncate the protein from 284 to 192 amino acids.

To test the functional consequence of this variant, we overexpressed tpm2 splice variant mRNA in zebrafish by microinjection into single-cell embryos, alongside wild-type tpm2 and GFP controls. Embryos were scored for phenotype severity at 5 days post-injection and examined by birefringence for muscle structure, Alcian Blue staining for craniofacial cartilage, and Calcein staining for bone mineralization. Splice variant mRNA microinjection led to a substantially higher frequency of morphological muscle defects than wild-type tpm2 or GFP controls, and produced significant craniofacial abnormalities, including altered Meckel-palatoquadrate angle and inter-palatoquadrate distance, connected to abnormal muscle development.

A locomotor assay is planned next, to test the effect of the TPM2 mutation on motor activity and muscle performance in zebrafish larvae. During an Erasmus+ research stay at the Sampaolesi Lab, KU Leuven, my work addressed muscle biology in a cancer cachexia model. Future work will test TPM2 misexpression in a second vertebrate model, the chick embryo, using the RCASBP retroviral system.

Key words: *TPM2, β -tropomyosin, zebrafish, congenital clubfoot, muscle development*

