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Zebrafish Models

Biomarkers & Pathology in Zebrafish Models

- COPD
- Obesity
- Ataxia-Telangiectasia
- NF1
- Melanoma
- Optic Neuritis

3 Great Questions in ALS Discovery by Dr. John Dirk Vestergaard Nieland

Methods & Models Microglia

Translation Lessons from the Past

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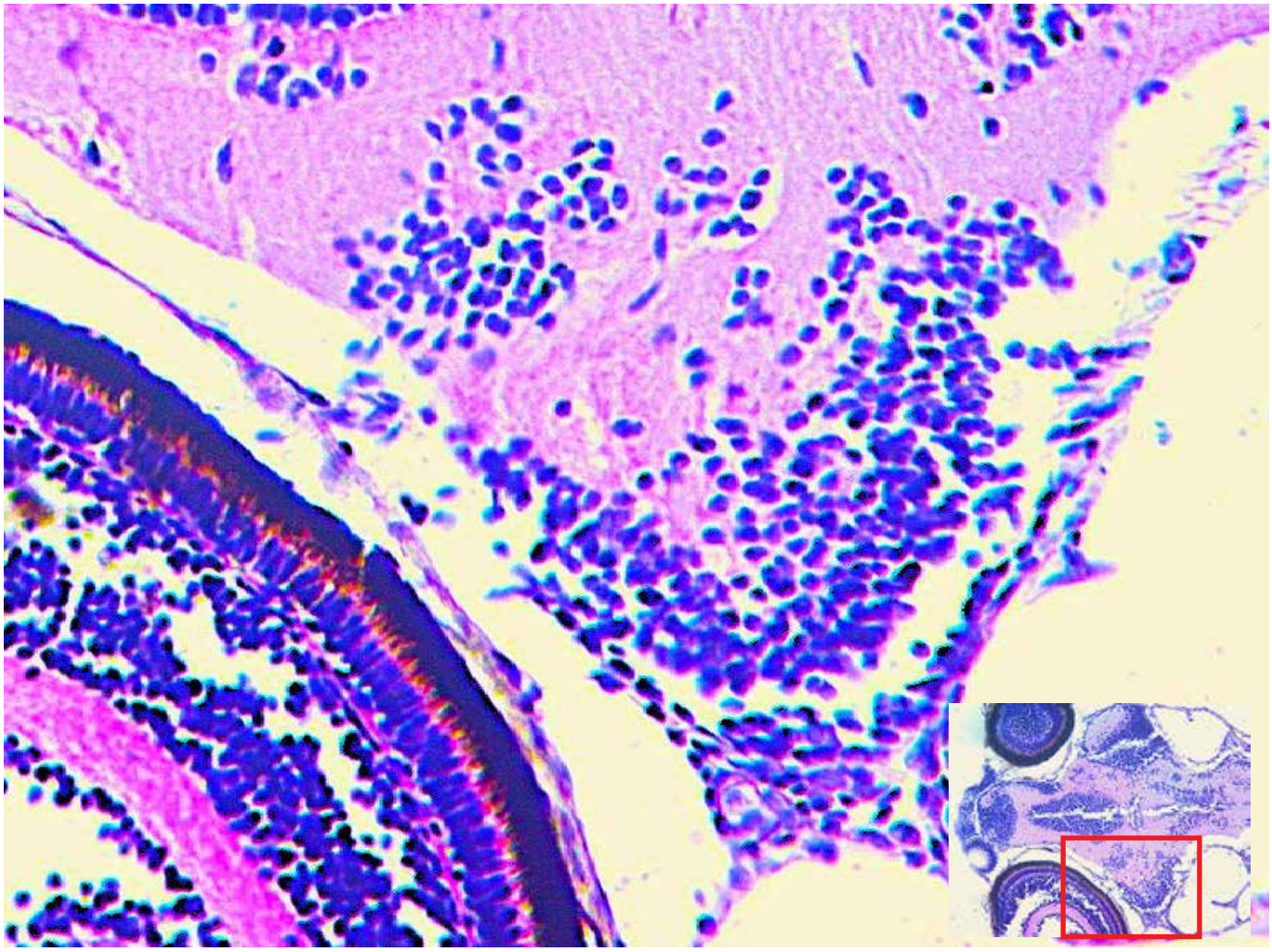

Histology section of ALS zebrafish model showing cytoplasmic vacuolization, a hallmark feature of human ALS pathology.

Monthly Exclusives from Pentagrit

FEATURED SCIENTIST *P3*

METHODS & MODELS - MICROGLIA *P6*

TRANSLATION LESSONS FROM THE PAST *P8*

BIOMARKERS & PATHOLOGY IN ZEBRAFISH MODELS *P11*

FEATURED SCIENTIST

Dr. John Dirk Vestergaard Nieland

Integrating findings across ALS models is essential for advancing translational research.

The Three Biggest Questions in ALS

Amyotrophic Lateral Sclerosis (ALS) is a progressive neurodegenerative disorder with a profound impact on patients and families. Despite decades of research, therapeutic options remain extremely limited. In Europe, only a single approved medication—riluzole, introduced nearly 30 years ago—offers a modest extension of life expectancy by approximately three months. In the United States, two medications are available, with the recent addition of a therapy targeting patients with SOD1 mutations. These realities underscore the formidable challenges inherent in ALS drug development. The persistent lack of effective, broadly applicable therapies highlights the urgent need for innovative approaches that address the underlying mechanisms of disease progression. Therefore, the first question is:

1. What causes ALS?

Approximately 10% of ALS cases are linked to genetic mutations, notably in SOD1, TDP-43, FUS, and C9ORF72. The remaining 90% are sporadic, with risk influenced by unidentified genetic factors and environmental stressors—psychological, physical, toxicological, pathological, and dietary. These stressors can trigger a shift from glucose to lipid metabolism in the brain, leading to mitochondrial dysfunction, oxidative stress, and inflammation. This disrupts myelin maintenance, impairs neuronal signaling, and accelerates neurodegeneration.

Importantly, new research displays that metabolic changes precede symptom onset by years, highlighting the need for early intervention targeting neurometabolic pathways in ALS.

2. Why is there no effective treatment for ALS?

Current ALS treatments focus on isolated symptoms such as hypoxia or neuronal loss, yet by diagnosis, multiple pathological processes including inflammation, protein misfolding, myelin degradation, signaling disruption, and mitochondrial dysfunction are already established. Targeting a single symptom is insufficient; effective therapy requires intervention at upstream disease mechanisms or simultaneous targeting of multiple pathways, ideally before clinical onset.

Metabolic inhibitors, particularly those modulating lipid metabolism, show promise. Neurometa Therapeutics is developing CPT1A inhibitors, inspired by the protective Arctic CPT1A mutation observed in both human and mouse studies. Early diagnosis remains challenging, but ongoing biomarker research is advancing this critical goal.

3. How can we more effectively test ALS drugs in pre-clinical models?

No single ALS model replicates the full spectrum of patient pathologies, necessitating the use of multiple models for comprehensive insight. Commonly used mouse models include those with SOD1, TDP-43, C9ORF72, and FUS mutations. SOD1 models lack TDP-43 aggregation but show downstream effects, while TDP-43 models exhibit aggregation yet miss key ALS features. C9ORF72 and FUS models often require environmental stressors to manifest disease, better reflecting sporadic ALS. Zebrafish models offer rapid disease progression and diverse pathology, complementing rodent studies. Integrating findings across these models is essential for advancing translational ALS research.

Microglia

BY SANDHIYA SEENIVASAN

Microglia, a specialized type of glial cell, serve as the primary immune defence mechanism within the central nervous system (CNS) and play a critical role in mediating neuroinflammatory responses. Zebrafish express similar mammalian microglial genes and are fully involved in addressing to detecting danger-associated signals, clearing apoptotic neurons, and modulating neuronal activity.

Methods:

Microglia in zebrafish larvae can be visualized using both **Hematoxylin and Eosin (H&E) staining** and **Immunohistochemistry (IHC)** techniques.

Zebrafish (Danio rerio) larvae at 9 days post-fertilization (dpf) are euthanized in 4% Tricaine methane sulfonate (MS-222) and immediately fixed in Dietrich's fixative for histological and immunohistochemical analysis of microglia. After fixation, tissues undergo a dehydration process through a graded ethanol series (70%, 80%, 95%, and 99%) followed by clearance in xylene to remove alcohol. The cleared tissues are then embedded in paraffin wax, oriented dorsal side up to optimize sectioning of neural tissues, such as the optic tectum and hindbrain regions. Serial 7 µm sections are cut using a rotary microtome.

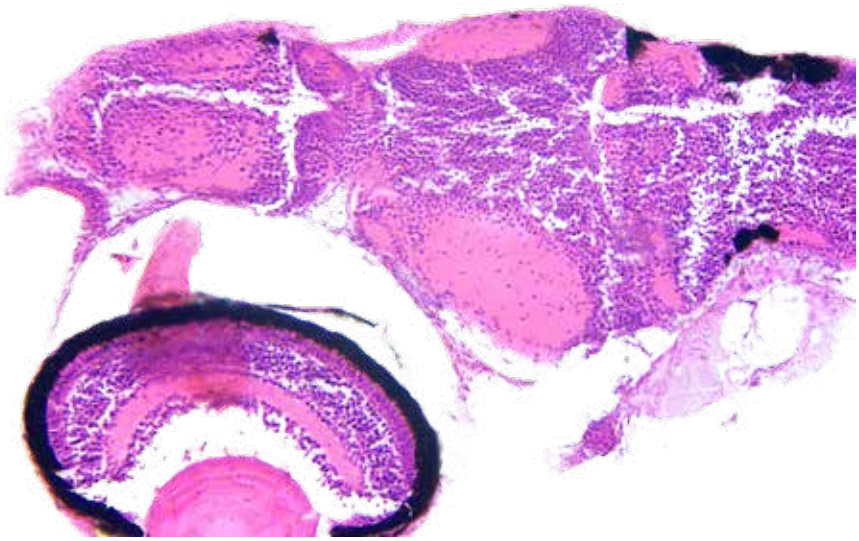

Figure 1 *presents a histological image of zebrafish larvae brain at 20X magnification under a light microscope, stained with Hematoxylin and Eosin*

Hematoxylin and Eosin (H&E) staining, paraffin-embedded sections are deparaffinized in xylene, rehydrated through a descending ethanol series, and then stained with hematoxylin for 30 seconds, followed by a 1-minute rinse in RO water and eosin for 2 minutes. After staining, the sections are dehydrated through an ascending ethanol series, cleared in xylene, and mounted using DPX mounting medium. Slides are then imaged under a light microscope at 20x and 40x magnification.

by incubating the sections with a blocking solution. The sections are then incubated at 4°C for 24 hours with an anti-Iba1 antibody (primary antibody), for microglia detection.

Following primary antibody incubation, the sections are washed with 1X TBS and then incubated with a secondary antibody, Donkey Anti-Goat IgG H&L, at room temperature for 30 minutes. After the secondary antibody incubation, the slides are washed and rehydrated using xylene. Finally, sections are imaged under a fluorescence microscope at 20X and 40X magnification, focusing on the central brain region as the primary area of interest.

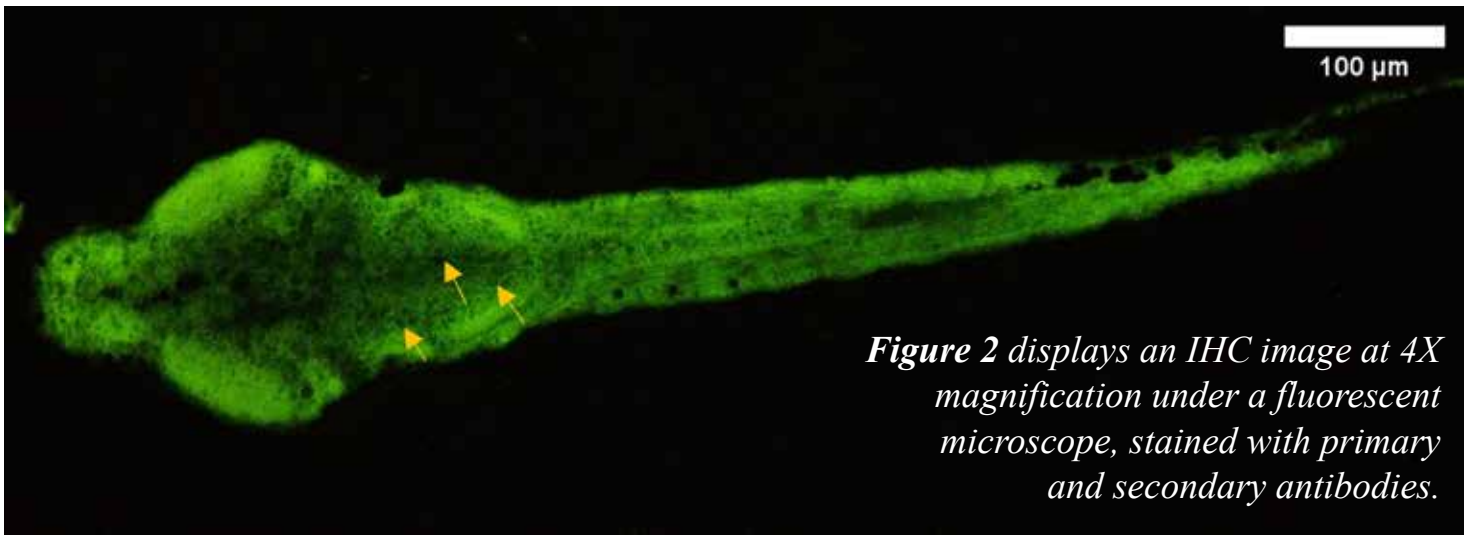

***Figure 2** displays an IHC image at 4X magnification under a fluorescent microscope, stained with primary and secondary antibodies.*

Immunohistochemistry (IHC) to detect microglia in zebrafish larvae is done first by deparaffinization of tissue sections and rehydration before proceeding with antigen retrieval. Heat-induced epitope retrieval (HIER) or enzymatic digestion is used to unmask antigenic sites.

Figure 3 *shows the experimenter observing the IHC slides of microglial cells under fluorescent microscope.*

About The Author

Sandhiya serves as a Research Assistant at Pentagrit Zebrafish CRO, actively working on neurological zebrafish models.

GET IN TOUCH

How can microglia quantification help my research?

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TRANSLATION LESSONS FROM THE PAST

BY KEERTHANA RAJENDRAN

Clinical Performance of Dalzanemdor:

Dalzanemdor, a positive allosteric modulator of the NMDA receptor, was developed to improve cognition in neuro- degenerative diseases such as Alzheimer's, Parkinson's, and Huntington's. Despite a rational and favourable safety profile, clinical trials consistently failed to demonstrate improvements on Symbol Digit Modalities Test (SDMT). SMDT is a neuropsychological screening tool that assesses cognitive processing speed, attention, visual scanning, and motor speed. Across all tested populations, dalzanemdor did not yield any measurable cognitive benefits. This outcome highlights that despite the use of a robust cognitive assessment tool the drug failed clinical trials. It does create the need for more predictive and functional evaluation in drug development.

Constraints in Translational Approaches:

The following key factors are likely to be contributing to dalzanemdor's lack of efficacy:

- **Endpoint Mismatch:** Clinical trials often measure limited cognitive functions, such as processing speed while leaving the functional deficits unassessed.
- **Assumptions About Cross-diseases:** NMDA receptor modulation was thought to provide uniform cognitive rescue for Huntington's, Parkinson's, and Alzheimer's diseases, ignoring the disease specific heterogeneity.
- **Limited Early Functional Evaluation:** The target engagement alone is not sufficient to measure all the cognitive outcomes, shows the importance of functional phenotype assessment in early stages of drug development.

FUNCTIONAL SCREENING IN ZEBRAFISH:

Cognitive and Behavioural Readouts:

Zebrafish models show different learning and memory behaviours, including **T-maze navigation, predator avoidance, novel object recognition, associative learning and spatial memory.**

These assays enable to evaluate diverse cognitive functions in real time. By utilziing these types of approaches to identify compounds that may show inconsistent effects on cognitive and executive functions such as learning and memory before advancing to clinical trials.

Disease-Relevant Genetic Models:

Genetic models representing key features of neurodegenerative diseases:

- APP and tau for Alzheimer's disease
- Pink1 for Parkinson's disease
- HTT mutants for Huntington's disease

Testing compounds by using these models allows evaluation of disease specific efficacy of drugs, which provides early translational insights.

Lessons from Dalzanemdor's Development:

Several key lessons emerge:

Future Directions/ Strategic Outlook:

To enhance the potential of preclinical screening for Cognitive drugs:

- Integrating zebrafish cognitive tests which includes multiple functional areas should be applied.
- Utilizing disease specific models to compare the efficacy of drugs across specific models such as Alzheimer's, Parkinson's and Huntington's diseases.
- Inclusion of zebrafish model that carries human receptors to measure target modulation

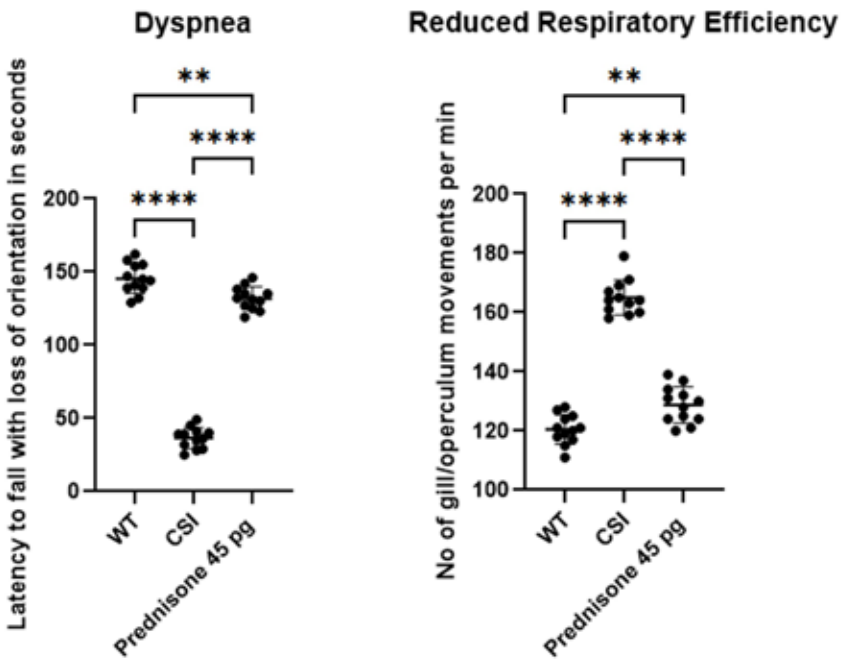
About The Author

Keerthana serves as a Research Assistant at Pentagrit Zebrafish CRO, actively working on translational research in zebrafish models.

THERAPEUTIC PATHOLOGY & BIOMARKERS In Zebrafish

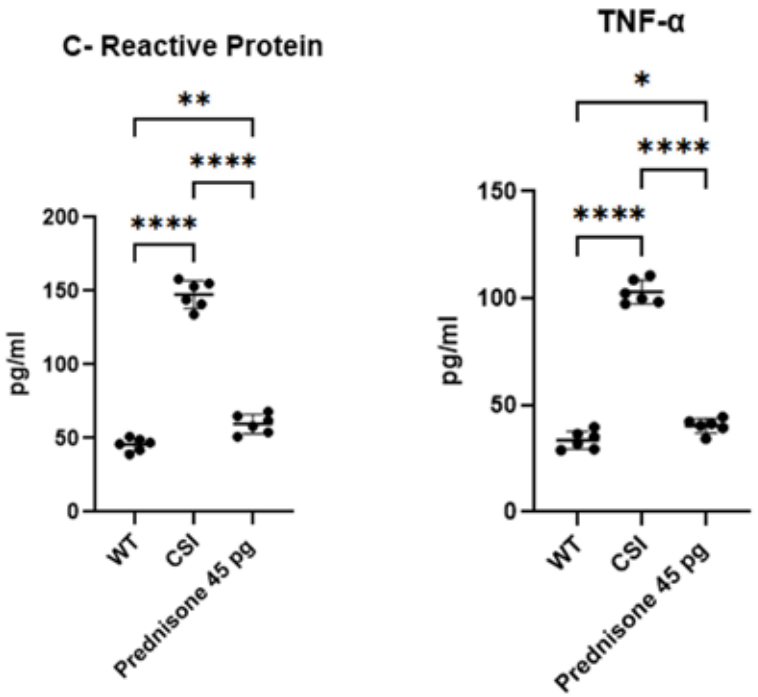
Inflammation - COPD

Model - Cigarette Smoke Induction



Pathology:

Zebrafish models with respiratory dysfunction exhibit **dyspnea** and impaired oxygen exchange, leading to **reduced respiratory efficiency.** Prednisone (45 pg) alleviates inflammation, improves gill function, and enhances oxygen uptake, restoring normal breathing patterns.

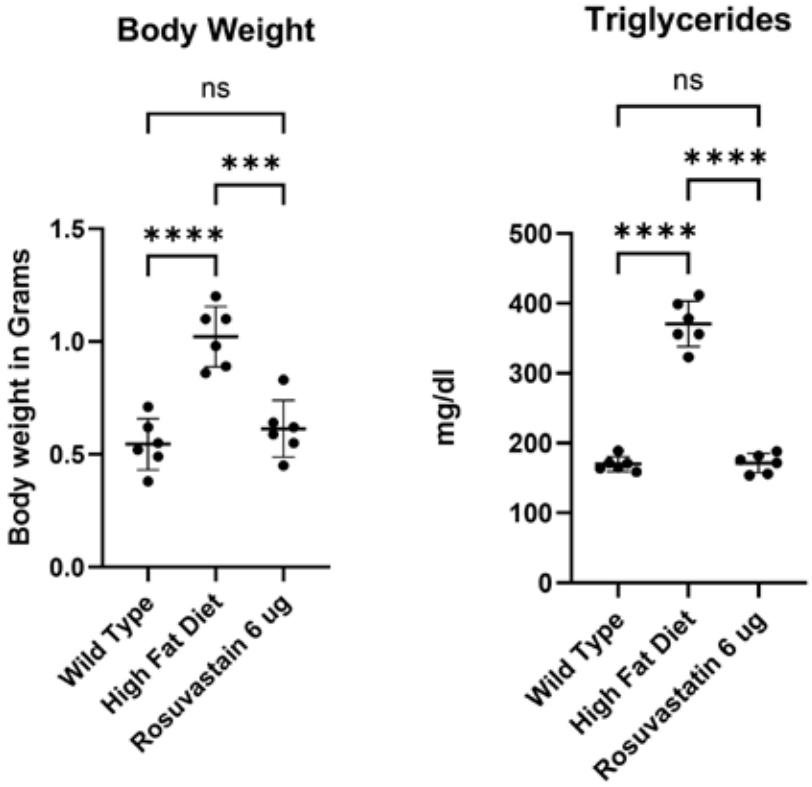


Biomarkers:

C-Reactive Protein (**CRP**) and **TNF-α** are elevated, promoting inflammation and tissue damage. Prednisone (45 pg) suppresses their expression, reducing inflammatory stress, stabilizing tissues, and improving overall respiratory function and breathing efficiency.

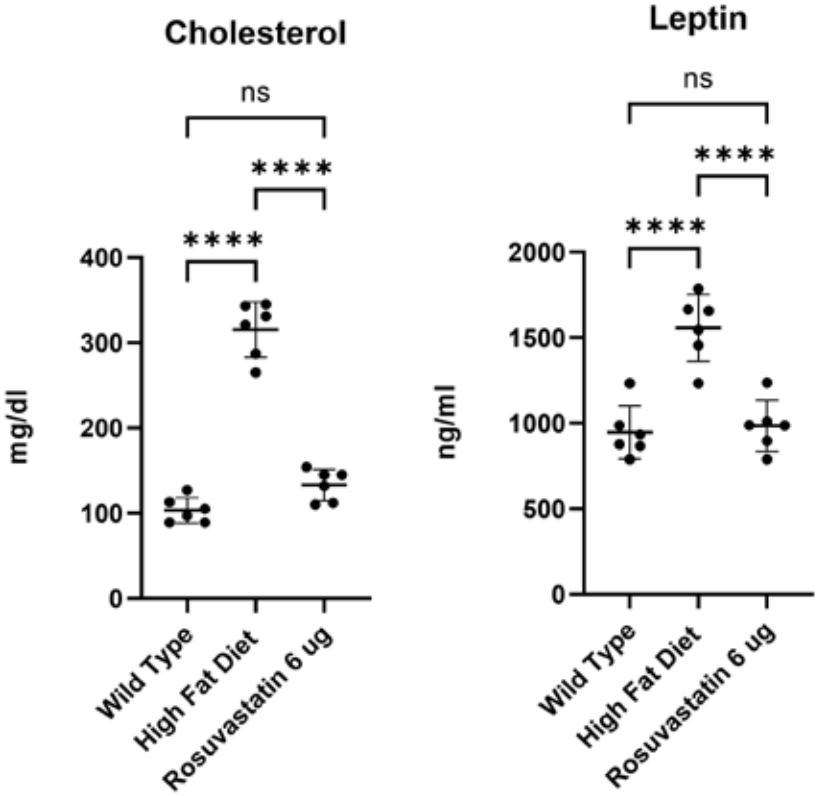
Metabolic - Obesity

Model - High Fat Diet



Pathology:

High-fat diet zebrafish show marked **body weight** gain due to excessive lipid accumulation and elevated **triglyceride** levels, reflecting disrupted lipid metabolism and metabolic stress. Rosuvastatin (6 µg) improves lipid handling, reduces adiposity, lowers triglycerides, and protects against obesity-related metabolic and cardiovascular complications.

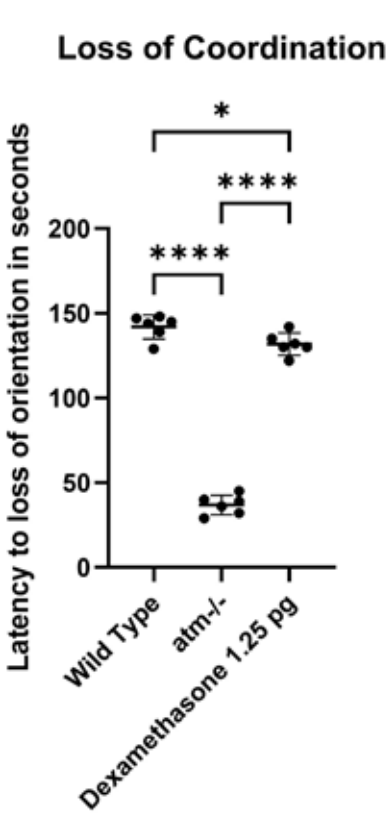


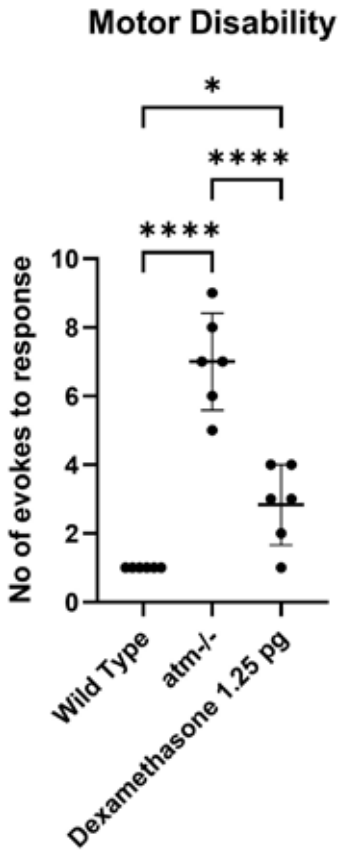
Biomarkers:

Cholesterol levels are elevated in high-fat diet zebrafish, contributing to lipid imbalance and metabolic dysfunction. **Leptin** levels rise due to increased adiposity, causing leptin resistance. Rosuvastatin (6 µg) reduces cholesterol synthesis, lowers leptin, restores leptin sensitivity, and improves overall metabolic health and energy balance.

Rare - Ataxia Telangiectasia

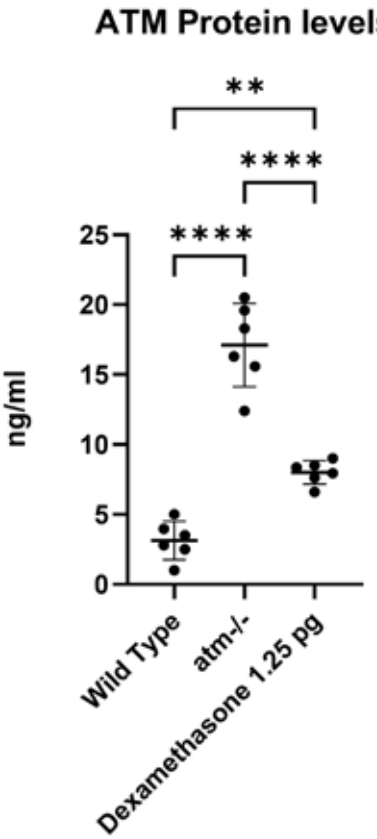
Model - Atm-/-

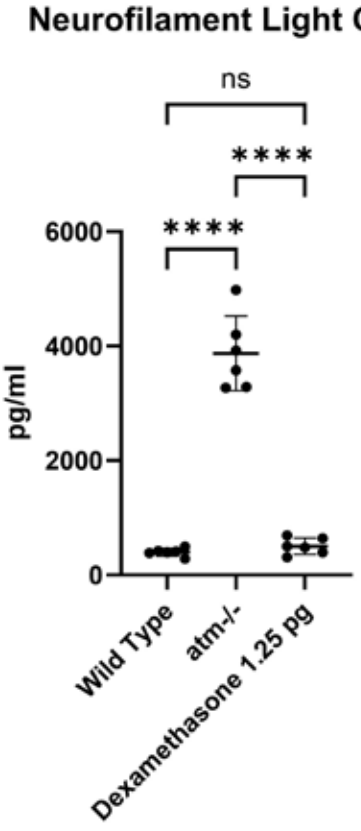




Pathology:

Atm-/- zebrafish exhibit impaired touch-evoked response due to neuronal degeneration and defective DNA repair, resulting in **loss of coordination** and **motor disability.** Dexamethasone (1.25pg) reduces neuroinflammation, supports neuronal survival, and partially restores motor function and sensory responsiveness in the model.



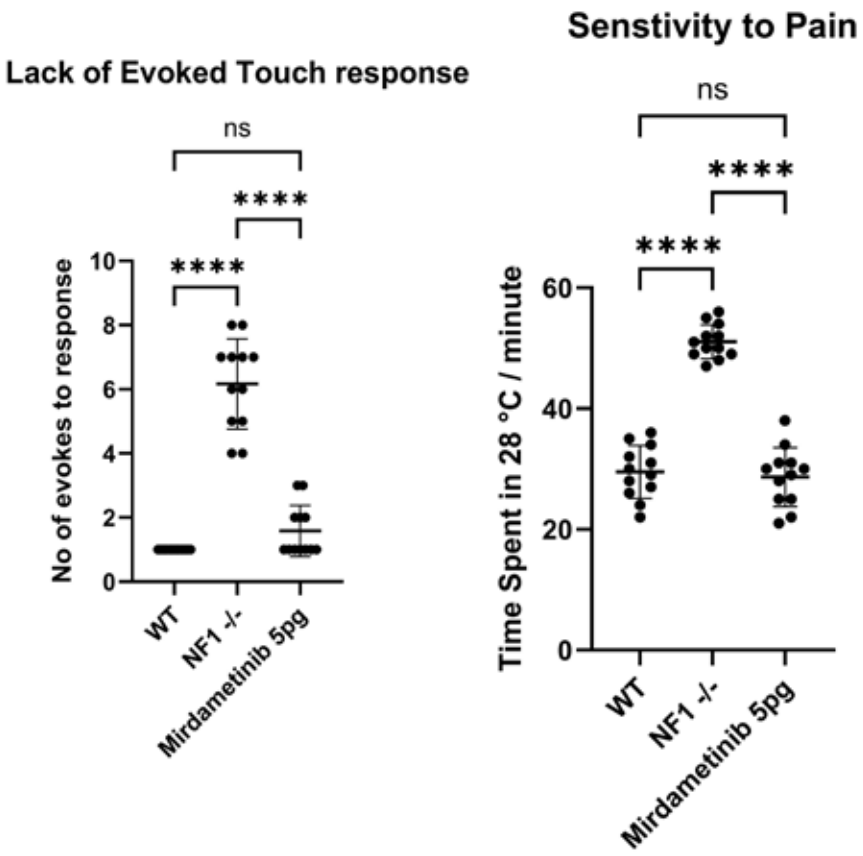


Biomarkers:

ATM protein levels are significantly reduced in Atm-/- zebrafish, impairing DNA repair and contributing to neuronal degeneration. **Neurofilament Light Chain** levels are elevated, indicating axonal damage. Dexamethasone (1.25pg) supports ATM activity, reduces neurofilament release, and protects neurons, improving motor and sensory function.

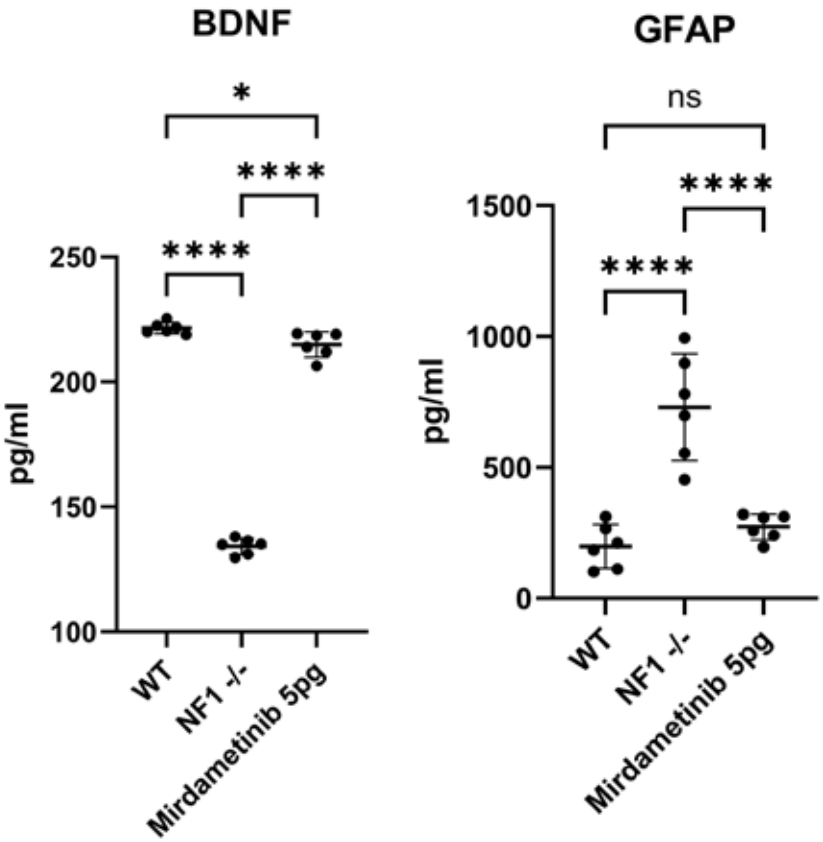
Neuro – Neurofibromatosis Type 1

Model - NF1-/-



Pathology:

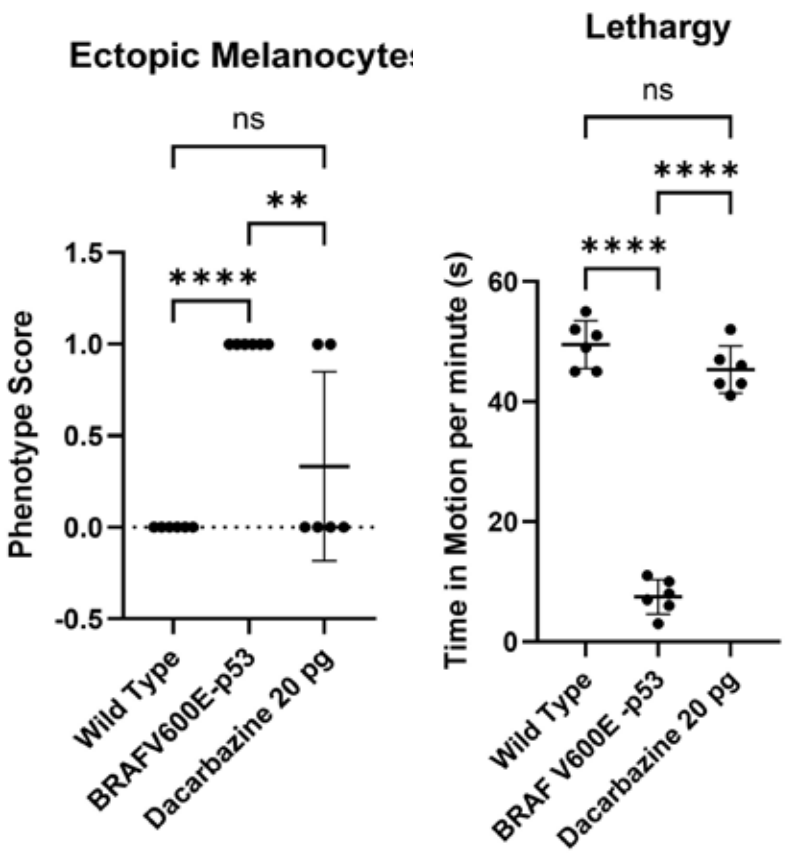
NF1-/- zebrafish exhibit impaired **touch-evoked responses** and heightened **pain sensitivity** due to neuronal hyperexcitability and MAPK/ERK pathway dysregulation. Mirdametinib (5pg) modulates signaling, reduces neuronal stress, and partially restores sensory function, improving tactile response and pain perception in the model.



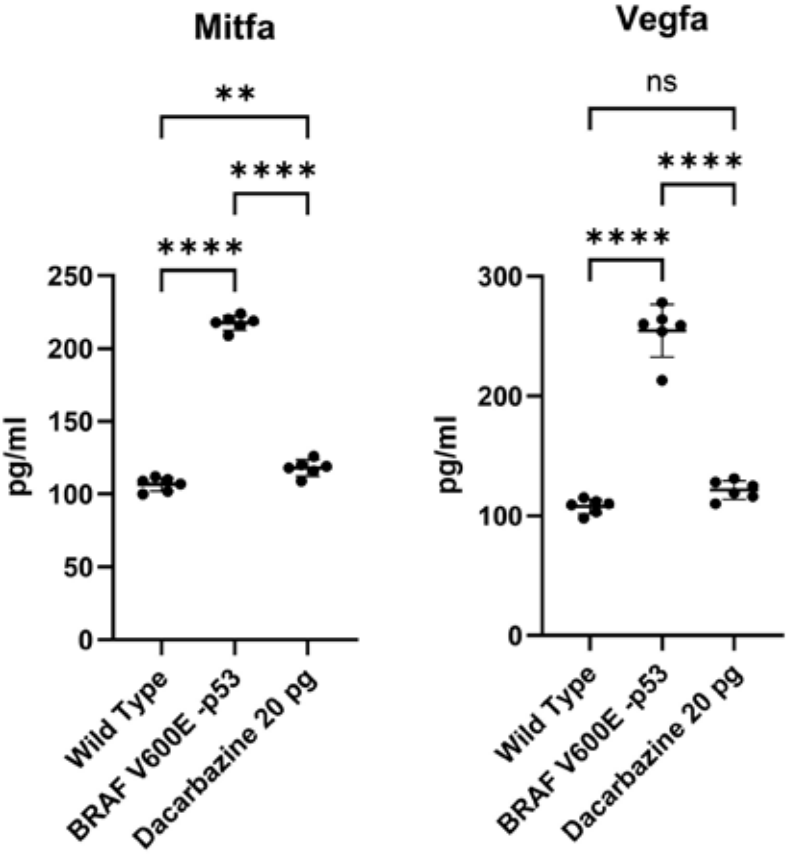
Biomarkers:

BDNF levels are dysregulated in NF1-/- zebrafish, impairing neuronal growth, survival, and synaptic function. **GFAP** is elevated, indicating glial activation and neuroinflammation. Mirdametinib (5pg) restores BDNF expression, reduces GFAP levels, and supports neuronal health, synaptic plasticity, and overall sensory function in the model.

Tumor - Melanoma
Model - BRAF^{V600E}-p53

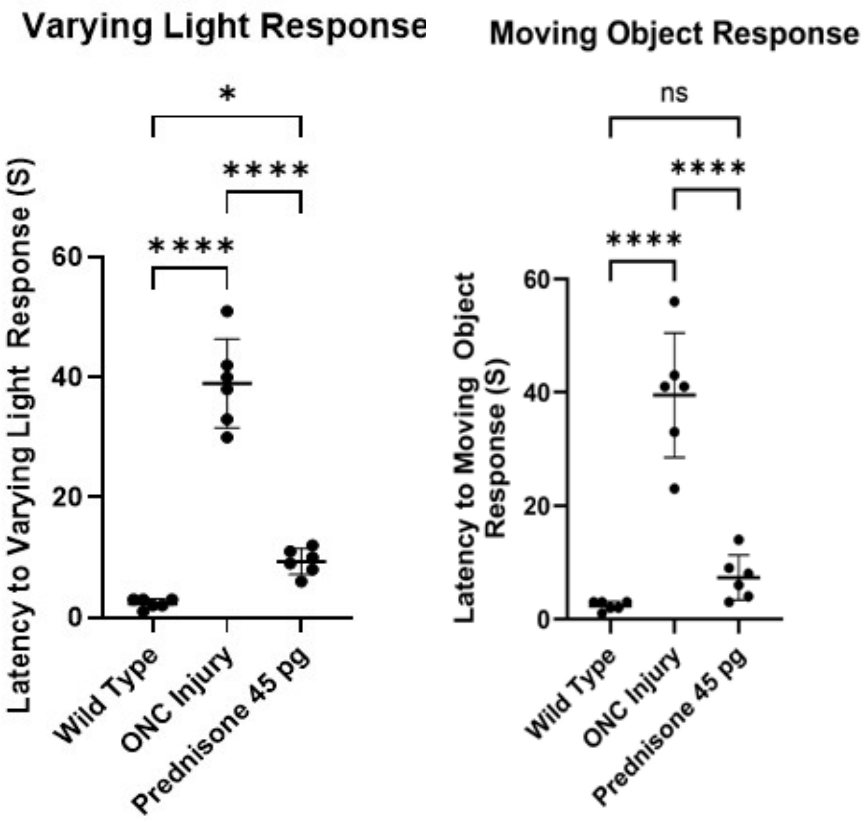


Pathology:
BRAF^{V600E}-p53 zebrafish exhibit **ectopic melanocyte** proliferation, disrupting tissue architecture, and reduced locomotor activity due to tumor burden and cellular stress. Dacarbazine (20pg) limits abnormal melanocyte growth, alleviates tumor-induced stress, and partially restores normal locomotion and energy levels in the model.

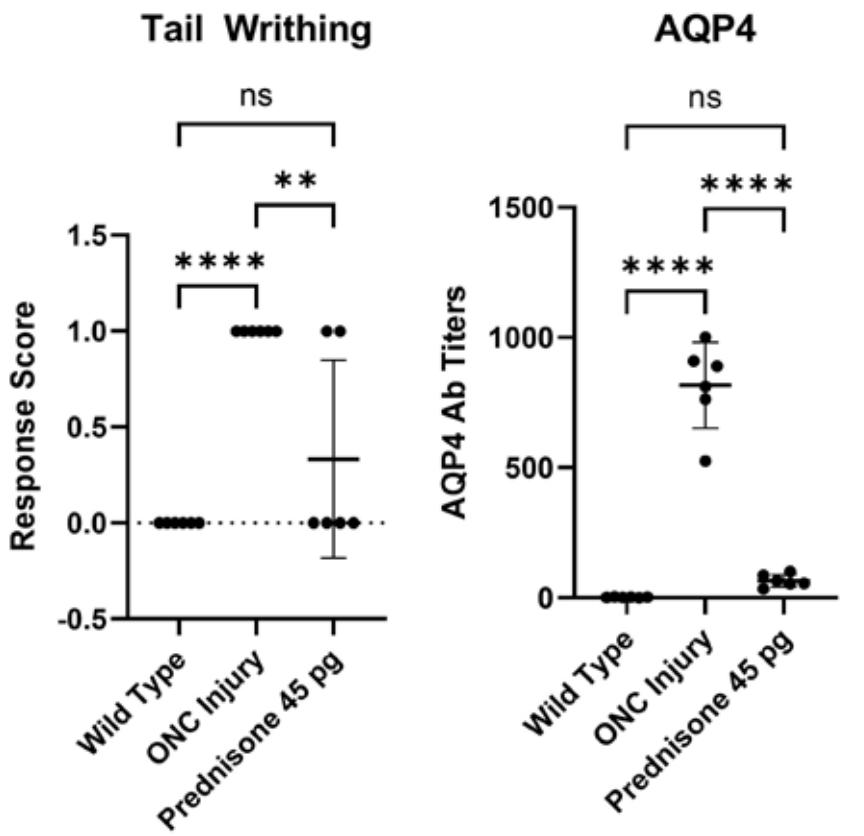


Biomarkers:
Mitfa expression is elevated in BRAF^{V600E}-p53 zebrafish, promoting melanocyte proliferation, while **VEGFA** is upregulated, driving angiogenesis and tumor progression. Dacarbazine (20pg) reduces Mitfa and VEGFA levels, limiting melanocyte overgrowth, inhibiting vascularization, and controlling tumor development in the model.

Eye – Optic Neuritis
Model - ONC Injury



Pathology:
ONC-injured zebrafish exhibit impaired **light sensitivity** and reduced **responsiveness to moving objects** due to retinal neuron and optic nerve damage. Prednisone (45pg) reduces inflammation, protects neurons, and partially restores visual function, improving both light perception and motion detection in the model.



Biomarkers:
Tail writhing phenotype reflects visual impairment and neuronal distress in ONC-injured zebrafish, while **AQP4** is upregulated, causing retinal edema and tissue stress. Prednisone (45pg) reduces tail writhing, normalizes AQP4 expression, alleviates inflammation, and supports retinal neuron survival and improved visual function.

CONTACT US

TOP 3 CSO Questions this Quarter.

1.How do we know long term safety of our drug and prevent an adverse effect scenario in clinical trials?

2.I got a few good compounds and need to select the best one for clinical development?

3.Can zebrafish tell me the outcome of a patient taking our drugs for more than 2 years?