The

6th Polish Zebrafish Society Workshop

Abstract Book

Kraków

14 - 16.02.2024

POLISH ZEBRAFISH SOCIETY MANAGEMENT COMMITTEE:

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http://www.zebrafish.org.pl

Abstract Book - Edited by Marta Migocka-Patrzałek

From the Chair's chair 😊

How is the time passing by….

We welcome you to already "**6th Polish Zebrafish Society Workshop**" organized by the **Polish Zebrafish Society** and held this time in Krakow, thanks to the support from Magda Chadzińska, Tomek Prajsnar and Krzysiek Rakus. Thank you so much for hosting us! Additionally, sincere thank you to the Director of the Institute of Zoology and Biomedical Research of Jagiellonian University for supporting our initiative.

The training is intended **for all researchers interested in using zebrafish** (*Danio rerio*) **as a model organism** or extending already existing skills. As a novelty, this time we will also have a satellite meeting specifically focused on the maintenance zebrafish facility and the art of zebrafish husbandry. This part is primarily dedicated to people directly taking care of the zebrafish, artemia, and rotifer cultures.

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., 14th Feb 2024- yes, the Valentine's Day). Stay with us after lectures to enjoy get together at the **Poster session**!

The lectures will be held in **Room 0.18** (ground floor) of the Institute of Zoology and Biomedical Research (Gronostajowa 9, 30-387 Krakow).

During the next two days, we will offer **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. The practicals will mainly take place in the Department of Evolutionary Immunology (third floor).

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.

Chair

Przemko Tylzanowski

LIST OF CONTENTS:

Introduction

The workshops will be held at the **Institute of Zoology and Biomedical Research of the Jagiellonian University** in Kraków. The training is intended for all scientists interested in research using **zebrafish** (Danio rerio) as a model organism.

During the training, there will be **open lectures**, which do not require registration, on research on human diseases using zebrafish as a model organism (1:00 - 5:00 p.m. on the first day of the Workshop, February 14, 2024).

Stay with us after lectures to enjoy get together at the Poster session!

The lectures will be held at the Institute of Zoology and Biomedical Research
Room 0.18 (ground floor) of the Jagiellonian University in Kraków
(ul. Gronostajowa 9, 30-387 Kraków, Poland).

Over the **next two days**, there will be **practical exercises** aimed at familiarizing participants with this model organism, its husbandry, possibilities of use in research, basic research techniques used in science, including toxicological tests, microinjections – also injections of cancer cells (zebrafish xenograft model), microscopic imaging using Light Sheet technology, behavioral analyses, use of gene editing technologies such as CRISPR-Cas9 method, genotyping and more.

The workshop will be held at the Institute of Zoology and Biomedical Research
of the Jagiellonian University in Kraków
(ul. Gronostajowa 9, 30-387 Kraków, Poland).

Satellite workshop dedicated to zebrafish facility staff (Spotkanie sekcji hodowli i dobrostanu danio pręgowanego)

SPOTKANIE SEKCJI HODOWLI I DOBROSTANU DANIO PRĘGOWANEGO
(English below)

Dzień I - 14.02.2024

Miejsce: Instytut Zoologii i Badań Biomedycznych, Sala 0.18 (parter), Uniwersytet Jagielloński, ul. Gronostajowa 9, 30-387 Kraków

Przewodniczący spotkania: **lek. wet. Piotr J. Korzeniowski**, Międzynarodowy Instytut Biologii Molekularnej i Komórkowej, Warszawa

10:00 - 10:30 - prezentacja przedstawiciela firmy diagnostycznej (Animal Lab). Podstawowe zagadnienia związane z ochroną zdrowia ryb w akwakulturze laboratoryjnej.
10:30 - 11:00 -"Podstawowe zasady i aspekty prawne hodowli danio pręgowanego w akwakulturze laboratoryjnej okiem pracownika" - **Magdalena Gral**, Zebrafish Core Facility, Międzynarodowy Instytut Biologii Molekularnej i Komórkowej, Warszawa
11:00 - 12:00 - wolna dyskusja na tematy hodowlane, w tym problemy z rozrodem i śmiertelnością narybku oraz żywienie i jakość wody jako podstawowe klucze do sukcesu w laboratoryjnej hodowli danio pręgowanego.

SATELLITE WORKSHOP DEDICATED TO ZEBRAFISH FACILITY STAFF

Day I - February 14, 2024
Place: Institute of Zoology and Biomedical Research, Room 0.18 (ground floor), Jagiellonian University, Gronostajowa 9 St., 30-387 Kraków

Meeting Chairman: **Piotr J. Korzeniowski, DVM**, International Institute of Molecular and Cell Biology, Warsaw

10:00 - 10:30 - Presentation by a representative of a diagnostic company (Animal Lab). Basic issues related to fish health protection in laboratory aquaculture.

10:30 - 11:00 - „Basic principles and legal aspects of zebrafish breeding in laboratory aquaculture from the perspective of an employee” **- Magdalena Gral**, Zebrafish Core Facility, International Institute of Molecular and Cell Biology, Warsaw

11:00 - 12:00 - free discussion on breeding topics, including problems with reproduction and mortality of fry, as well as nutrition and water quality as the basic keys to success in laboratory

Program of the 6th Polish Zebrafish Society Workshop

Plan of Lectures

DAY I (open to the public)

13.00-17.30 LECTURES

13:00 -13:10 - "Welcome and Introduction" - **Przemko Tylżanowski** - Chairmen of Polish Zebrafish Society, Medical University of Lublin, KU Leuven, Belgium
13:10 - 13:40 - "The zebrafish as a model for scientific research" - **Marta Migocka-Patrzałek** -University of Wroclaw
13:40 - 14:10- "Zebrafish xenografts in cancer research - High-content screening to identify drug combinations effective against Ewing sarcoma"- **Martin Distel**- Medical University of Vienna
14:10 - 14:40 - "Zebrafish model to study brain development and diseases" – **Justyna Zmorzyńska** - The International Institute of Molecular Mechanisms and Machines
14:40 - 15:10 - "Zebrafish as a Model Organism for Toxicity Studies: Exploiting Advantages in Biomedical Research" - **Barbara Budzyńska** - Medical University of Lublin
~ 20 min break ~
15:30 - 16:00 -"Zebrafish in the lens of behavioral research"- **Małgorzata Potoczna** – Transpharmation Poland, University of Warmia and Mazury in Olsztyn
16:00 - 16:30 - "Zebrafish larvae infection model to study host-pathogen interactions" - **Magdalena Widziołek-Pooranachandran** - Jagiellonian University
16:30 - 17:00 - "Modeling of viral infections in fish:from fundamental to applied science" - **Krzysztof Rakus** - Jagiellonian University
17:00 - 17:30 "Evolution of genome editing in zebrafish: from origins to advances and integration into translational medicine" - **Anna Sarosiak** -*Institute of Physiology and Pathology of Hearing*

17.30 - 20.00 Poster session/get-together - stay with us after lectures!

NOTE: Authors with odd numbered posters will present them between 17.30-18.30. Those with even numbered posters are asked to remain at their posters between 18.30-19.30.

Practical parts schedule

DAY II

8:00 - 8:15: Registration + welcome

Time	Group I	Group II	Group III	Group IV
8:15 - 10:15	Toxicity screening using zebrafish embryos (LiCl) - Part I *(Natalia Kasica)* + Dissection of organs from adult zebrafish, finclips and swabbing *(Joanna Dybalska, Mikołaj Mazur)*	Basics of imaging and sample preparation - BF/fluorescence *(Piotr Podlasz, Anna Sarosiak)*	Microinjections (basics) *(Przemko Tylzanowski, Tomasz Prajsnar)* + Zebrafish xenograft model *(Anna Boguszewska-Czubara)*	Behaviour *(Agnieszka Michalak, Małgorzata Potoczna)* + Visit in the zebrafish facility *(Dariusz Gajdziński)*
10:15 - 10:30	Short break	Short break	Short break	Short break
10:30 - 12:30	Behaviour *(Agnieszka Michalak, Małgorzata Potoczna)* + Visit in the zebrafish facility *(Dariusz Gajdziński)*	Toxicity screening using zebrafish embryos (LiCl) - Part I *(Natalia Kasica)* + Dissection of organs from adult zebrafish, finclips and swabbing *(Joanna Dybalska, Mikołaj Mazur)*	Basics of imaging and sample preparation - BF/fluorescence *(Piotr Podlasz, Anna Sarosiak)*	Microinjections (basics) *(Przemko Tylzanowski, Tomasz Prajsnar)* + Zebrafish xenograft model *(Anna Boguszewska-Czubara)*
12:30 - 14:00	Lunch & Discussion	Lunch & Discussion	Lunch & Discussion	Lunch & Discussion
14:00 - 16:00	Microinjections (basics) *(Przemko Tylzanowski, Tomasz Prajsnar)* + Zebrafish xenograft model *(Anna Boguszewska-Czubara)*	Behaviour *(Agnieszka Michalak, Małgorzata Potoczna)* + Visit in the zebrafish facility *(Dariusz Gajdziński)*	Toxicity screening using zebrafish embryos (LiCl) - Part I *(Natalia Kasica)* + Dissection of organs from adult zebrafish, finclips and swabbing *(Joanna Dybalska, Mikołaj Mazur)*	Basics of imaging and sample preparation - BF/fluorescence *(Piotr Podlasz, Anna Sarosiak)*
16:00 - 16:15	Short break	Short break	Short break	Short break

16:15 - 18:15	Basics of imaging and sample preparation - BF/fluorescence *(Piotr Podlasz, Anna Sarosiak)*	Microinjections (basics) *(Przemko Tylzanowski, Tomasz Prajsnar)* + Zebrafish xenograft model *(Anna Boguszewska-Czubara)*	Behaviour *(Agnieszka Michalak, Małgorzata Potoczna)* + Visit in the zebrafish facility *(Dariusz Gajdziński)*	Toxicity screening using zebrafish embryos (LiCl) - Part I *(Natalia Kasica)* + Dissection of organs from adult zebrafish, finclips and swabbing *(Joanna Dybalska, Mikołaj Mazur)*

Note: Coffee/tea/sweets will be available at all times. Help yourself at the time convenient for you.

18:15 - 20:00/21:00 - Social event

DAY III

Time	Group I	Group II	Group III	Group IV
9:00 - 10:00	Toxicity screening using zebrafish embryos (LiCl) - Part II *(Natalia Kasica)*	Introduction to cell sorting *(Katarzyna Kłak)*	Visualisation of xenografts and transgenic zebrafish (confocal microscopy) *(Piotr Podlasz)*	CRISPR/Cas9 design *(Anna Sarosiak), note:* ***personal laptop required***
10:00 - 11:00	CRISPR/Cas9 design *(Anna Sarosiak), note:* ***personal laptop required***	Toxicity screening using zebrafish embryos (LiCl) - Part II *(Natalia Kasica)*	Introduction to cell sorting *(Katarzyna Kłak)*	Visualisation of xenografts and transgenic zebrafish (confocal microscopy) *(Piotr Podlasz)*
11:00 - 11:15	Short break	Short break	Short break	Short break
11:15 - 12:15	Visualisation of xenografts and transgenic zebrafish (confocal microscopy) *(Piotr Podlasz)*	CRISPR/Cas9 design *(Anna Sarosiak), note:* ***personal laptop required***	Toxicity screening using zebrafish embryos (LiCl) - Part II *(Natalia Kasica)*	Introduction to cell sorting *(Katarzyna Kłak)*
12:15 - 13:15	Introduction to cell sorting *(Katarzyna Kłak)*	Visualisation of xenografts and transgenic zebrafish (confocal microscopy) *(Piotr Podlasz)*	CRISPR/Cas9 design *(Anna Sarosiak), note:* ***personal laptop required***	Toxicity screening using zebrafish embryos (LiCl) - Part II *(Natalia Kasica)*
13:15 - 14:45	Lunch & Summary	Lunch & Summary	Lunch & Summary	Lunch & Summary

Note: Coffee/tea/sweets will be available at all times. Help yourself at the time convenient for you.

Abstracts

Lectures

1. The zebrafish as a model for scientific research – Marta Migocka-Patrzalek

Marta Migocka-Patrzalek

Department of Animal Developmental Biology, University of Wrocław, Sienkiewicza 21,50-335 Wrocław, Poland. e-mail: marta.migocka-patrzalek@uwr.edu.pl

Many different animal species such as worms, fruit flies, mice, and zebrafish have important roles as experimental model organisms in advanced biomedical research. The great anatomical and physiological similarities between humans and animals are the reason why researchers can use models to perform research and apply the results to understanding biological processes in humans.

Zebrafish, a small tropical fish with five stripes on its side, is one of the leading research models because of its exceptional features. This fish, like other model organisms, has a sequenced genome, is easy to breed and its maintenance is relatively inexpensive. What's more, the high fecundity is combined with external fertilization and development. These aspects together with the fact that the embryo is transparent, allow for easy, noninvasive observations of almost all early developmental processes.

The high quality of genome assembly shows that approximately 70% of human genes have at least one zebrafish orthologue. It is a reason why zebrafish are very often used to generate mutants mirroring human diseases. To date, more than 10,000 strains with mutations in protein-coding genes have been generated. A wide range of genetic, molecular, and bioinformatic tools are available too. Taken together, the use of zebrafish allows for the investigation of disease pathomorphology and the search for novel, effective therapies. A large amount of offspring enables also the high-throughput screening of potentially therapeutic compounds.

Although the zebrafish model has its limitations, such as being different from the human respiratory and reproductive system, it is an important biomedical model in (almost) every aspect of biology, and the scientific network of teams working with zebrafish is growing worldwide.

- References: Danio adventure. Developmental biology of the zebrafish in science popularization (2022) M Dubińska-Magiera, M Migocka-Patrzałek, A Cegłowska. Journal of Biological Education 56 (3), 245-255. doi.org/10.1080/00219266.2020.1776752

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Keywords: zebrafish, model animals, model organisms, modeling of human diseases

2. Zebrafish xenografts in cancer research - Martin Distel

Martin Distel

St. Anna Children's Cancer Research Institute, Innovative Cancer Models & Zebrafish Platform Austria for preclinical drug screening, Zimmermannplatz 10, 1090 Vienna, Austria

Xenotransplantation of human cancer cells in zebrafish larvae provides an assay complementing classical mouse xenograft experiments for drug testing. The main advantages of larval zebrafish xenografts are the achievable throughput, the ease of compound administration, the phenotypic readout by live imaging, the low costs and the short duration of the assay, which in principle would allow one to apply zebrafish avatars for instructing patient treatment.

Not surprisingly, zebrafish xenografts have become a popular model in cancer research over the last years and many different protocols to establish such xenografts exist.
To best leverage the strengths of zebrafish xenografts for drug testing, we set out to automate key steps in the compound screening workflow and established the Zebrafish platform Austria for preclinical drug screening (ZANDR). Specifically, we tried to improve and standardize current xenotransplantation techniques and developed methods for high-content imaging and automated analysis for zebrafish xenografts being applied in drug screening.

We utilized our established setup to screen for combinations of compounds, which are effective against Ewing sarcoma, a malignant pediatric bone and soft tissue tumor. We identified dual inhibition of MCL-1 and BCL_{XL}, two anti-apoptotic proteins, to be a specific vulnerability for Ewing sarcoma.

However, the use of zebrafish xenografts is not restricted to testing small compounds. We provide a proof-of principle that zebrafish xenografts can be applied for preclinical testing of immunotherapies. We show that the killing efficacy of CAR T cells directed against CD19-positive leukemia cells can be quantified by live imaging in zebrafish xenografts.

Keywords: zebrafish xenografts, high-content screening, Ewing sarcoma, CAR T cells

3. Zebrafish model to study brain development and diseases - Justyna Zmorzyńska

Justyna Zmorzyńska
International Institute of Molecular Mechanisms and Machines, PAS

Appreciation of zebrafish as a model organism to study pathogenesis of brain diseases is growing. Zebrafish is a small fish which develops externally what makes live imaging of the brain and environmental manipulation easier than in placentals. Zebrafish gives hundreds of eggs in one mating that offers a possibility of population-like research. What is more, it exhibits a plethora of complex behaviours including early developing social behaviour and it is genetically similar to humans with 70% of human genes having their zebrafish orthologues. These features make zebrafish well-suited for studies of the conserved mechanisms of brain development and diseases. Moreover, state-of-the-art tools for genetic manipulation and well characterized behaviours, together with advanced tests for learning and memory is beneficial. On top of that, the zebrafish brain connectivity has been recently resolved making brain research on zebrafish even more powerful. With the ease of live imaging of morphology and function of the developing brain, coupled to specific complex behaviours, zebrafish offers a unique possibility to study conserved neuronal circuits on a molecular level in the context of the whole brain and specific behaviours.

Keywords: zebrafish, brain development, neurodevelopmental diseases, brain diseases

4. Zebrafish larvae infection model to study immunity and host-pathogen interactions - Magdalena Widziołek-Pooranachandran

Magdalena Widziołek-Pooranachandran

Institute of Zoology and Biomedical Research, Jagiellonian University, Kraków, Poland

Zebrafish has become a well appreciated *in vivo* real-time experimental system for studying infection and immunity, and testing new therapeutic strategies aimed at reducing inflammatory and infectious diseases.

The immune system of zebrafish is highly similar to that of humans, although in zebrafish the maturation of adaptive immunity happens much later. This allows us to study the innate immune system separately during early larval stages. The innate immune system, including macrophages and neutrophils, is crucial in inflammatory and infectious diseases.

These phagocytes are well-conserved between humans and the zebrafish, which has emerged as a powerful animal model to study inflammation and host-pathogen interactions. This is mainly due to (i) larval optical transparency (ii) availability of transgenic lines/mutants (iii) high throughput system for anti-inflammatory drugs.

This lecture will provide an overview of zebrafish immunity, methods employed to study inflammatory processes, and insights into pathogen interactions with the zebrafish host. I will also present our research conducted at the Department of Evolutionary Immunology, Jagiellonian University using zebrafish infection model.

Key words: immunity, infection, phagocytes, pathogens

5. Zebrafish as a Model Organism for Toxicity Studies: Exploiting Advantages in Biomedical Research - Barbara Budzyńska

Barbara Budzyńska

Independent Laboratory of Behavioral Studies, Medical University of Lublin

Zebrafish (Danio rerio) has emerged as a prominent model organism for toxicity and safety evaluations of investigational drugs. Various testing methods have been established, including large-volume screening platforms utilizing multi-well plates to assess chemical impacts on embryo development and malformations across concentration gradients, e.g. OECD 236 guideline. Due to their transparency, embryos are particularly preferred for toxicity examinations, allowing for facile observation of developmental phases and evaluation of endpoints. This provides technical and economic advantages over rodent models. Small molecules can be introduced into the water for uptake by diffusion, or injected directly into the yolk sac, enabling investigations toxicants. Zebrafish embryos also offer insights into organogenesis, with major organ systems such as the heart, liver, brain, and pancreas forming within five days post-fertilization. The optical clarity of zebrafish embryos aids in phenotypic screening and toxicity endpoint determination, which is particularly beneficial for mutagenesis screening and investigating heart development and functional effects of toxicants. Thus, zebrafish embryos represent a valuable model for toxicity assessment in drug development, offering insights into developmental processes and organ function while minimizing animal usage and experimental costs. Furthermore, despite the anatomical disparities between the nervous systems of zebrafish and mammals, zebrafish possess essential structures similar to those found in mammalian brains. They harbor fundamental neurotransmitters, receptors, transporters, and enzymes crucial for synthesizing and metabolizing signaling molecules. Dysfunction in these components underlies various central nervous system disorders in mammals. Consequently, Danio rerio presents a promising model for investigating the etiology of diseases associated with neurological dysfunctions.

Keywords: toxicity, organogenesis, neurotransmitters

6. Zebrafish in the Lens of Behavioural Research - Małgorzata Potoczna

Małgorzata Potoczna [1,2]

[1] *Transpharmation Poland Ltd., ul. Michała Oczapowskiego 13/105D, 10-719 Olsztyn, Poland*

[2]*Department of Pathophysiology, Forensic Veterinary Medicine and Administration, Faculty of Veterinary Medicine, University of Warmia and Mazury in Olsztyn, ul. Michała Oczapowskiego 13, 10-719 Olsztyn, Poland*

Zebrafish (*Danio rerio*) has emerged as a prominent model in behavioural research, valued for its small size, cost-effectiveness, and short generation interval. During the lecture, we explore the model's unique advantages and delve into essential behavioural facets, encompassing activities from embryos and larvae to advanced assays and equipment for observing adult behaviours.

Exhibiting behaviours similar to humans and rodents, zebrafish provide an excellent paradigm for studying diverse aspects of behaviour. The exploration extends to behaviours exhibited by zebrafish embryos and larvae, revealing distinctive actions such as tail flicks and coilings. These behaviours emerge as crucial indicators in toxicological and pharmacological studies, providing valuable insights.

Critical behavioural assays, including the Light-Dark Challenge Assay and the Light-Dark Preference Tests, are analysed for their utility in screening potential anxiolytic drugs and assessing developmental neurotoxicity. Thigmotaxis research nuances are addressed, emphasizing the correlation between this behaviour and stress.

Furthermore, this presentation delves into the diverse range of equipment essential for behavioural observations, encompassing tools applicable to embryos, larvae, and adult zebrafish. This comprehensive exploration underscores the versatility of zebrafish in behavioural research, offering a roadmap for researchers utilizing this model to unravel the intricacies of behaviour.

Keywords: zebrafish behaviour, behavioural assays, anxiolytic drug screening, high throughput

7. Modeling of viral infections in fish: from fundamental to applied science - Krzysztof Rakus

Krzysztof Rakus

Department of Evolutionary Immunology, Institute of Zoology and Biomedical Sciences, Faculty of Biology, Jagiellonian University, Krakow, Poland

In recent decades, aquaculture has become the fastest growing food production sector in the world, with fish providing an important source of nutrients for humans that can be produced with a low carbon footprint. However, in many aquaculture production systems fish are bred in high stocking density and are exposed to a combination of diverse abiotic and biotic stressors, which may lead to an increase in disease susceptibility, risk of potential pathogen spread and finally disease development. Therefore, prevention and control of fish diseases are crucial to maintain a sustainable aquaculture, both economically and environmentally.

There are many fish viruses that are responsible for high losses in various farmed fish species. Identification of the underlying mechanisms associated with antiviral response could lead to the better protection of the fish against viral infections by discovering new therapeutic targets, development of new drugs and vaccines or identification of prophylactic strategies, which are highly needed in modern aquaculture. Moreover, since the essential immune mechanisms, receptors and pathways are often well conserved in vertebrates, fish represent a relevant model for the study of the core immune mechanisms activated by viral infections.

During my lecture I will present two models of viral infection in fish that can be used for both basic and applied research: common carp – CyHV-3 (cyprinid herpesvirus 3) and zebrafish – TiLV (tilapia lake virus).

Keywords: fish viruses, antiviral response, common carp, zebrafish

8. Evolution of genome editing in zebrafish: from origins to advances and integration into translational medicine – Anna Sarosiak

Anna Sarosiak[1], Justyna Jędrychowska[2], Dominika Oziębło[1], Marcin Leja[1], Nina Gan[1], Natalia Bałdyga[1], Henryk Skarżyński[3], Vladimir Korzh [2], Monika Ołdak[1]

[1]*Department of Genetics, Institute of Physiology and Pathology of Hearing, Warsaw, Poland*
[2]*International Institute of Molecular and Cell Biology in Warsaw, Warsaw, Poland*
[3]*Oto-Rhino-Laryngology Surgery Clinic, Institute of Physiology and Pathology of Hearing, Warsaw/Kajetany, Poland*

Genomic editing allows precise manipulation of DNA sequences within an organism's genome. In the 1980s, zebrafish (Danio rerio) emerged as a powerful model organism suitable for genomic editing due to its high fecundity, external insemination, rapid embryo development, and genetic similarity to humans. This review explores the evolution of genomic editing tools in zebrafish research, from the advent of chemical mutagenesis to the development of nuclease-based methods such as Zinc Finger Nucleases (ZFNs) and Transcription Activator-Like Effector Nucleases (TALENs). However, the recent breakthrough in genomic editing techniques came with the introduction of CRISPR/Cas9, which has swiftly become the method of choice due to its simplicity, efficiency, and availability.

In zebrafish, CRISPR/Cas9-mediated genome editing has become particularly popular for elucidating the role of particular genes in various biological processes, their involvement in molecular pathophysiology, and modelling human diseases. As an example of this approach we present our research on the *TBC1D24* gene, linked to autosomal dominant hearing loss (ADHL). In this study we demonstrated that the CRISPR/Cas9-based knock-out of *tbc1d24* leads to impaired mechanotransduction in hair cells and results in a reduction in the number of hair cells and neuromasts in zebrafish.

Furthermore, current enhancements on CRISPR/Cas9-based genome editing methods allow for precise introduction of single nucleotide variants and epigenome editing. This technology holds great promise for the development of novel therapeutic approaches, as evidenced by its successful application in screening of potential drug candidates.

Keywords: genome editing, CRISPR/Cas9, disease models, hearing loss

Posters

1. Development of a novel zebrafish SCID model - Karolina Czapla

Karolina Czapla
Department of Biochemistry and Molecular Biology, Medical University of Lublin, Lublin

Over the past few years, it has become increasingly apparent that no two patients' cancers are identical. Due to the heterogeneity of the disease, selecting the most effective treatment method for each individual patient remains a significant challenge. Recently, a novel strategy has emerged, utilizing animal Patient Derived Xenografts (PDX) models for xenotransplantation of human cancer cells. PDX offers an approach to the development of personalized medicine based on in vivo studies that allow for the monitoring of human tumor growth and progression.

This strategy proves valuable in mimicking patients' tumor biology and predicting their drug response by directly comparing drug responses in patients with those in their corresponding xenografts. However, the use of PDX models is constrained by the strong rejection of transplanted cells by the host. To address this issue, new models with a suppressed immune defense system in the host are continually being developed, such as Severe Combined Immunodeficiency (SCID).

Our designed SCID model will be generated using the CRISPR genome editing method. Through targeted knock-in techniques, immune deficiency induction will be achieved using a well-established system based on nitroreductase (NTR) and metronidazole (Mtz). The target gene for genome editing in zebrafish is the L plastin-coding gene, a protein that exhibits high expression in fish leukocytes. Using the SCID model eliminates the risk of tumor rejection during transplantation, and the induced reduction in immune response ensures that until that fish remain in good health and do not require a sterile environment.

Keywords: zebrafish, PDX, SCID, L-plastin

2. Zebrafish as a model for assessing the impact of bioactive substances - Sebastian Knaga

Sebastian Knaga[1], Elzbieta Pietrzak[1]. Karolina Stasiak[2]

[1] *Bydgoszcz University of Science and Technology, Faculty of Animal Breeding and Biology, Department of Animal Biotechnology and Genetics, 85-796 Bydgoszcz, Poland*

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Bioactive compounds are essential and non-essential compounds (e.g., vitamins or polyphenols) that occur in nature, are part of the food chain, and can be shown to have an effect on human or animal health. The assessment of the effects of bioactive substances on living organisms is crucial for understanding their potential positive (health-promoting and therapeutic) as well as negative effects.

This is typically conducted using model species, such as the zebrafish (*Danio rerio*). It can be utilized as a biological system to assess the effect of these substances on blood biochemical parameters, microbiological profile, gene expression, and histomorphology. Measurements of biochemical parameters, such as concentrations of enzymes, hormones, or metabolites, allow for the evaluation of the metabolic functions of the organism under study. Meanwhile, the analysis of the zebrafish microbiome enables an understanding of changes in the composition of gut microorganisms in response to administered bioactive substances. Changes in gut microbiota can influence gene expression along the gut-liver-brain axis. Histological analyses provide insight into the impact of bioactive substances on tissue and organ structure and morphology, serving as a significant indicator of their condition.

Bioactive substances can significantly influence various aspects of zebrafish biology, which has implications for the health and behavior of these organisms and can provide insights into potential benefits or risks for human or animal species' health. Utilizing the zebrafish as a research model allows for understanding the mechanisms of action of those substances and their potential effects on living organisms.

Keywords: bioactive compounds; microbiome; histomorphology; gene expression; model organism

3. The impact of differences in the structure of the analog of fenoterol and their potential inhibitory effect on MAPK signaling - Robert Kostecki

Robert Kostecki[1], Jakub Wójcik[2], Artur Wnorowski[2], Krzysztof Jóźwiak[2]

[1]*Student Science Club "Molecular Pharmacology"*
2 Department of Biopharmacy, Medical University of Lublin, Lublin, Poland

Background
The β2-adrenergic receptor (β2AR) plays a vital role in cancer development, influencing processes like cell proliferation, apoptosis, and angiogenesis. Fenoterol, a robust scaffold for studying β2AR activity, has been explored through analogs like (*R*,*R*′)-4′-amino-1-naphtylfenoterol ((*R*,*R*')-ANF) and (*R*,*R*′)-2-naphtylfenoterol ((*R*,*R*')-NF). This study aims to evaluate their inhibitory effects on ERK signaling in melanoma cells and correlate structural variations with mitogen-activated protein kinase (MAPK) pathway inhibition, measured by half-maximal inhibitory concentrations (IC_{50}). Understanding fenoterol derivatives mechanisms could aid in developing more effective cancer therapies.

Material and methods
Human UACC-647 melanoma cells were treated for 20 min. with an increasing concentration of fenoterol derivates or vehicle (DMSO, 0.1%). Phosphorylation of ERK signaling node was studied by western blotting.

Results
The IC_{50} values of the two derivatives of fenoterol (*R*,*R*')-ANF [IC_{50}=83.5pM] and (*R*,*R*')-NF [IC_{50}=77.9pM] compared to (*R*,*R*')-fenoterol ((*R*,*R*')-F) [IC_{50}=63,5pM] show that expanding phenyl ring to naphthyl ring and changed hydroxy group for amino group decreased inhibition of ERK signaling.

Conclusions
In summary, structural disparities between (*R*,*R*')-F and its analogs (*R*,*R*')-ANF and (*R*,*R*')-NF highlight a nuanced relationship between these disparities and their respective activities in inhibiting the MAPK signaling pathway. These findings shed light on the crucial role played by specific modifications in enhancing the efficacy of these analogs in modulating cancer-related signaling pathways.

Acknowledgments
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Keywords: fenoterol, β2-adrenergic receptor, structural analogues,

4. Revealing the molecular mechanism of endocrine disruptive effect of mycotoxins in humans - Karolina Kowalska

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Mycotoxins, secondary metabolites of fungi concern both economic and health care issues. Although it seems that the toxicity of the best known mycotoxins like deoxynivalenol (DON) or zearalenone (ZEN) is already known, little is known about the molecular mechanism of its action in human cells, especially concerning their endocrine disruptive effect in cells. The significance of the research concerning endocrine disruptive chemicals (EDC) has changed during last decade, revealing that many EDC might trigger a significant effect, which might be exacerbated by other environmental factors.

Thus, in our research we broaden the scope of classic toxicological studies to understand in detail how mycotoxins might affect the main molecular signaling pathway in cells, if and how might be useful in clinics and last but not least, if they might interact with the other environmental factors, both EDC as well as pathological conditions like obesity or cancer.

So far, we showed that DON, ZEA and alternariol (AOH) might possess an endocrine disruptive effect in cells via modulation of steroidogenesis process (DON), induction of oxidative stress (DON, ZEA, AOH), DNA damage (AOH), cell cycle modulation (DON, ZEA, AOH) and cell death (DON, ZEA, AOH). We evaluated the involvement of nuclear estrogen receptors (ERs) in their effect (ZEA. AOH) and postulated main molecular pathways modulated by them (DON, ZEA, AOH). Now, we are going further and investigate the interaction of mycotoxins with obesity (ZEA, AOH), chemotherapeutics (DON) and immune system (ZEA, AOH) to gain a broader scope of mycotoxins' action in hormone-sensitive cells.

Keywords: mycotoxins, endocrine disruptive chemicals, cancer, oxidative stress

5. Zebrafish as a model in novel ERMS therapies development - Damian Lewandowski

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Rhabdomyosarcomas (RMSs) are a heterogeneous group of malignant myogenic tumours. These tumours originate from undifferentiated primary mesenchymal cells with the potential to differentiate into skeletal muscles. Embryonal rhabdomyosarcoma (ERMS) is one of the most frequent types of RMSs in children. Clinically ERMS is treated with a combination of surgery, chemotherapy, and radiation therapy (RT) adjusted to the stage of the disease. It has been shown that chemotherapy and RT in ERMSs treatment remain fundamental. However, the ERMSs chemotherapy has many disadvantages such as induction of multidrug resistance proteins activity (MDR) and the appearance of toxic side effects. Almost all anti-cancer agents affect not only cancer but also healthy cells. Moreover, the majority of cytostatics are only approved for the treatment of adults but not for paediatric cancers. The RT application is also very problematic for young children and infants due to its high toxicity.

The aim of the project is an assessment of the therapeutic, anti-cancer potential of selected natural substances in the treatment of ERMS in zebrafish xenograft models. In our studies, we will analyse biochanin a, caffeic acid phenethyl ester, and cucurbitacin E as novel, natural, poorly known in ERMS therapy, and anti-cancer agents. Furthermore, vinorelbine and daunorubicin will be used as a positive control. The results obtained during the project implementation will broaden the knowledge about the novel, natural chemotherapeutics mechanism of action and cellular response. The novel data can potentially revolutionize the planning of anti-cancer, less toxic strategies for ERMS treatment, especially for a paediatric patients.

Acknowledgements: This work was supported by the „Excellence Initiative – Research University” for the years 2020-2026 for the University of Wrocław

Keywords: zebrafish, xenotransplantation, rhabdomyosarcoma

6. Can Porphyromonas gingivalis accelerate Alzheimer's disease (AD) progression? - Magdalena Marcinkowska

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Porphyromonas gingivalis (*Pg*) is a keystone pathogen in the aetiology of chronic periodontitis, however, recent evidence suggests that this bacterium is also able to cross blood-brain barrier, induce neuroinflammation and neurodegeneration. To support this, *Pg*-derived DNA and gingipains, the main virulence factors of this pathogen, were found in the brains of AD patients. In addition, *Pg* have been identified as a risk factor for the development of amyloid-beta (Aβ) senile plaques characteristic for AD pathology. Interestingly, in cases of mild AD, the periodontal therapy seems to be promising approach to support cognitive function. Here, using zebrafish larvae infected systemically with the wild-type *Pg* (W83) or gingipain-deficient mutant strain (*ΔK/R-ab*), we demonstrated: (i) presence of *Pg* in the brains of infected larvae, (ii) gingipain-dependent activation of microglia and (iii) *Pg*-induced neuroinflammation manifested as increased gene expression of pro-inflammatory mediators.

Next, we plan to verify whether intraventricular administration of the most toxic form of Aβ (Aβ1-42) induces microglia activation, neuroinflammation and neurodegeneration in zebrafish larvae. Subsequently, we will examine how Aβ1-42 administration effects zebrafish larvae behaviour. Finally, we will determine the impact of systemic *Pg* infection in these Aβ-induced processes.

Our data indicate that zebrafish larvae can by successfully used for the host-*Pg* interaction studies, especially in the context of central nervous system (CNS) immune homeostasis disruption that can lead to neuroinflammation. In the future it can open the way for discovering new therapeutics for neuroinflammatory and/or neurodegenerative disorders.

Keywords: neurodegeneration, *Porphyromonas gingivalis*, microglia, zebrafish larvae

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7. Modeling of human melanoma in Zebrafish larvae xenografts - Aleksandra Mordzinska-Rak

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Cholesterol is a fundamental component of animal cell membranes important for maintaining their proper fluidity, permeability and homeostasis. Several experimental data indicate that changes in the chemical composition and spatial organization of the plasma membrane are directly related to the development, progression and invasiveness of cancer. Moreover, in some cancers cells contain higher cholesterol levels, what often correlates with the more aggressive type of tumors.

One of the main goals of our project is to check various parameters of melanoma cells such as proliferation, migration and metastasis in context of the activity of ATP-binding cassette (ABC) transporter A1 (ABCA1) which is responsible for removing of excess cholesterol from cells. We performed *in vitro* and *in vivo* (xenografts of melanoma cell lines) studies using human melanoma cell lines derived from patients.

Currently, we focus our attention on the pharmacological inhibition of the activity of ABCA1 protein in some selected human melanoma cell lines using probucol which is known as antyhiperlipidemic drug. I have been working on two-days old zebrafish larvae model and checking the level of proliferation and the ability of melanoma cells to form a metastasis.

Keywords: melanoma, cholesterol, ABCA1, probucol

8. Impact of Cocaine on Heart Rate and Locomotor Activity in Danio rerio: Exploring Metal-Organic Frameworks as Potential Interventions – Weronika Mrozek

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Psychoactive substance use and poisoning pose a serious threat to health and life. One of the most commonly used psychoactive substances is cocaine (COC), originally derived from the *Erythroxylum coca*. It is a widely abused compound known for its potent effects including sensations of increased confidence, energy, and mental alertness, accompanied by a temporary sense of well-being and euphoria, which is caused by inhibiting the reuptake of dopamine, serotonin, and norepinephrine. However, it induce serious cardiovascular complications, neurological impairments, and psychiatric disorders and addiction. Chronic use leads to enduring changes in the brain's reward circuitry, fostering compulsive drug-seeking behaviors and tolerance development. Withdrawal from COC is marked by dysphoria, fatigue, and intense cravings, complicating sobriety efforts.

Current detoxification methods for COC dependence rely on behavioral interventions and pharmacotherapy, but they often yield suboptimal outcomes and fail to address the complexity of addiction. Our research explores the potential of porous metal-organic frameworks (MOFs) – NU-1000, UiO-67, MOF-808, which show promise as drug adsorbents, including for cocaine, in acute overdose scenarios. In vivo experiments, particularly using zebrafish as a model due to their anatomical and neurotransmission similarities, are used to assess MOFs' efficacy in drug removal. Our studies confirmed that COC increased heart rate at the concentrations: 50 μM, 100 μM, 150 μM, 250 μM, and 500 μM. In the larvae incubated in the MOF solutions, we did not observe changes in observed parameters. Also, MOFs did not influence COC-increased heartbeat. However, in our study, MOF reduced locomotor activity in larvae, which was significantly elevated by COC at the concentration 50 μM.

Thus we may suggest that MOFs alleviate COC induced effects in vivo, aid recovery and can potentially be used in the pharmacotherapy of intoxication or addiction.

Keywords: behavioral pharmacology, cocaine, metal-organic framework, toxicity.

The research is supported by National Science Center, Poland (2021/43B/NZ7/00827).

9. Is Zebrafish an alternative model to study mycotoxin influence on gut function? - Piechota-Polanczyk Aleksandra

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Inflammatory bowel diseases (IBD) encompass a group of chronic inflammatory conditions of the gastrointestinal (GI) tract, including Crohn's disease and ulcerative colitis. These conditions are characterized by inflammation, immune dysregulation, and damage to the digestive tract. The link between mycotoxins like Deoxynivalenol (DON) and Zearalenone (ZEA) and inflammatory bowel diseases is an area of ongoing research. Some studies suggest that exposure to mycotoxins may contribute to the development or exacerbation of inflammatory bowel diseases. The mechanisms are not fully understood, but it's believed that mycotoxins may disrupt the intestinal barrier function, induce inflammation, and modulate the immune response.

The use of zebrafish in toxicology and disease research provides valuable insights into the potential mechanisms of mycotoxin-induced toxicity and its relevance to human health. However, it's important to note that findings from zebrafish studies should be extrapolated cautiously to human conditions, as there can be differences in physiology and immune response between species.

In our study we plan to verify the effect of DON and ZEA on GI transit in zebrafish with or without chemically-induced enterocolitis. We will use spectrophotometry method for measuring zebrafish larvae GI transit time and immunofluorescent analysis of infiltration of inflammatory cells.

Keywords: mycotoxins, intestinal transit, enterocolitis

10. New possibilities of using Danio Rerio models in pharmacological and toxicological research - Lucyna Pomierny-Chamioło

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Zebrafish may be a rapid and cheap animal model used for the initial screening of new chemical structures as potential drugs.

The research we plan to perform will focus on assessing the impact of new chemical compounds and substances of plant origin on the cardiovascular system. Setting up appropriately validated models of cardiac arrhythmias will allow us to test compounds with potential anti-arrhythmic effects. Independently, the analysis of ECG records will allow us to initially assess the cardiological safety of the tested compounds.

The use of transgenic *Danio rerio* larvae will also enable the assessment of angiogenesis and the impact of the tested chemicals on this process.

Validation of behavioral models correlated with imaging tests at the molecular level using Light Sheet technology, will allow testing of compounds with potential anti-depressive, anti-anxiety, anti-psychotic, neuroprotective and pro-cognitive effects.

11. Assessment of the anxiolytic effects of citral using the Danio rerio model - Monika Rudkowska

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Anxiety disorders are the world's most common mental disorders, affecting 301 million people in 2019. Pharmacotherapy plays a crucial role in the treatment of this disorder and in reducing the risk of comorbid mood disorders. Selective serotonin reuptake inhibitors (SSRIs), serotonin and norepinephrine reuptake inhibitors (SNRIs), and benzodiazepines are the main classes of drugs used in the treatment of anxiety disorders. Unfortunately, therapy with these drugs is not always effective and can lead to drug abuse, delayed therapeutic effects, dependence, and tolerance.

Therefore, there is a need to search for new psychoactive substances with rapid action and fewer side effects. Monoterpenoids are plant secondary metabolites that exert various neuropharmacological effects, including improvement of learning and memory, antidepressant, anxiolytic, analgesic, anticonvulsant, and neuroprotective actions, indicating the promising potential of these compounds.

In the present study, we evaluated the anxiolytic activity of citral (CIT), a monoterpenoid present in the essential oils of several medicinal plants. The effects of CIT on the locomotor activity of zebrafish larvae at 5 days post-fertilization were explored under different illumination conditions. Additionally, the acute embryotoxicity of CIT to the early life stages of zebrafish was evaluated using the Fish Embryo Acute Toxicity (FET) Test method according to OECD guideline 236.

Based on our research, we found that CIT at doses of 1, 2.5, and 7.5 mg/l exhibits anxiolytic activity. The lethal concentration 50 (LC_{50}) was 6.509 mg/L. Therefore, CIT at lower doses can be considered a promising candidate for the treatment of anxiety disorders and related conditions.

Keywords: citral, anxiety, toxicity, monoterpenoids.

12. Multiparameter assessement of tyrosine kinase inhibitors effect on endothelium in zebrafish model - Emilia Seta

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Despite growing rate of success in cancer treatment, the increasing number of severe side effects on cardiovascular system has been observed among patients. The mechanisms of such vascular toxicity of many chemotherapeutic compounds are largely unknown.
Tyrosine kinase inhibitors (TKIs) of BCR-ABL, a class of chemotherapeutic agents, are being successfully used in therapy against chronic myeloid leukaemia. However, it has been shown in the clinical trials that treatment with TKIs could contribute to adverse cardiovascular events, such as myocardial infarction or stroke.

In this study, we use the zebrafish model that is currently gaining popularity in biomedical research as it combines the low cost, ease of use or amenability to high throughput screening of in vitro systems with complexity or genetic and functional similarity of mammalian systems. Firstly, we explored the cardiotoxicity of TKIs from every generation. Our further experiments involved microinjections of dextran conjugated with fluorescein to the bloodstream of transgenic zebrafish larvae with fluorescently tagged endothelium at 2 days post fertilization. Subsequently, the injected larvae were treated with TKI by immersion and their peripheral or cerebral vasculature was finally imaged by confocal microscopy. In order to quantify the extravasation of dextran, the fluorescence intensity within areas inside and outside a dorsal aorta was measured in each individual. Moreover, measurements of blood vessel diameters were taken.

We observed oedema, vasoconstriction and enhanced vascular leakage in ponatinib- and nilotinib-treated larvae in comparison to vehicle controls. Therefore, we believe zebrafish is a valuable model for studying endothelial toxicity in vivo.

Keywords: tyrosine kinase inhibitors, endothelium, vascular permeability, cardiovascular toxicity

13. Visualizing high-resolution FRET images detects specific effects of GABA transporters on Ca2+ and PKA dynamics in discrete plasma membrane microdomains - Marta Sobolczyk-Prawda

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The phenomenon known as astrocytic Ca^{2+} excitability is a highly orchestrated process that generates spatiotemporal oscillations with a significant impact on physiological and pathophysiological changes in the nervous system. Although astroglial modulation of GABAergic transmission has been widely reported, the sequence of events following GABA uptake and the consequences on gliotransmission remain largely unresolved. By using new targeted FRET biosensors allowing for measurement of calcium changes and PKA activation in the astrocytic lipid rafts, we showed that GABA-mediated Ca^{2+} entry into C6 astrocyte-like cell line is primarily mediated by GABA transporter type 3 (GAT-3). Visualization of GAT-3 demonstrated its predominant location to lipid rafts and a strong colocalization with plasma membrane Ca^{2+}-ATPase isoform 4 (PMCA4), the main PMCA isoform found in C6 astrocytic cells. Interestingly, this interaction is specific for lipids rafts as depletion of cholesterol from these structures using β-cyclodextrin irreversibly disrupted GAT-3/PMCA4 complex. Our functional single-cell imaging with unimolecular FRET biosensors showed that interaction of both proteins with lipid rafts and with each other regulate local Ca^{2+} handling and PKA activity in these plasma microdomains and is crucial for secretory activity of astroglia. Our findings provide novel insight into the role of membrane microdomains in the compartmentation of plasma membrane events in astrocytes evoked by GABA-dependent signal transduction. Understanding of how astroglia responds to inhibitory events mediated by GABA in the central nervous system is of paramount importance for developing an efficient therapeutic approach for many brain diseases.

Keywords: calcium, protein kinase A (PKA), plasma membrane microdomains, Fluorescence resonance energy transfer (FRET)

14. Study of immunomodulatory effects of oxysterols, 25-hydroxycholesterol and 7α, 25-di-hydroksycholesterol using zebrafish (*Danio rerio*) and Nile tilapia (*Oreochromis niloticus*) - Justyna Starzyk

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Cholesterol is one of the main lipid components of animal cells. During the antiviral response cholesterol is metabolized to the oxysterol 25-hydroxycholesterol (25HC) and further to the 7α,25di-hydroksycholesterol (7α,25diHC). In mammals, 25HC and 7α,25diHC have immunomodulatory properties affecting the activation of cells of the innate and adaptive immune system. However, the role of oxysterols in antiviral response in fish is poorly understood. The aim of this project is to study the role of 25HC and 7α,25diHC in the antiviral response of fish and to check their immunomodulatory potential and the possibility of using them as adjuvants in vaccines.

The experiments will be conducted using two animal models: zebrafish and Nile tilapia. At first, zebrafish larvae will be stimulated with 25HC or 7α,25diHC prior infection with various fish viruses: TiLV, SVCV, VHSV, CyHV-3, and CSV. In parallel, zebrafish knock-out mutants for *ch25h*$^{-/-}$ (gene encoding enzyme producing 25HC) and *cyp7b1*$^{-/-}$ (gene encoding enzyme producing 7α,25diHC) will be created using CRISPR/Cas9 technology. Mutant larvae will be infected with above mentioned viruses. In both approaches, survival of fish, viral load and innate antiviral response will be studied. Moreover, using adult zebrafish mutants, activation of the adaptive response will also be studied upon viral infection.

Finally, adult Nile tilapia will be vaccinated with DNA vaccine against TiLV, with addition of 25HC or 7α25HC as adjuvants. The effectiveness of the adjuvants will be tested upon TiLV infection. This study will allow for a better understanding of the role of oxysterols in antiviral response in fish.

Keywords: zebrafish, oxysterols, viral infection, DNA vaccine

15. Zebrafish as a new animal model of McArdle disease – Ewa Stefanik

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McArdle's disease is a genetic disorder caused by a mutation in the gene that controls the muscle isoform of glycogen phosphorylase (PYGM), a crucial enzyme in glycogenolysis. This disorder, inherited in an autosomal recessive manner, hinders the muscles' ability to break down glycogen, the primary source of energy, leading to difficulty in engaging in physical activities.

Our project aims to establish a novel animal model for human McArdle 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).

It is recognized that animal models may not fully replicate all aspects of human diseases, yet even if the mutants exhibit some symptoms resembling those observed in patients, our Zebrafish model could enhance our understanding of the underlying pathological mechanisms and potential treatments for McArdle disease.

In summary, our goal is to develop a new strain of Zebrafish with a knock-out of the muscle form of glycogen phosphorylase (*pygm-/-*), creating an animal model that accurately represents human McArdle disease. This reliable model will serve as a valuable tool in advancing research towards effective treatment options.

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Keywords: McArdle disease; metabolic disorder; skeletal muscle; disease model

16. Zebrafish xenograft models in oncology - Łucja Walczak-Nowicka

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Introduction: The discovery of anticancer drugs requires multiple screening tools including *in silico*, *in vitro*, and *in vivo* studies. *Danio rerio* is an *in vivo* model that is suitable as a platform for drug candidates screening, making it an attractive model when it comes to drug research.

The aim of the study: Present the current knowledge regarding zebrafish xenograft models.

Materials and Methods: A literature review was conducted using PubMed and GoogleScholar search services.

Results: Zebrafish xenograft models can be divided into two main types: the first is created using commercially available cell lines, and the second is a xenograft model known as ZTX, in which cancer cells are transplanted directly from the patient. The ZTXs model has found application in predicting the prognosis and treatment of the following cancers: breast cancer, gastric cancer, non-small cell lung cancer, glioblastoma, and bladder cancer. The use of the zebrafish as a xenograft model is supported by its several unique features. These include: the translucency of the embryo and, providing a set of orthotopic organs and tissues shortly after fertilization. *Danio rerio* has innate immune cells but lacks an acquired immune system early in life. The small size makes breeding simpler and cheaper and fewer cells are needed for transplantation. *Danio rerio* prefers an ambient temperature of 28°C, but can survive at 32 to 36°C.

Conclusion: Xenografts contribute to the understanding of cancer biology and the discovery of new drugs. ZTXs may be a step forward towards precision and personalized medicine in the field of cancer research.

Key words: zebrafish; cancer; oncology; xenograft

17. Zebrafish as a model organism for studying genetic defects causing inner ear malformations - Krystyna Żyżyńska-Galeńska

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One of the possible causes of congenital hearing loss is inner ear malformations (IEMs) caused by genetic abnormalities in known genes as well as unknown mutations. The most common IEM is an enlarged vestibular aqueduct (EVA), which may be associated with incomplete partition type 2 (IP2). The gene frequently implicated in these defects is SLC26A4. However, causative variants are found in only 25% of EVA/IP2 patients. A group of specific polymorphisms, known as the CEVA haplotype, have been found in the region upstream of this gene and are common to many heterozygous patients. Developmental studies of mammalian ear development are difficult due to the inaccessibility of the inner ear.

Since the zebrafish genome was sequenced, it has become a model for many human genetic diseases. Studying hearing and balance development in zebrafish has advantages due to the external development and transparency of the larvae and the availability of various methods to manipulate fish embryos. The development of CRISPR/Cas9 gene editing technology has accelerated genetic studies of the role of specific genes.

This project aims to analyze the role of the SLC26A4 gene in development of hearing and it's regulation. The hypothesis of the role of the CEVA haplotype in IEMs will be tested using the zebrafish model.

Keywords: hearing loss; slc26a4; inner ear malformations; coding and noncoding causative variants

PTZ webpage

www.zebrafish.org.pl