

**MARCH**

**2026**

Vol. 3 No.1

# **Zebrafish** Models

## **Phenotype & Biomarkers in Zebrafish Models**

Asthma

Type 2 Diabetes

Pompe Disease

Multiple Sclerosis

Acute Myeloid Leukemia

Stargardt Disease

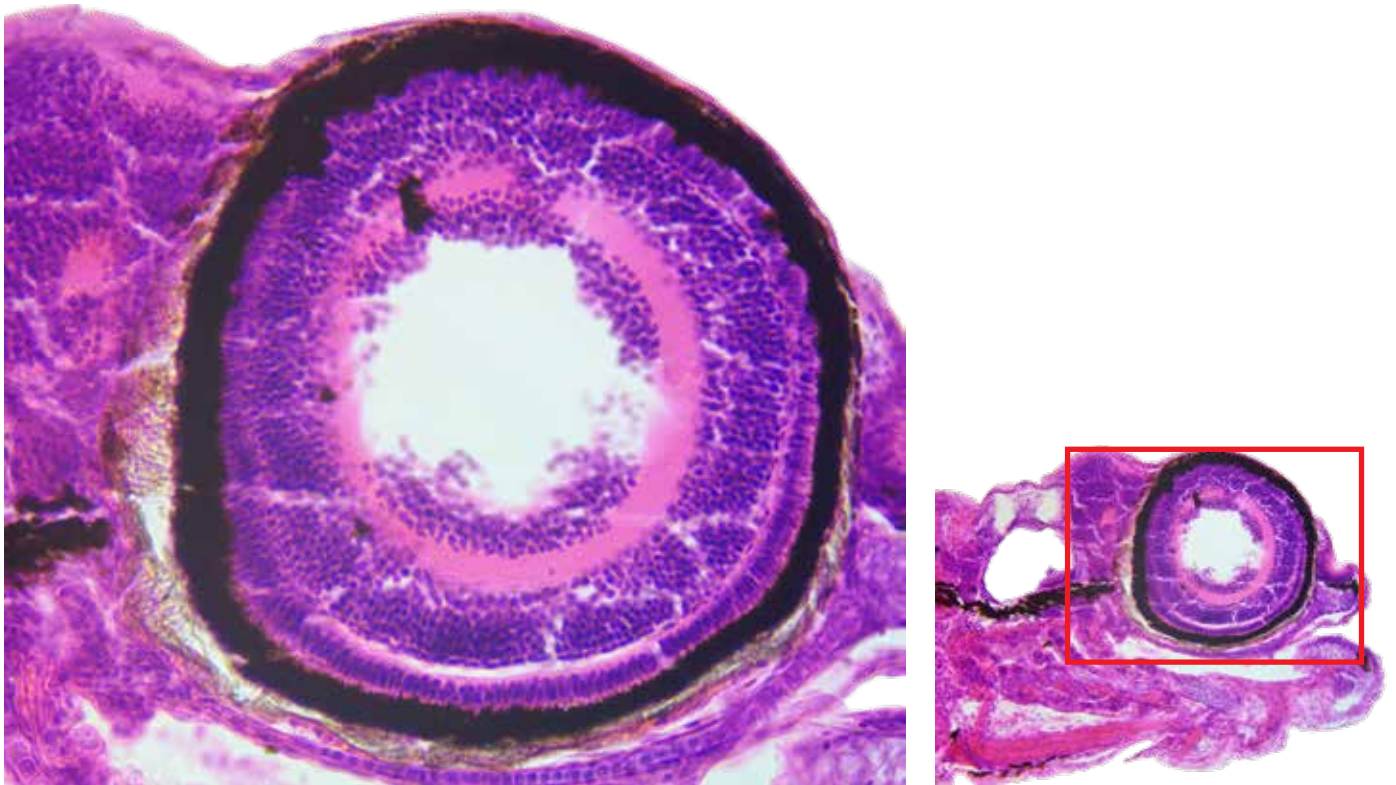
**3 Great Questions in Lung Cancer Biomarkers  
by Dr. Uppala Radhakrishnan, PhD**

## **Methods & Models Black Shade Identification**

## **Translation Lessons from the Past**

# **PENTAGRIT**

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Histology section of eye in zebrafish model showing presence of abnormal RPE cells (right, zoomed out) along with degeneration and thinning of the photoreceptor layer, majorly the outer nuclear layer (left, zoomed in).

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## Monthly Exclusives from *Pentagrit*



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# FEATURED *SCIENTIST*



**R**esistance is the shadow that follows every breakthrough. It is not only written in genes, it is etched in epigenetics, shaped by the environment, and woven into the tumor's living ecosystem.

**- Dr. Uppala Radhakrishnan, PhD**

# The Three Great Questions in Lung Cancer Biomarkers

Lung cancer is a highly heterogeneous malignancy that continues to impose a significant global health burden on healthcare systems. In many regions, standard treatments including chemotherapy, radiation and targeted therapy offer only limited improvements in overall survival, often accompanied by the development of resistance. Recently, the introduction of immunotherapies has improved outcomes for a subset of patients. However, these benefits are not universal and depend on specific molecular and immunological profiles. The persistent variability in treatment response and the lack of effective therapies highlight the urgent need for innovative strategies that address tumor heterogeneity, resistance mechanisms and disease progression. Therefore, the first question is:

## **Q1. Advancing lung cancer biomarkers: what's next?**

Lung cancer remains a relentless opponent, evolving faster than our ability to track its shifting biology. Established biomarkers such as EGFR, ALK, ROS1, KRAS, and PD L1 have guided therapy for years, yet they reveal only fragments of a larger, more dynamic story. Traditional biopsies provide static snapshots while the tumor adapts in real time.

The future lies in continuous, multidimensional profiling. Liquid biopsies that capture circulating DNA, exosomal RNA, and proteins

are reshaping practice, though sensitivity and breadth must advance. True progress will come from weaving together multiple layers of insight including methylation, fragmentomics, proteomics, metabolomics, and single cell analysis, ideally from a single, minimally invasive blood draw. This approach creates a living portrait of tumor evolution rather than isolated moments.

Beyond science, the promise is deeply human. Real time biomarker intelligence can reveal resistance before symptoms appear and guide therapy before cancer

## About the Featured Scientist

**Dr. Uppala Radhakrishnan, PhD**, cancer biomarker and precision medicine expert based out of United States, with extensive experience in translational oncology research. His work focuses on the discovery and validation of molecular biomarkers that enable early cancer detection, prognosis, and prediction of therapeutic response.

He has been actively involved in developing biomarker driven strategies that support targeted therapies and improve clinical outcomes. His research aligns with the growing emphasis on precision medicine, where disease specific molecular insights are used to optimize diagnosis and therapy.

adapts. By anticipating the tumor's moves, we edge closer to making lung cancer predictable, controllable, and outmaneuverable.

### **Q2. Cracking adaptive resistance with biomarkers.**

Resistance is the shadow that follows every breakthrough. It is not only written in genes, it is etched in epigenetics, shaped by the environment, and woven into the tumor's living ecosystem. Today, cutting edge technologies such as single cell RNA sequencing, spatial transcriptomics, and nanopore methylome profiling are peeling back these layers, reveal

ing lung cancer not as a static disease but as a system in constant motion.

One of the most striking revelations is the way biomarkers shift under therapeutic pressure. The tumor microenvironment does not simply endure treatment, it pushes back. After PD1/PDL1 therapy, immune suppression markers surge. Under KRAS inhibition, stromal signatures rise like a defensive wall.

Tracking resistance in real time lets clinicians see it before it appears in the clinic. The challenge is not discovery but translation,

turning insights into predictive models that guide therapy. The promise is clear: resistance becomes a forecast we can act on, not a surprise.

### **Q3. When biomarker-guided therapies fail: what's next?**

When targeted therapies such as EGFR, KRAS, ALK inhibitors and immunotherapies falter, it is rarely because the science was wrong. It is because cancer found another path. Emerging clones bypass the original biomarker, and current assays often detect them only after progression has already taken hold.

What is missing is relentless surveillance and predictive modeling. Ultra sensitive ctDNA monitoring, full length RNA profiling, and machine learning driven mutation forecasting can illuminate resistance before it strikes. Yet detection alone is not enough.

We must also validate function through organoid cultures, ex vivo drug screens, and AI powered drug target predictions to ensure every mutation translates into actionable therapy.

The future is multiplexed targeting with bi specific and tri specific therapies, adaptive regimens that evolve alongside the tumor, and treatment algorithms guided by continuous biomarker monitoring. This is not just science, it is strategy. It is the difference between reacting to cancer and staying ahead of it.

## Black Shade Identification

BY SANDHIYA SEENIVASAN



Black Shade Identification assay is employed to assess visual perception, contrast sensitivity, and exploratory behavior in zebrafish larvae. This behavioral paradigm evaluates the integrity of the visual system by leveraging the innate preference of larvae for high-contrast environments and their natural avoidance of dark zones (negative phototaxis). The light/dark preference test has been validated as an anxiety measure in zebrafish larvae.

### 1. Subject Preparation

Zebrafish larvae are maintained under standard laboratory conditions ( $27 \pm 1$  °C, 14:10 h light/dark cycle) in larvae medium. For the screening N=24 larvae randomly selected and collected per experimental group.

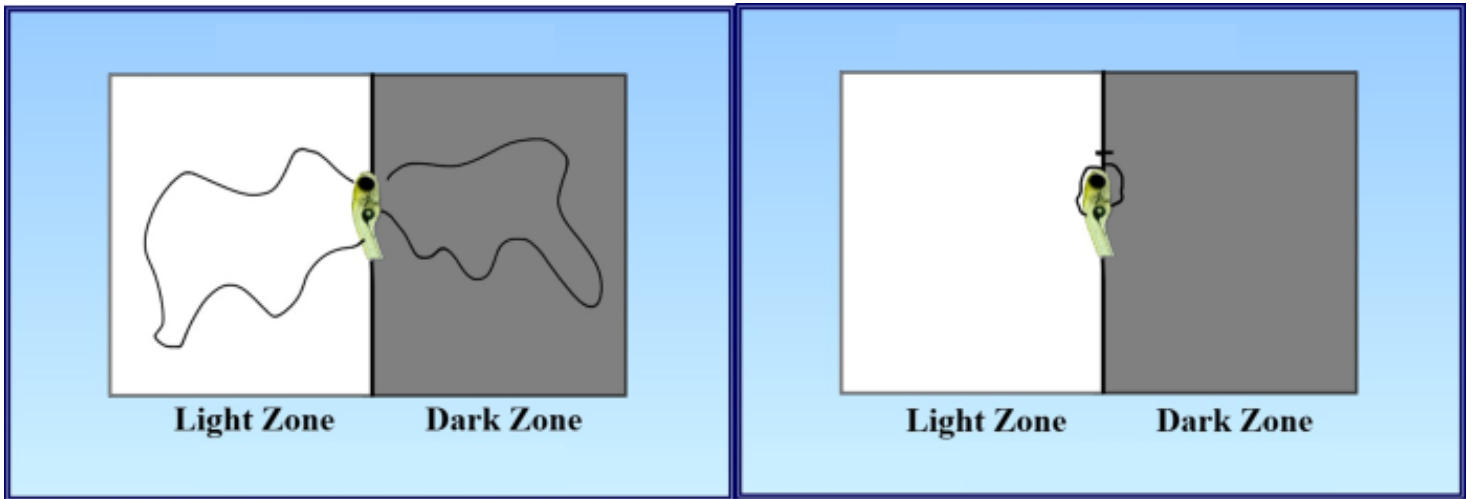
### 2. Experimental Tank set up

A behavior recording tank (spherical shaped) is prepared with clearly demarcated black and white zones to assess the larvae's ability to distinguish and respond to varying contrast levels.

### 3. Behavioral Procedure

The assay is conducted in two distinct phases:

- **Acclimation Phase:** Larvae are individually placed in the centre of the experimental tank for a 1-minute acclimation period. During this time, external stimuli (noise, vibration, and ambient light fluctuations) are maintained to allow the subjects to orient themselves to the environment.
- **Test Phase:** Following acclimation, larvae are allowed to freely explore the black and white zones for a 2-minute recording period and observed for zone preference.



*Figure 1 shows the Schema of Phenotype Screening Set up for Black Shade Identification Assay*



*Figure 2 shows the Experimenter performing the Black Shade Identification Assay in Zebrafish Larvae*

## Behavioral Observation and Interpretation

**White Zone Preference** - Indicates intact visual processing, normal contrast sensitivity, and functional negative phototaxis.

**Random Movement or more time in dark zone** - Suggests a failure to distinguish contrast boundaries, often associated with optic nerve damage or retinal degeneration.

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### ABOUT THE AUTHOR

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

#### GET IN TOUCH

How do you determine rescue of vision defects in black shade identification ?

EMAIL US AT:  
sandhiya@pentagrit.com

# TRANSLATION LESSONS FROM THE PAST

BY KEERTHANA RAJENDRAN

## Clinical Performance of Latozinemab



**L**atozinemab, monoclonal antibody, was developed to increase progranulin levels for the treatment of Frontotemporal Dementia (FTD) and other neurodegenerative diseases.

Although, the drug has a favorable safety profile, the clinical trials did not show any improvements on cognitive and functional endpoints which includes the Clinical Dementia Rating Sum of Boxes.

Latozinemab successfully increased plasma and cerebrospinal fluid progranulin levels, but this biochemical effect did not reflect in cognitive rescue. This outcome highlights the limitations of dependency on biomarker modulation without functional evaluation in drug development.

### Constraints in Translational Approaches:

The following key factors are likely to be contributing to Latozinemab lack of efficacy:

- **Biomarker and Endpoint mismatch:** While progranulin levels increased, the clinical endpoints measured are mostly limited which does not capture disease modifying effects.
- **Disease heterogeneity:** FTD can be genetically heterogeneous, yet Latozinemab was thought to provide uniform benefits regardless of underlying mutation and disease subtype.

- **Limited early functional assessment:** Initial drug development focused on target engagement rather than functional rescue and behavioral phenotypes which highlights the need of early functional assessment.

## FUNCTIONAL SCREENING IN ZEBRAFISH:

### Cognitive and Behavioural Readouts:

**Z**ebrafish models demonstrate wide range of learning and memory associated behaviors: **T-maze navigation, novel object recognition, social preference, predator avoidance, spatial learning and associative learning.** Utilizing these assays to identify compounds that show inconsistent effects on cognitive and behavioral can provide more predictive preclinical screening before advancing to clinical trial.

These assays provide quantifiable endpoints such as latency, accuracy and exploration time allowing precise assessment of subtle cognitive changes. Many of these assays are high throughput and automated, making them suitable for early stage drug screening.

In addition, zebrafish exhibit reproducible behavioral responses, enabling detection of both therapeutic effects and potential off target effects, further supports tracking of disease progression and treatment response over time, improving the translational relevance of preclinical findings.

### Disease Relevant Genetic Models:

**G**enetic zebrafish models relevant for progranulin associated neurodegenerative diseases include:

- **Grn knockdown or knockout** for progranulin deficiency in FTD.
- **Tau and TDP 43 models** for pathological protein aggregation.

# Lessons from Latozinemab's Development:

Several key lessons emerge:



## Future Directions :

To improve potential of preclinical screening for FTD and other neurodegenerative diseases:

- Integrating zebrafish cognitive and social behavior assays which includes multiple functional domains.
- Utilizing disease specific genetic models (Grn, Tau, TDP-43) to test efficacy across different pathological mechanisms.

- Inclusion zebrafish model that carries humanized receptors to directly assess target modulation alongside functional read-outs.

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### ABOUT THE AUTHOR

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

### GET IN TOUCH

Learn how zebrafish can help to classify your in vitro compounds?

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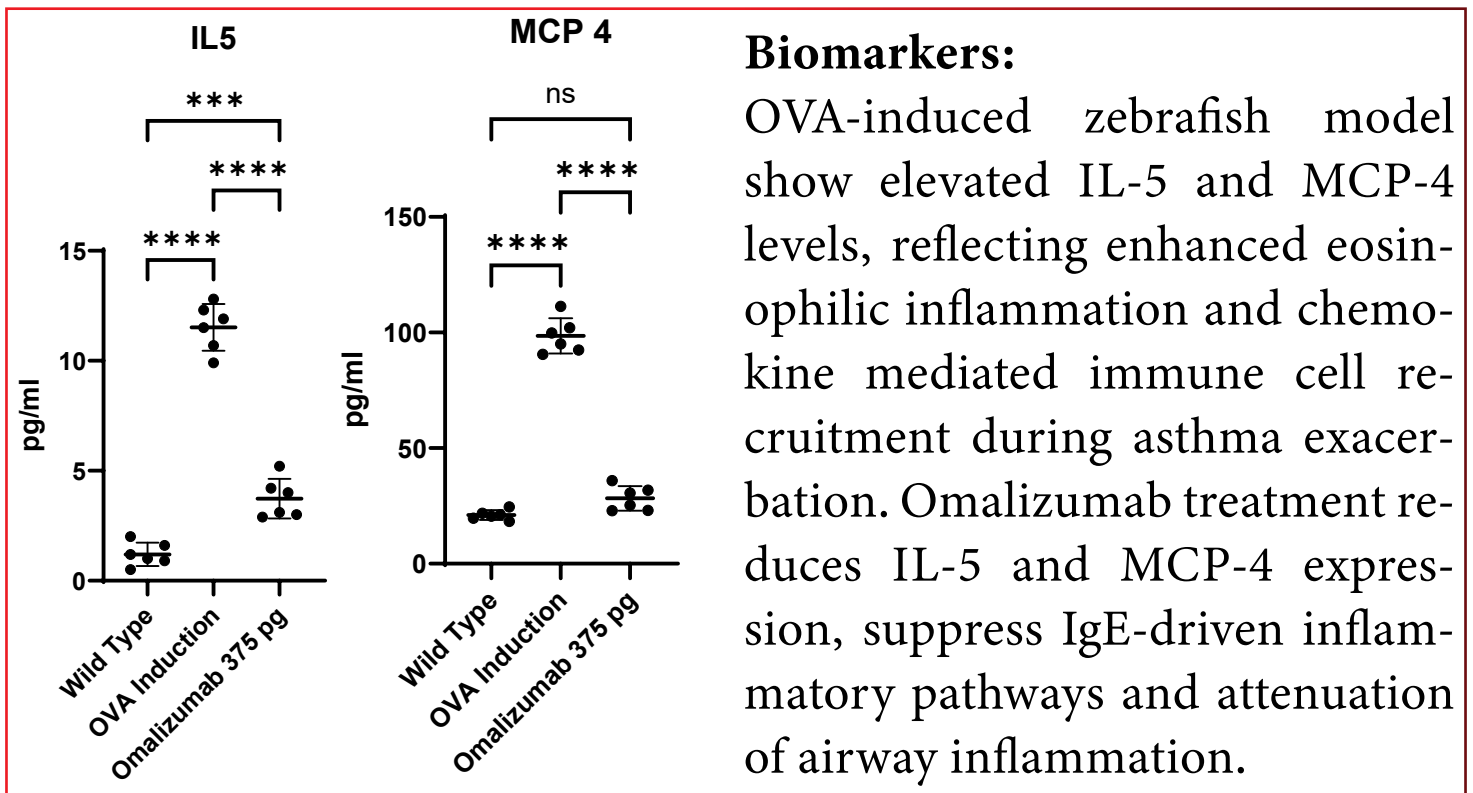
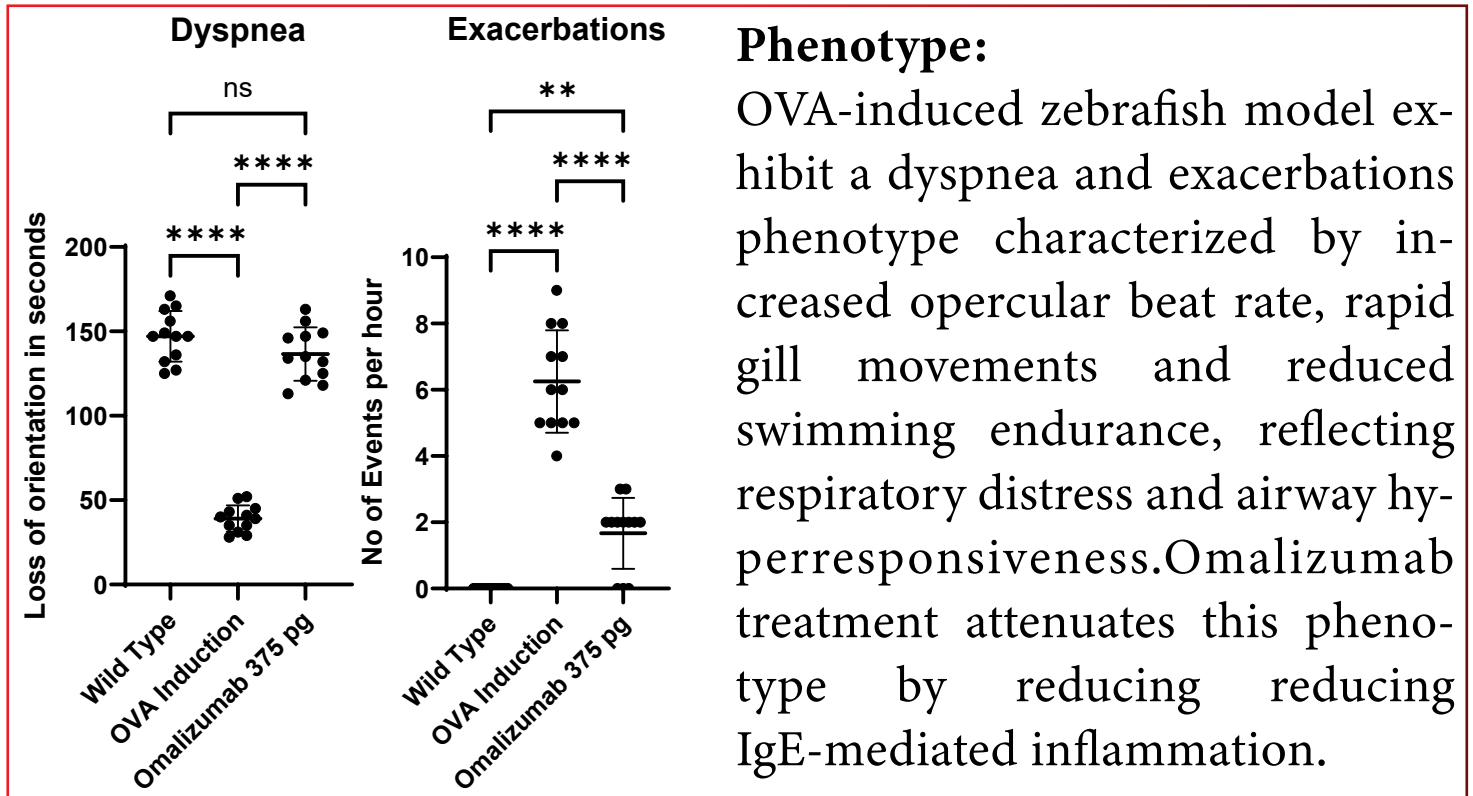
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# THERAPEUTIC PHENOTYPE & BIOMARKERS

## In Zebrafish

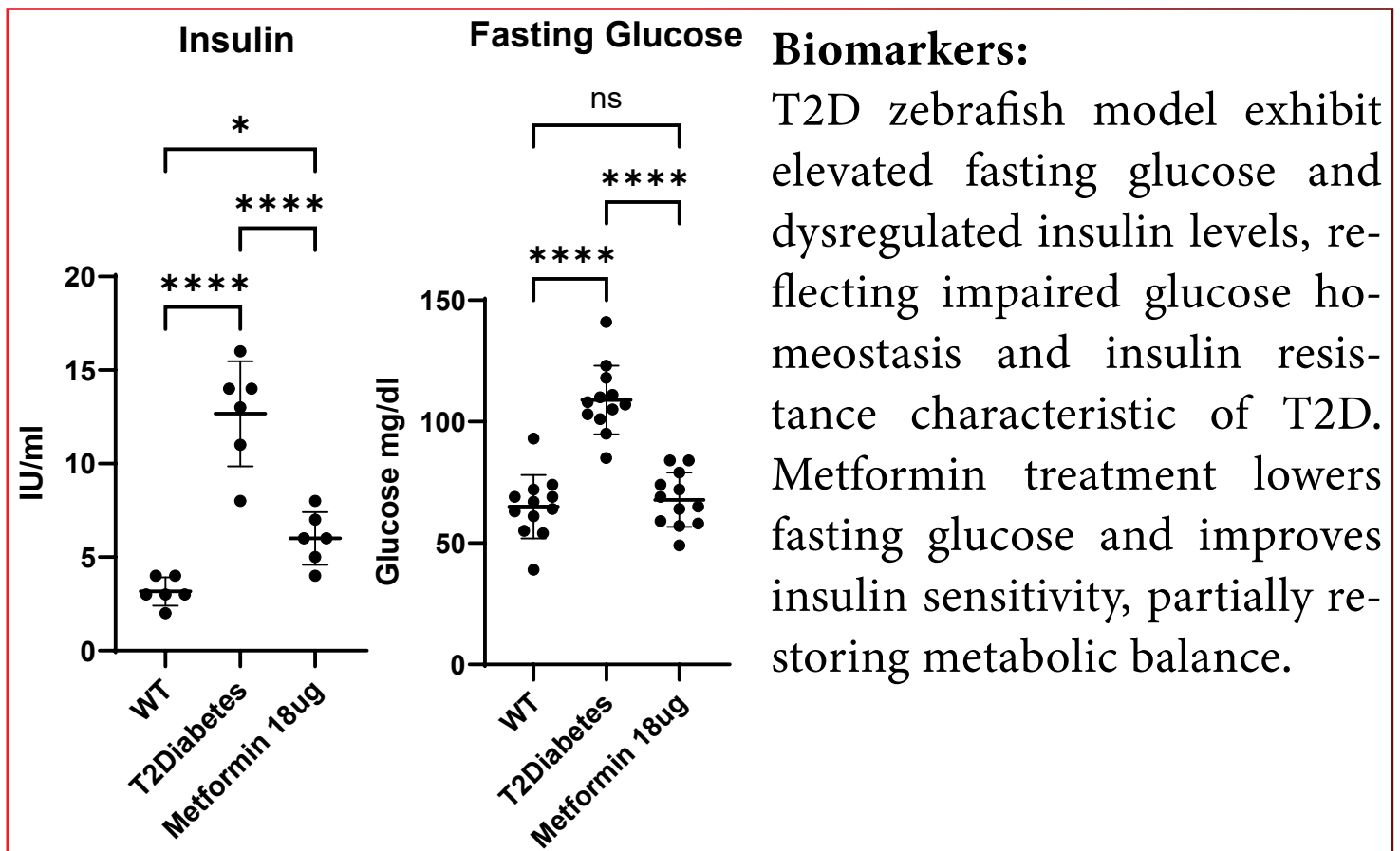
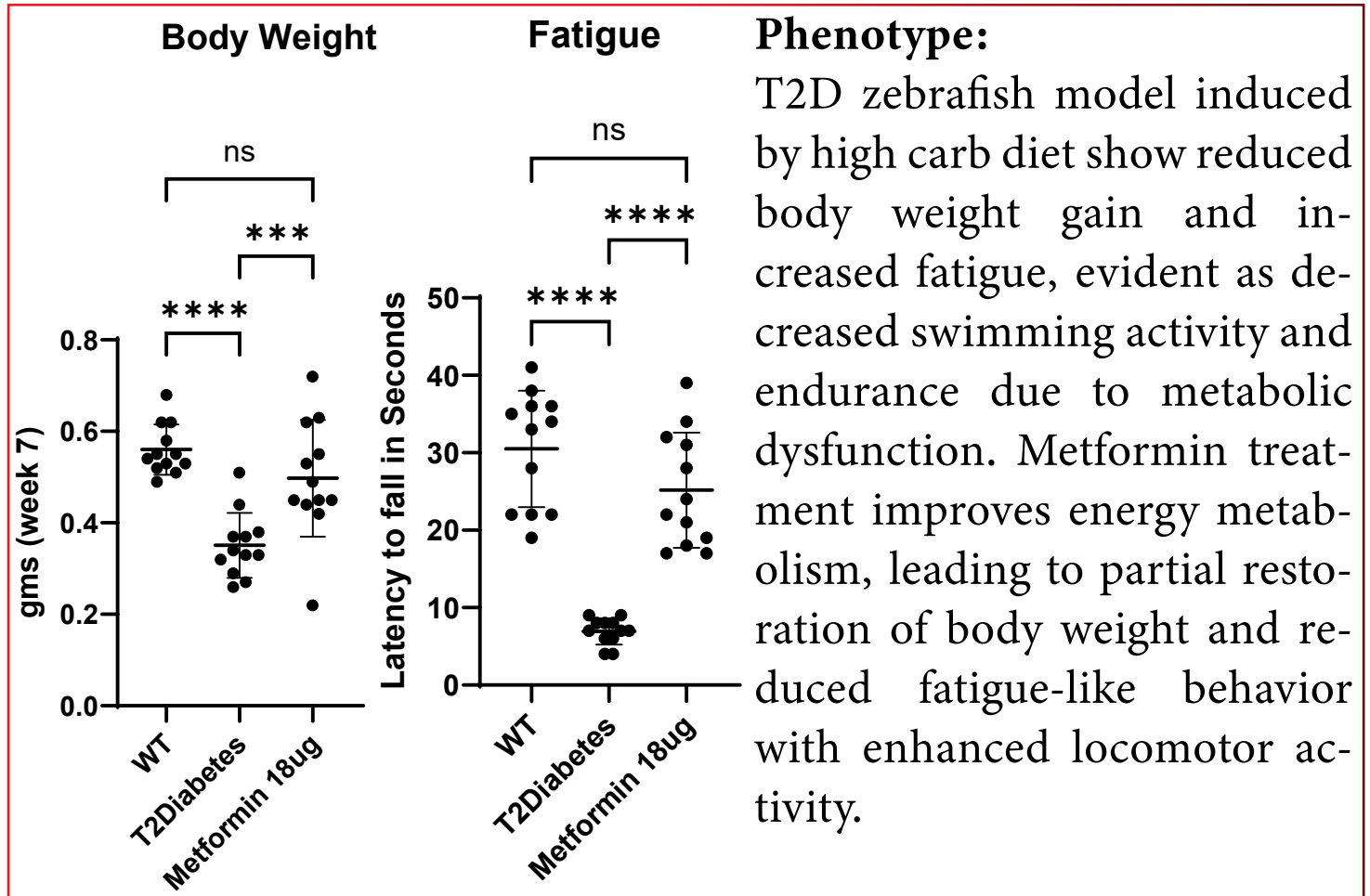
### Inflammation - Asthma

### Model - Ovalbumin Induction



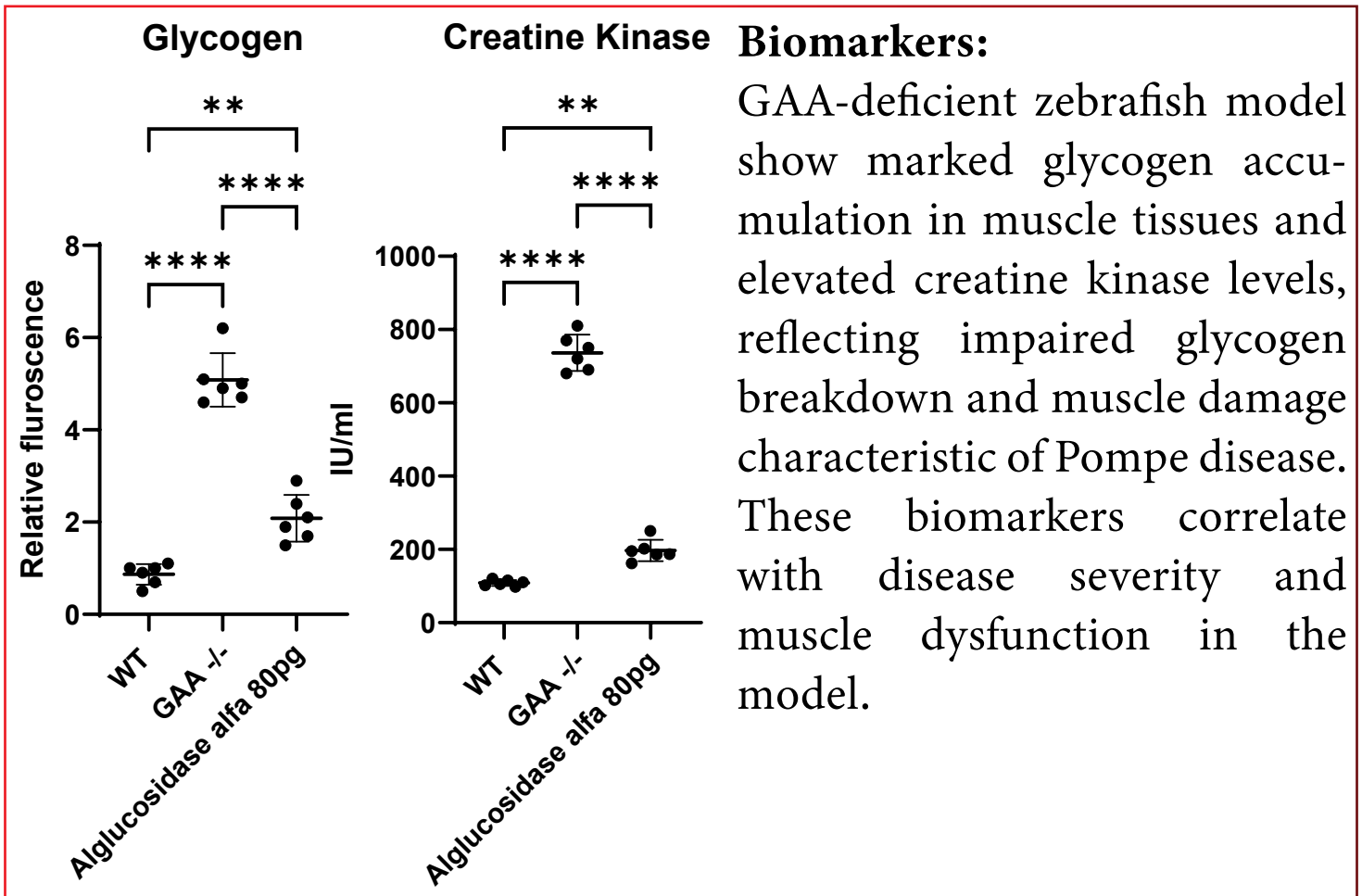
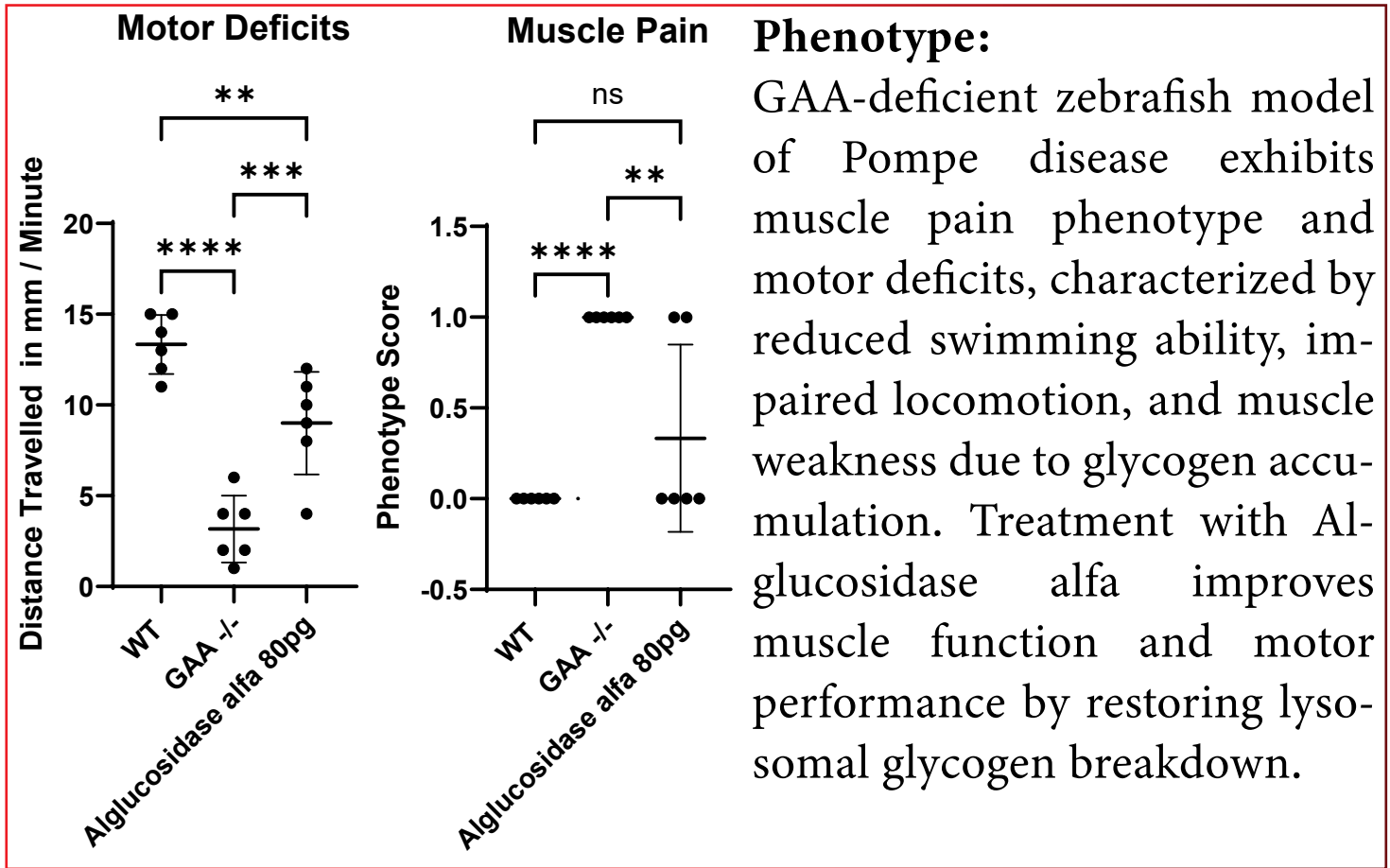
# Metabolic - Type 2 Diabetes

## Model - High Carb Diet



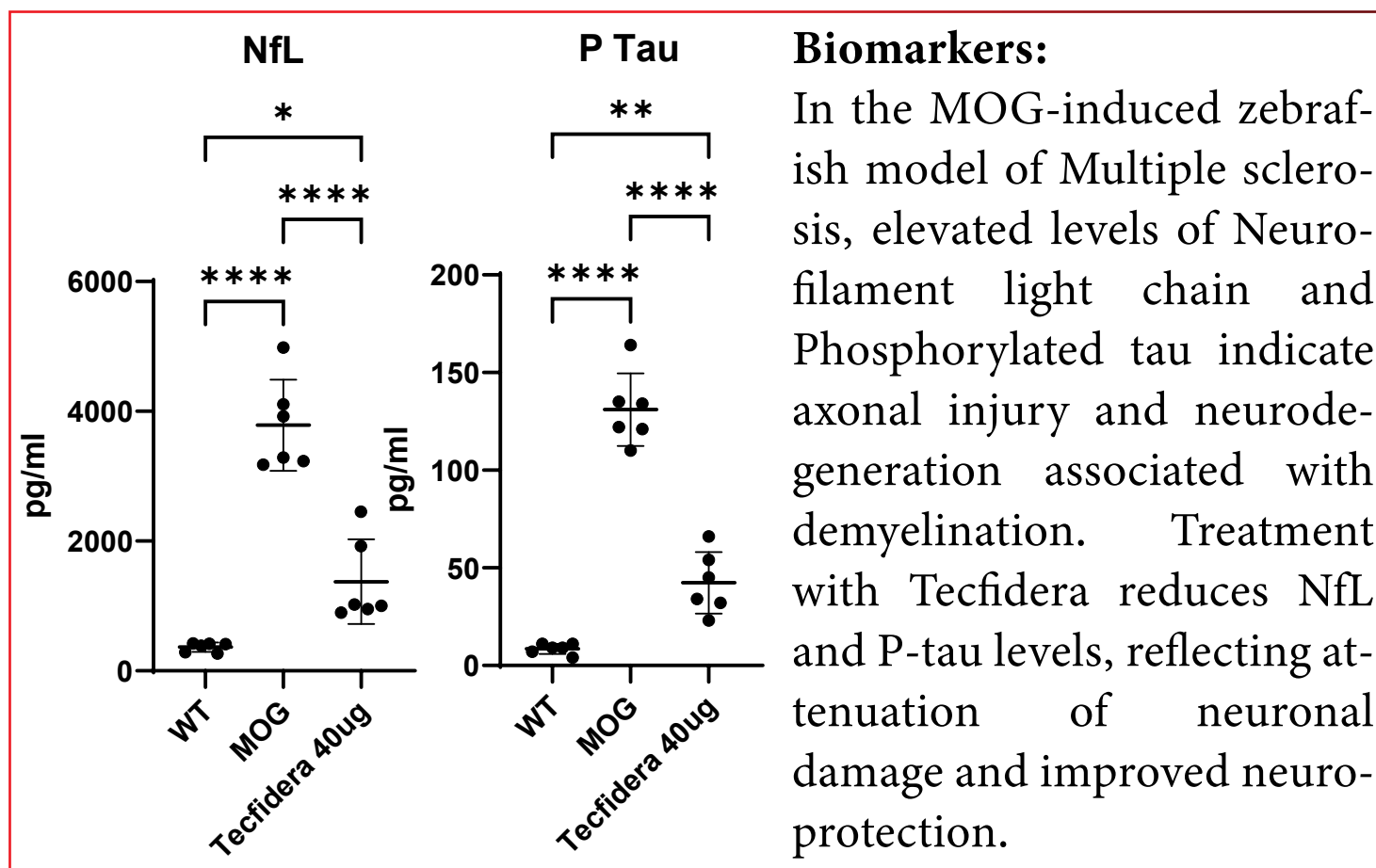
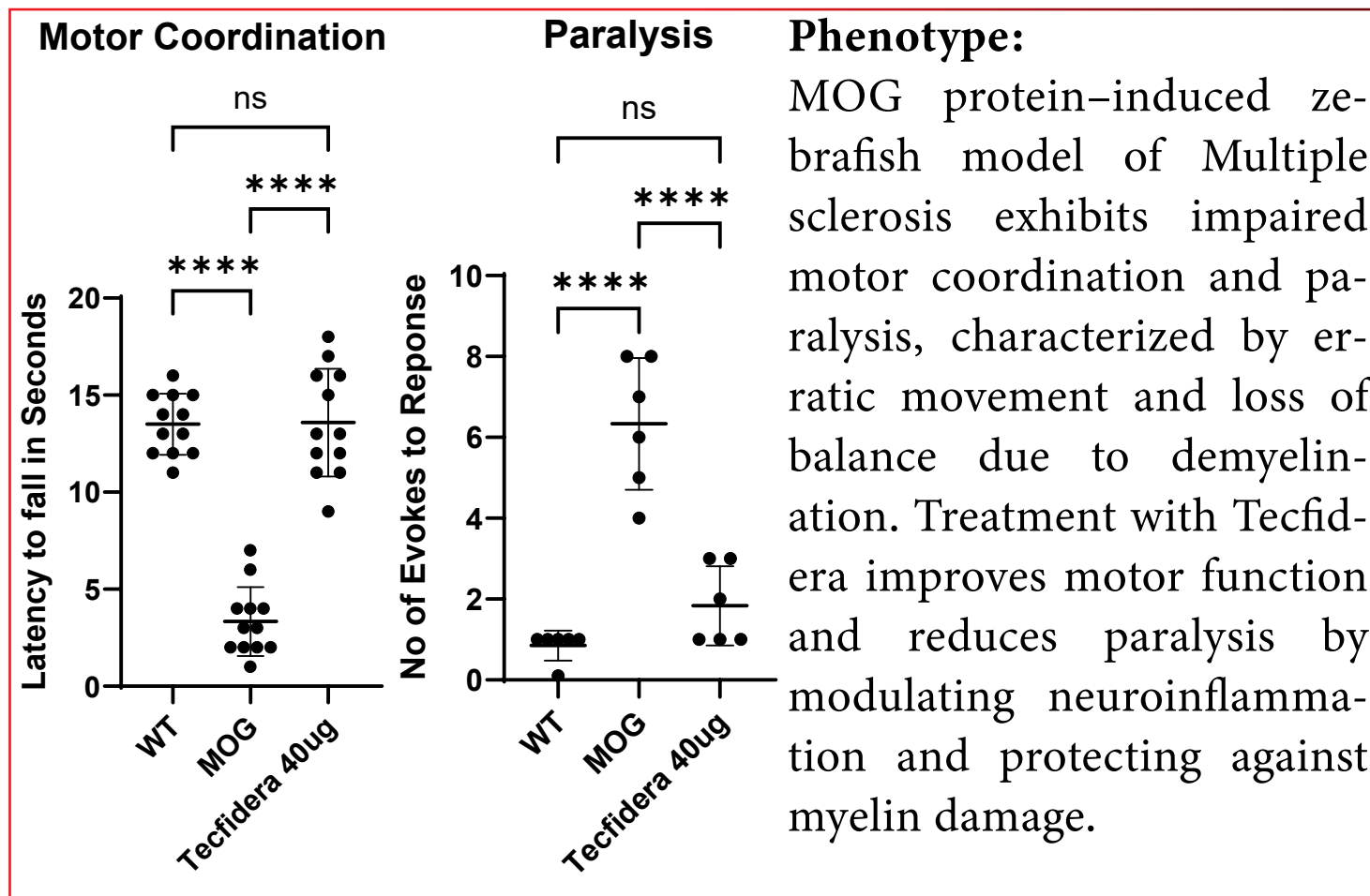
# Rare - Pompe Disease

## Model - GAA -/-



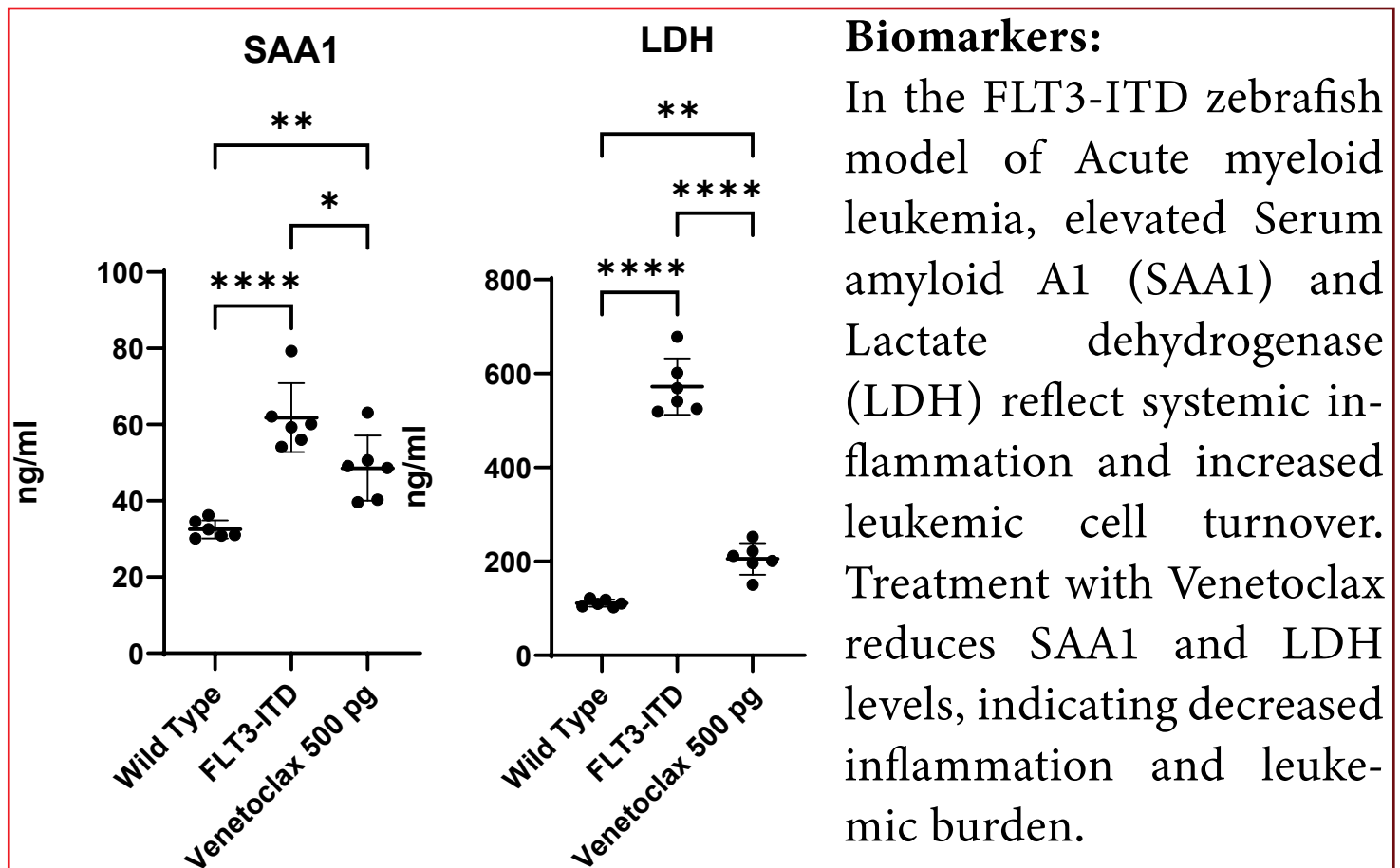
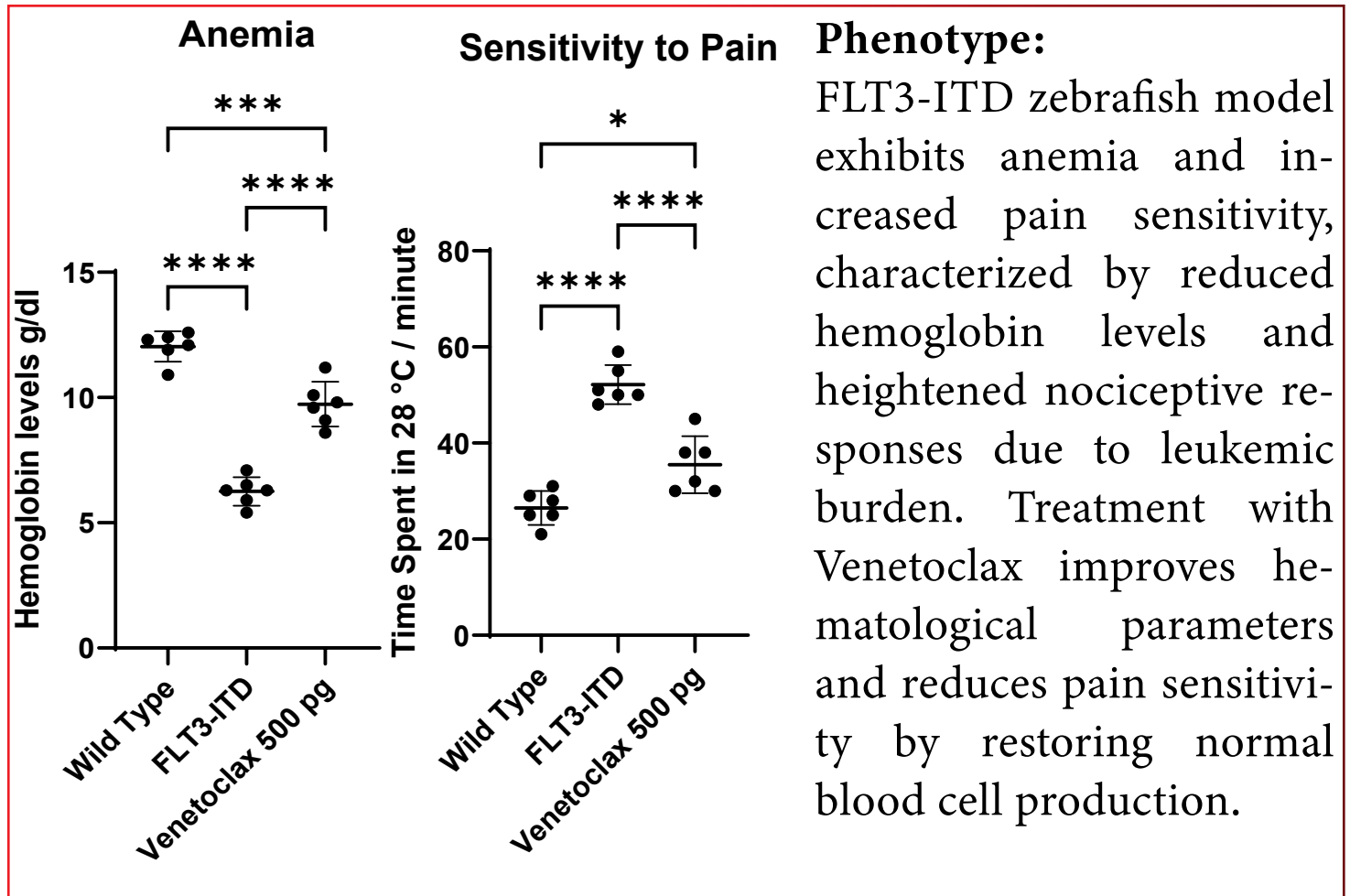
# Neuro – Multiple Sclerosis

## Model - MOG Protein



# Tumor - Acute Myeloid Leukemia

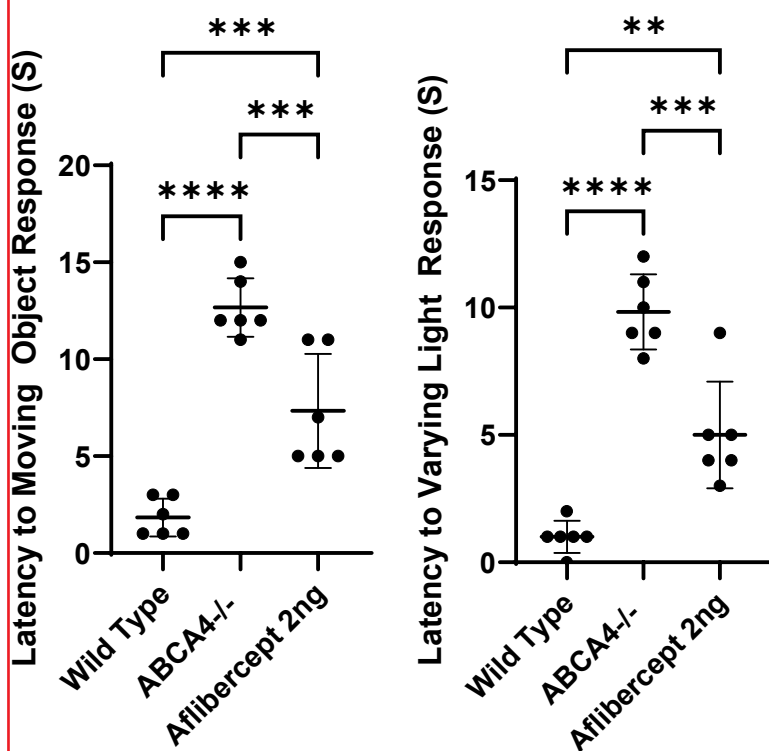
## Model - FLT3-ITD



# Eye – Stargardt Disease

## Model - ABCA4<sup>-/-</sup>

### Visual Acuity Test Varying Light Response Phenotype:

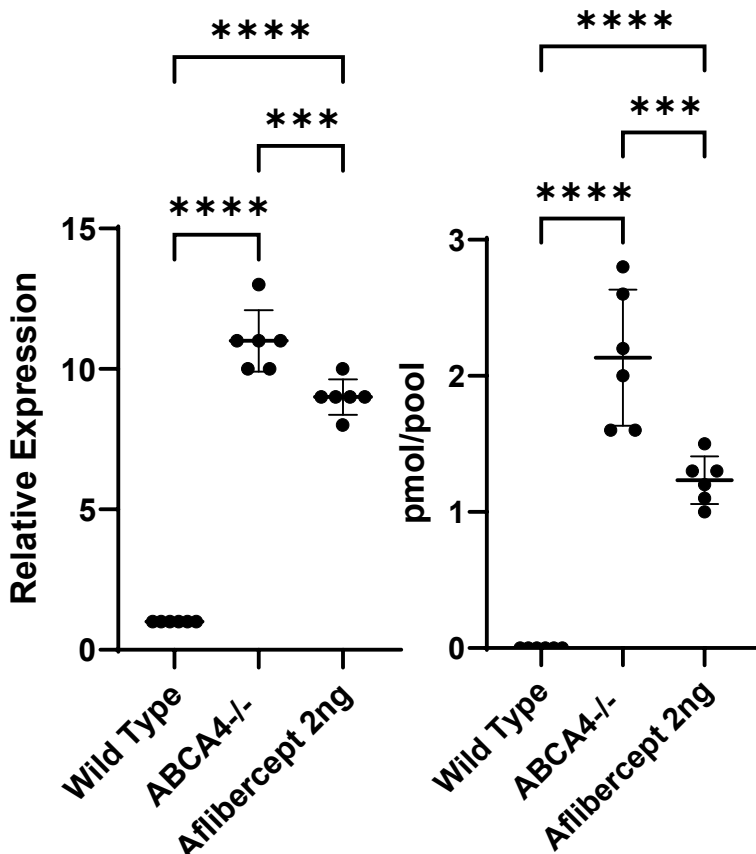


ABCA4 zebrafish model exhibits reduced visual acuity and altered light response, characterized by impaired optokinetic response and photoreceptor dysfunction due to lipofuscin accumulation. Treatment with Aflibercept improves retinal function and stabilizes visual behavior by reducing retinal stress and secondary degeneration.

### IL6

### Lipofuscin (A2E) levels

### Biomarkers:



In the ABCA4 zebrafish model of Stargardt disease, increased Interleukin-6 (IL-6) and accumulation of Lipofuscin reflect retinal inflammation and photoreceptor degeneration. Treatment with Aflibercept reduces IL-6 levels and limits lipofuscin-associated retinal damage, indicating attenuation of inflammatory and degenerative processes.

# CONTACT *US*

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## ***TOP 3 CSO Questions this Quarter.***

1. What types of assays are available for High-throughput screening (HTS) in metabolic disease models?
2. How can therapeutic rescue be effectively assessed in chronic conditions such as type 2 diabetes, MASH, and obesity?
3. Are there zebrafish lines that model and recapitulate immunosuppressive cell populations, such as Tregs, MDSCs, and TAMs?



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**Chennai, India - 600 100.**