

Abstract book of the



Polish Zebrafish Society

2nd virtual meeting

15 September 2021

Organizers:
The Polish Zebrafish Society

Organizing committee:

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Program of the 2nd Polish Zebrafish Society Virtual meeting

Part I (9.00 – 10.30)	General Assembly of PTZ members	Przemko Tylżanowski – PTZ Chair, Department of Development and Regeneration, University of Leuven
Part II (10.45 – 12.15)	Invited speakers' presentations	- The European Zebrafish Society (EVS) – an introduction Stefan Schulte-Merker – Director of the Institute Cardiovascular Organogenesis and Regeneration, Westfälische Wilhelms-Universität Münster, Germany NOTE: because of an unforeseen emergency the speech will be given by Stephan Neuhauss, the Vice President of the European Zebrafish Society - Zebrafish models of human diseases with shortened lifespan Máté Varga - Eötvös Loránd Tudományegyetem Biológiai Intézet, Budapest, Hungary
	Members' presentations	1. Optimising two-photon microscopy for investigating neural activity in the larval zebrafish eye and brain Filip Janiak, Sussex Neuroscience, School of Life Sciences, University of Sussex, UK 2. Spin probe and spin trap toxicity for zebrafish (<i>Danio rerio</i>) embryos Katerina Makarova, Department of Physical Chemistry, Faculty of Pharmacy, Medical University of Warsaw, Poland 3. The Good, the Bad and the Ugly – histopathology in zebrafish Karolina Naumowicz, Department of Pathophysiology, Forensic Veterinary Medicine and Administration, Faculty of Veterinary Medicine, University of Warmia and Mazury in Olsztyn, Poland

Coffee break (12.15 - 12.30)		
Part III (12.30 – 14.00)	Members' presentations	4. Voltage-gated potassium channels as developmental regulators of inner ear Justyna Jedrychowska, International Institute of Molecular and Cell Biology in Warsaw, Warsaw, Poland 5. Role of antisense oligonucleotide-induced intronic miRNA on axonal morphogenesis Savani Anbalagan, Institute of Molecular Biology & Biotechnology, Faculty of Biology, Adam Mickiewicz University, Poznań, Poland. 6. Knockdown of tubgcp2 results in small head and cell cycle disruption of brain neural progenitor cells in zebrafish embryos Dawidziuk Mateusz, Department of Medical Genetics, Institute of Mother and Child, Warsaw, Poland 7. Neuroinflammation and sickness behavior in zebrafish infected with Tilapia lake virus Miriam Mojzesz, Department of Evolutionary Immunology, Institute of Zoology and Biomedical Research, Faculty of Biology, Jagiellonian University, Krakow, Poland 8. Modelling chemotherapy-associated cardiotoxicity in zebrafish: Pharmacological evaluation of doxorubicin-induced heart failure Monika Maciag, Department of Biopharmacy, Medical University of Lublin, Lublin, Poland 9. Screening of endosulfan and its degradation products toxicity using the <i>Danio rerio</i> embryos

		Patrycja Jesionkowska, Department of Physical Chemistry, Faculty of Pharmacy, Medical University of Warsaw, Warsaw, Poland 10. Application of zebrafish model to unravel the involvement of tbc1d24 gene in the development of autosomal dominant hearing loss Anna Sarosiak, Department of Genetics, Institute of Physiology and Pathology of Hearing; Postgraduate School of Molecular Medicine, Medical University of Warsaw, Warsaw, Poland
Closing remarks (14.00 - 14.15)		

Abstracts

1. Optimising two-photon microscopy for investigating neural activity in the larval zebrafish eye and brain - Filip Janiak

Filip Janiak¹, Phillip Bartel¹, Michael Bale¹, Takeshi Yoshimatsu¹, Mingyi Zhou¹, Emilia Komulainen¹, Kevin Staras¹, Lucia Prieto-Godino², Thomas Euler^{3,4}, Miguel Maravall¹, Tom Baden^{1,3}

1: Sussex Neuroscience, School of Life Sciences, University of Sussex, UK; 2: The Francis Crick Institute, London, UK; 3: Institute of Ophthalmic Research, University of Tuebingen, Germany; 4: Centre for Integrative Neuroscience, University of Tuebingen, Germany.

In vertebrate vision, signals are pre-processed in the retina and projected to diverse brain areas to ultimately drive behaviour. However, how different brain areas integrate different streams of visual information, and how they are connected, remains an area of active research. For example, many retinal output axons do not simply innervate a single brain target, but instead bifurcate to simultaneously drive two or more distinct areas based on the same original spike train. Is this bifurcation simply a duplication of original message, or might there be additional, asymmetric modification of the message in each output branch? To understand these types of questions, we would ideally like to simultaneously monitor neuronal activity in the eye and multiple brain areas.

We present our progress towards achieving this imaging capability using scanning 2-photon scanning microscopy which allows imaging inside the live retina without strongly exciting photoreceptors. Our ultra-low-cost solution only requires optical modifications of a typical commercial microscopes' optical pathway and the addition of an electrical tunable lens.

By simplifying and tuning a standard Sutter-MOM 2-photon setup into a noncollimated design, we can extend our x-y field of view up to 3.5 mm diameter using a standard x20 objective. In hand, our z-range covers 0.6 mm within 2 ms without strongly distorting the excitation volume or efficiency. Our design also provides for millisecond excitation power adjustment via a Pockels cell, as required. Finally, we achieve arbitrary spectrum optical/optogenetic stimulation by synchronizing our light sources with the scan retrace. Taken together, we can create arbitrary scan-paths with a maximal travel time of 2 ms during arbitrary spectrum optical stimulation. Our approach can be easily adapted to experiments on different animal models, like mouse, rats and drosophila.

2. Spin probe and spin trap toxicity for zebrafish (*danio rerio*) embryos - Katerina Makarova

Katerina Makarova¹, Katarzyna Zawada¹, Malgorzata Wiweger²

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Electron Paramagnetic Resonance (EPR) is a technique that allows direct detection of free radicals. In most cases, probes and spin traps are used to determine the redox status with the EPR technique [1]. Spin probes such as TEMPO, TEMPOL, 5-DSA, OX063 are paramagnetic chemical compounds that can be directly observed by EPR spectroscopy and give information concerning the physicochemical parameters of microenvironment. On the other hand, spin traps (e.g. DMPO, PBN, POBN) are compounds that only after capturing free radicals give a specific EPR signal. Thus, it is possible to perform a qualitative and quantitative analysis of individual free radicals which is important e.g. in the studies of oxidative stress.

Models based on zebrafish embryos are used in oxidative stress studies [2]. Additionally, the zebrafish is one of the few laboratory animals whose size allows to perform analyzes using standard EPR resonators. In *in vivo* studies, both probes and spin traps should be administered to the animals before the start of the actual experiment, at concentrations high enough to obtain an EPR signal and safe for the animal at the same time.

This study investigated the toxicity of TEMPO, TEMPOL, and 3-carbamoyl-PROXYL spin probes and PBN, POBN, and DMPO spin traps for wild-type *D. rerio* embryos at short (30 minutes) and long (2 days) exposure. The aim was to determine the concentration range allowing the evaluation of oxidative stress with the EPR technique in the *in vivo D. rerio* embryo model.

Acknowledgement: The research was performed under grant “Innovator incubator 2.0” from Technology Transfer Office, Medical University of Warsaw.

3. The Good, the Bad and the Ugly – histopathology in zebrafish - Karolina Naumowicz

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Faculty of Veterinary Medicine, University of Warmia and Mazury in Olsztyn, Poland

Histopathology in fish, especially in the zebrafish field, is often troublesome and its results can be more than confusing. It is considered a key endpoint in animal studies, the importance of which is often overlooked by research teams. This is explained by the common but erroneous statement that the basic techniques of pathology are completely standardized compared to modern molecular, biochemical, genetic and "omic" methods. In fact, many variables can influence the outcome of even "routine" pathological techniques, and failure to define these factors makes it impossible to properly evaluate the results.

The main traps of histopathology in zebrafish are inadequate experiment design, improper histological processing, inadequate data analysis, misinterpretation of the diagnosis, peer review by persons with insufficient specialist knowledge of the test species, model system or methods used, non-compliance with existing guidelines, and last but not least increasing pressure to publish quickly.

To increase the reliability of the histopathological assessment widely available guidelines were created. The international standards include guidelines for research planning "Animal Research: Reporting In Vivo Experiments" (ARRIVE), the "Gold Standard of the Publication Checklist" (GSPC), the Minimum Information for Publication of Experimental Pathology Data (MINPEPA), the global open Registry Nomenclature Information System (goRENI), the International Harmonization of Nomenclature and Diagnostic Criteria for Lesions (INHAND), the Registry of Industrial Toxicology Animal-data (RITA). These guidelines comprehensively cover study planning, publication elements and detailed technical aspects of the histopathological assessment.

4. Voltage-gated potassium channels as developmental regulators of inner ear - Justyna Jedrychowska

Justyna Jedrychowska, Eugene Gasanov, Vladimir Korzh

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Voltage-gated potassium channels selectively regulate transport of K^+ along electrochemical gradient in plasma membrane. They are involved in a variety of biological processes including mechanotransduction, protein transport, neuronal impulse, etc. The zebrafish *Kcnb1* encodes the electrically-active subunit of the slow-inactivating delayed rectifier (IK) Kv2.1, a member of the Kv2 subfamily of Kv channels expressed in the mammalian, *Xenopus*' and zebrafish's inner ear. Moreover, it is known that of all bodily fluids, a fluid of the inner ear – endolymph, has the highest concentration of K^+ . *Kcnb1* is expressed in cells lining the cavity of developing inner ear of zebrafish (Shen et al., Development, 2016). Hence, Kv2.1 could be important for ear development, where it may be required for proper hearing and spatial orientation. Using the loss-of-function *kcnb1* mutant, a role of this gene was studied during zebrafish development and a link between deficiency of *Kcnb1* and abnormal development and functioning of the inner ear has been shown. Ear of developing zebrafish *kcnb1*^{-/-} and its hearing stones, otoliths are small. The orientation of kinocilia of mutants' mechanosensory cells is affected. All these phenomena taken together could be responsible for behavioral defects in hearing and balance detected in *Kcnb1* morphants and mutants. *Kcnb1* plays an important role during development and function of zebrafish ear.

5. Role of antisense oligonucleotide-induced intronic miRNA on axonal morphogenesis - Savani Anbalagan

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Monogenic diseases due to haploinsufficiency are caused by reduced expression or deficient proteins. A promising approach to restore gene expression is via antisense oligonucleotides (ASO) targeting non-productive splice sites that can increase targeted gene expression. There are 1246 disease-associated genes whose expression can be potentially modulated via ASO's targeting non-productive splice sites. Although this is a straightforward approach, a major issue arises when the targeted gene is embedded with intronic miRNA. Whether ASO's targeting host genes embedded with intronic miRNA affect miRNA expression and function is unknown. While studying how glial cells regulate axonal morphogenesis in larval zebrafish, we made a serendipitous observation that ASO targeting host gene splice site disrupt developmental axonal morphogenesis and the phenotype can be rescued only by intronic miRNA knockdown. To the best of our knowledge, this is the first time an indirect effect of ASO-induced intronic miRNA dysregulation is observed. Our results call for a careful evaluation of ASO's when targeting host genes embedded with intronic miRNA.

Key words: Intronic miRNA, antisense oligonucleotides, axonal morphogenesis

6. Knockdown of tubgcp2 results in small head and cell cycle disruption of brain neural progenitor cells in zebrafish embryos - Dawidziuk Mateusz

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Nine year old boy with microcephaly and delayed psychomotor development was admitted to the Genetic Counselling of the Department of Medical Genetics at IMC. Exome sequencing revealed bi-allelic variants in TUBGCP2 gene encoding a protein that is part of a multiprotein complex responsible for microtubules nucleation. However, the involvement of this gene in the development of the central nervous system is yet unknown. Using GeneMatcher we found three additional families carrying possibly pathogenic variants in TUBGCP2 with similar phenotypes. This allowed for delineation of previously undescribed neurodevelopmental disorder. To investigate molecular mechanisms responsible for disruption of proper growth of the human brain caused by mutations in this gene, we investigated homologs of human TUBGCP2 in model organisms, *Danio rerio* and *Drosophila melanogaster*. Knockdown of tubgcp2 by morpholino revealed significant head reduction in 3 days post fertilization zebrafish embryos. This phenotype was rescued in more than 30% of embryos by coinjection of corresponding mRNA. Additionally, pH3 staining, similar to TUBGCP3 published data, revealed higher number of dividing cells in the brain of morphants in comparison to the wild type that suggests block of the cell cycle in the M phase. Also, developmental aberrations including larval brain were observed in *Drosophila melanogaster* mutant for either hypomorphic or amorphic alleles of Grip84, a homolog of TUBGCP2. Our data confirms that TUBGCP2 and its homologs, are necessary for proper brain development in human as well as zebrafish and fly, and that disruption of mitotic cell division is the likely cause of smaller head phenotype.

7. Neuroinflammation and sickness behavior in zebrafish infected with Tilapia lake virus - Miriam Mojzesz

Miriam Mojzesz¹, Magdalena Widziolek¹, Mikolaj Adamek², Urszula Orzechowska¹, Piotr Podlasz³, Tomasz Prajsnar¹, Niedharsan Pooranachandran¹, Anna Pecio⁴, Anna Michalik⁵, Win Surachetpong⁶, Magdalena Chadzinska¹, Krzysztof Rakus^{1*}

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In mammals, relationship between immune system and behavior is widely studied. In fish, the knowledge about the brain immune response and behavioral changes during viral infection in the brain is very limited. To investigate this subject, we used, developed in our laboratory, model of tilapia lake virus (TiLV) infection of larvae and adult zebrafish (*Danio rerio*). Our results demonstrated that zebrafish brain in both developmental stages is susceptible to TiLV infection. Moreover, we demonstrated that TiLV persist in the brain of adult zebrafish for at least 90 days, even when the virus is not detectable in other peripheral organs such as spleen, kidney and liver and induces pathological changes in the brain morphology. The gene

expression study demonstrated that TiLV induces strong anti-viral immune and inflammatory response and up-regulation of microglia/macrophages markers in the brain. Furthermore using zebrafish larvae and in vivo microscopy, we showed the activation of microglia during TiLV infection. Finally, we found that during TiLV infection zebrafish show clear sickness behavior: decreased locomotor activity and food intake and tend to stay in the bottom zone of aquaria. This is the first study presenting a comprehensive analysis of the brain immune response connected with microglia activation and subsequent sickness behavior during systemic viral infection of zebrafish. We believe that the extended knowledge of evolutionary conservation of important ligands and receptors involved in the regulation of the immune response within nervous system and in inducing behavioral changes will support hypothesis about the functional importance of these.

This work was supported by the National Science Centre of Poland under Sonata Bis 5 project (Grant number UMO-2015/18/E/NZ6/00516).

8. Modelling chemotherapy-associated cardiotoxicity in zebrafish: Pharmacological evaluation of doxorubicin-induced heart failure - Monika Maciag

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Although doxorubicin belongs to one of the most effective anticancer drugs, its use leads to life-threatening heart failure. Cardiotoxic properties of doxorubicin have been thoroughly studied in both rodent models and human patients. However, it remains unknown how the zebrafish heart responds to this well-known cardiotoxic compound. Modelling cardiac diseases in zebrafish has many advantages compared to the traditional murine model. Optical transparency and rapid development allow for visualisation of the circulatory system within individual animals even at the embryonic stages. Validation of drug-dependent cardiotoxicity profile is essential for further translation of zebrafish data to humans.

To determine whether both existing and novel therapies attenuate the doxorubicin-induced cardiotoxicity in zebrafish, we investigated the protective effects of β -blockers (metoprolol and carvedilol), angiotensin receptor blocker (valsartan), and dexrazoxane (the only clinically approved cardioprotective agent used in anthracycline-based anticancer therapy) against doxorubicin.

Zebrafish were exposed to both cardiotoxic and cardioprotective drugs from 24 to 96 hours post fertilisation. The compounds were compared regarding the elicited changes in cardiovascular parameters and morphological alterations using DanioScope (Noldus).

The zebrafish model reflects key aspects of doxorubicin-induced cardiotoxicity, i.e. reduction in heart rate, blood flow and vasoconstriction. The functional dysfunctions are accompanied by reactive oxygen species overproduction. Additionally, we show that the cardiotoxicity is mitigated by dexrazoxane and pharmacological inhibition of the angiotensin system, and to a lesser extent, the β -adrenergic system. In conclusion, our results support the value of the zebrafish model for studying cardiovascular disease, including in the cardio-oncology field.

The authors acknowledge financial support from the National Science Centre, Poland (Grant 2017/25/B/NZ7/02654).

9. Screening of endosulfan and its degradation products toxicity using the *Danio rerio* embryos – Patrycja Jesionkowska

Patrycja Jesionkowska, Katerina Makarova, Katarzyna Zawada, Paweł Siudem

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Endosulfan, an organochlorine insecticide, is an environmentally persistent pesticide. It poses health problems for people and aquatic animals. Endosulfan is still used in many countries, it is applied to crops like cashew or cotton in India and China despite the official ban.

In the present study *Danio rerio* (wild type, ABxTL) embryos were used as a model to compare xenoestrogenic properties of endosulfan alpha and beta, endosulfan sulfate, lactone, ether, alcohol, 1-hexene and methylcyclohexane (concentration from 1 to 100 µg/L). Embryos were exposed to the tested compounds from 24 to 72 hours post-fertilization. Endosulfan lactone 2,5 µg/l and 5 µg/l caused the biggest mortality, endosulfan beta 10 µg/l led to the biggest delay in the hatching rate. Endosulfan and its metabolites did not lead to significant differences in length of the 72 hpf larvae

We used molecular docking (*in silico*) to study the receptor-binding affinities of tested compounds to a number of zebrafish and human receptors (hER α , hER β , fER α , fERRyA, fERRyB, hGABA-A and hGABA-B). It revealed relatively low binding affinity of all studied compounds to fish estrogen receptors. However, all studied compounds have an affinity to GABA receptors, with the highest binding affinity obtained for the endosulfan alcohol and 1-hexene. Molecular docking results suggest that endosulfan and its metabolites induce toxicity through a different mechanism.

Zebrafish *in vivo* tests and *in silico* works gave complementary results in the assessment of toxicity and estrogenicity of the studied compounds and could be applied for toxicological studies of other xenoestrogens.

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10. Application of zebrafish model to unravel the involvement of *tbc1d24* gene in the development of autosomal dominant hearing loss – Anna Sarosiak

Sarosiak A.^{1,2}, Jędrychowska J.³, Oziębło D.^{1,2}, Leja M.^{1,2}, Gan N.¹, Bałdyga N.¹, Skarżyński H.⁴, Korzh V.³, Ołdak M.¹

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TBC1D24 genetic variants are causally involved in the development of both autosomal recessive hearing loss and epilepsy syndromes, and autosomal dominant hearing loss (ADHL). So far, our group revealed and published four novel ADHL-causative *TBC1D24* probably pathogenic variants by performing high-throughput genetic testing in families with ADHL. We aim to use zebrafish model for deciphering the pathogenicity of novel *TBC1D24* variants. Different methodological approaches were used to investigate the function of *tbc1d24* orthologue and its involvement in hearing in zebrafish, including (i) expression studies by whole mount *in situ* hybridization (WISH), qPCR and cryosections, (ii) assessment of the hair cell morphology by high-resolution imaging and (iii) behavioral studies in knock-down, overexpression and rescue *tbc1d24* models. The expression pattern of *tbc1d24* revealed by WISH in 24 hpf zebrafish embryos consists of clusters of primary neurons in the brain and spinal cord. Noteworthy is *tbc1d24* expression in the sensory cranial ganglia. In the later development, *tbc1d24* expresses more broadly in the brain and neural retina. The additional experiments will clarify the details of *tbc1d24* expression in the ear and adjacent neural ganglia. The morpholino-mediated knock-down and overexpression of *tbc1d24* caused no significant ear-specific morphological changes. The behavioral studies revealed changes in the locomotor activity upon *tbc1d24* morpholino-mediated knock-down at 24 hpf and 5 dpf. In contrast, the detrimental effect of overexpression of *TBC1D24* p.Asp185Asn variant on locomotor activity of 24 hpf larvae was detected. Further studies are needed to understand the role of *tbc1d24* in the zebrafish ear and nervous system.

PTZ webpage

www.zebrafish.org.pl