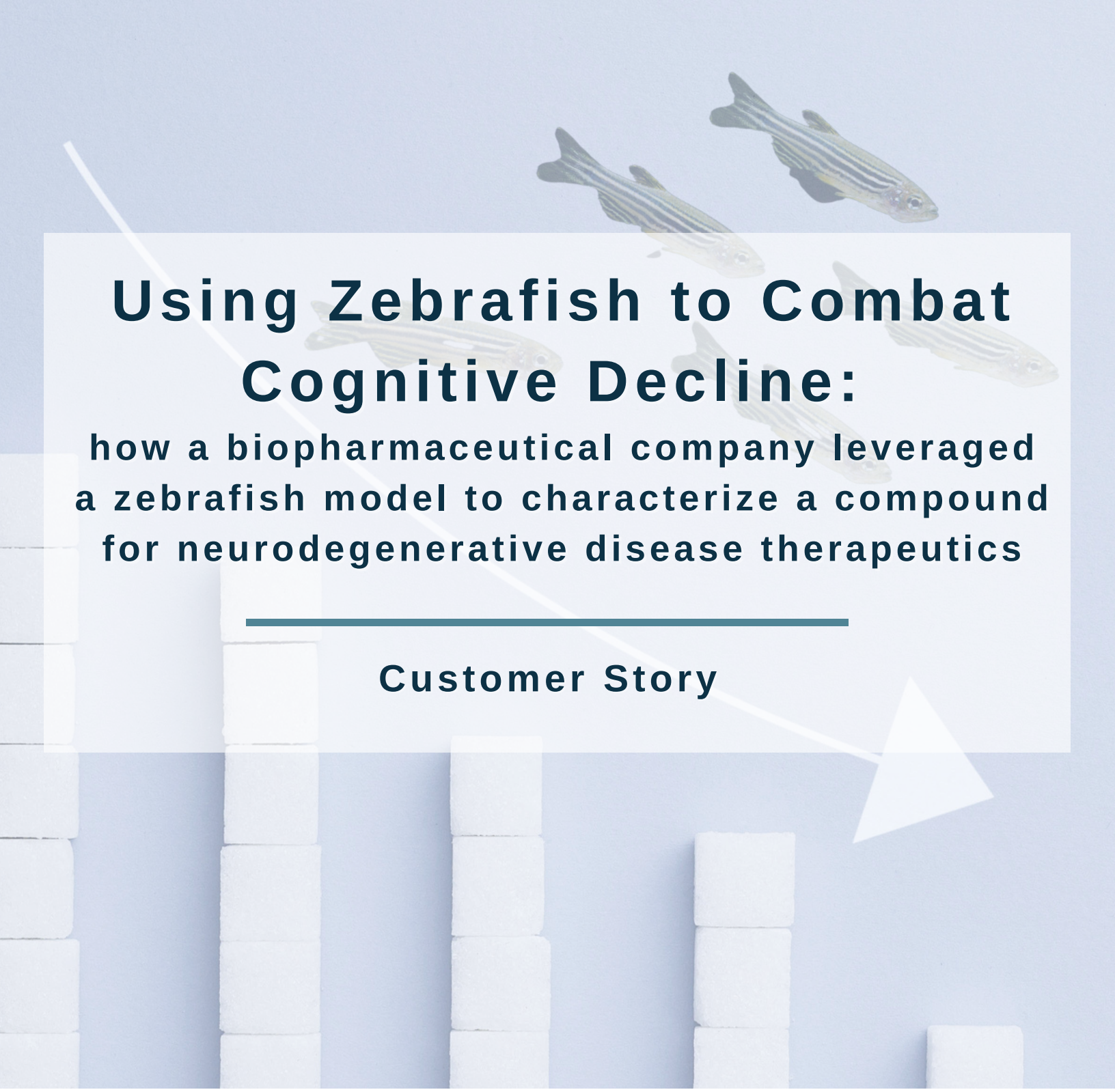

Using Zebrafish to Combat Cognitive Decline:

how a biopharmaceutical company leveraged a zebrafish model to characterize a compound for neurodegenerative disease therapeutics

Customer Story

InVivo Biosystems

InVivo Biosystems

Overview:
A Massachusetts biopharma company developing therapeutics for neurodegenerative diseases such as Alzheimer's and Parkinson's disease was able to accelerate their pre-clinical development by partnering with InVivo Biosystems to generate in vivo data in zebrafish. By using zebrafish, they were able to fast track the data generation needed for patent filings, grant applications, and follow on investments.

Challenge:
A Massachusetts biopharmaceutical company approached InVivo Biosystems wanting to know how they could use zebrafish as a model organism to comprehensively test the effects of their neurodegenerative therapeutics. They needed to gather robust data to validate their compound's neuroprotective potential, with the ultimate goal of using it as a therapeutic for neurodegenerative disorders.

Solution:
Partnering with InVivo Biosystems, the Massachusetts biopharma took a multistep approach to their project. First, InVivo Biosystems utilized a chemical model in order to mimic the effects of neurodegeneration targeted by the Massachusetts biopharma's therapeutic, Compound X. We then carried out an experiment in the zebrafish model to test the neuroprotective effects of Compound X. Using this paradigm, InVivo Biosystems was able to rapidly examine the molecular effects of the Massachusetts's compound and generate robust data within weeks.

> This gives us a hint of how effective the compound is...and very impressive results were gained
>
> -Director of the Massachusetts biopharma company

The Director of the biopharma company shared their excitement about the zebrafish model's contribution to their drug development: "Based on [this] information, we will seek more investment and grant funding. With our strengthened case [thanks to the new data], we are confident in our ability to get more capital which, in turn, will allow us to do further experiments and studies. Ultimately, we believe this compound could act as a therapeutic to slow the progressive cognitive decline in the neurodegenerative diseases."

Benefits:
By collaborating with InVivo Biosystems, the Massachusetts biopharma successfully assessed the effects of their drug candidate on oxidative stress and neurodegeneration using zebrafish as a model organism. This breakthrough allowed them to continue their testing in a faster, more cost effective way.

Further, the partnership with InVivo Biosystems provided the Massachusetts biopharma with critical data on the neuroprotective effects of their therapeutic. This data strengthened their grant and patent applications by demonstrating the compound's efficacy.

Since InVivo Biosystems used a chemical induction of disease states, the Massachusetts biopharma avoided any licensing issues associated with using models created by an academic institution.

Background:
Historically, drug development has been performed in mouse and rat models. But there has been a recent push for researchers and drug companies to move to alternative animal models due to time and cost constraints. For example, by using these mammalian models, developing a new drug can cost between 1 and 2 billion USD (Congressional Budget Office , 2021). In December 2022, the FDA signaled their support of the move to alternative animal models by instating the Modernization Act 2.0, which announced that therapeutic interventions no longer need to be tested in two specific animal models (typically one rodent and one large animal such as dog or ape) before moving to human clinical trials.

Due to this act, companies are now able to cost-effectively capitalize on our expertise in developing human disease models in zebrafish and *C. elegans* with CRISPR/Cas9. In turn, we have seen a shift of clientele as we have begun partnering with more pharmaceutical companies who are interested in leveraging our alternative animals to act as a fast, practical first step to test their compounds.

> We were interested in partnering with InVivo Biosystems because people [that have the neurodegenerative diseases we are looking to treat] don't have all the time on the planet to wait for these things
>
> -Director of the Massachusetts biopharma company

The Massachusetts biopharma was one of these companies; they had no prior experience using a zebrafish model to test their compounds, so their first question was - can we use fish? One of the benefits they saw in a zebrafish model, was that as a small vertebrate, the zebrafish enables a *whole animal examination* of a compound's effect, unlike a cell culture which can only test the effect on a single aspect of an organism's system. This was particularly important to The Massachusetts biopharma as their compound is looking to mitigate the effects of oxidative stress on the brain, and thus, act as a therapeutic for neurodegenerative disorders such as Parkinson's and Alzheimer's disease.

The Study:

Oxidative stress occurs when the body's production of reactive oxygen species (ROS) overpowers its ability to neutralize these reactive molecules. ROS are damaging to cells, proteins, lipids, and DNA. In particular, oxidative stress is a key contributor to the neurodegenerative diseases that The Massachusetts biopharma is looking to treat, such as Parkinson's and Alzheiemer's diseases (Kim, Kim, Rhie & Yoon, 2015). The herbicide paraquat (PQT) is often used to induce oxidative stress. The Massachusetts biopharma approached InVivo Biosystems asking whether our experts could design an experiment using a chemical zebrafish model. First, our scientists needed to determine the correct dosage of the chemical toxin as PQT can be lethal at high concentrations.

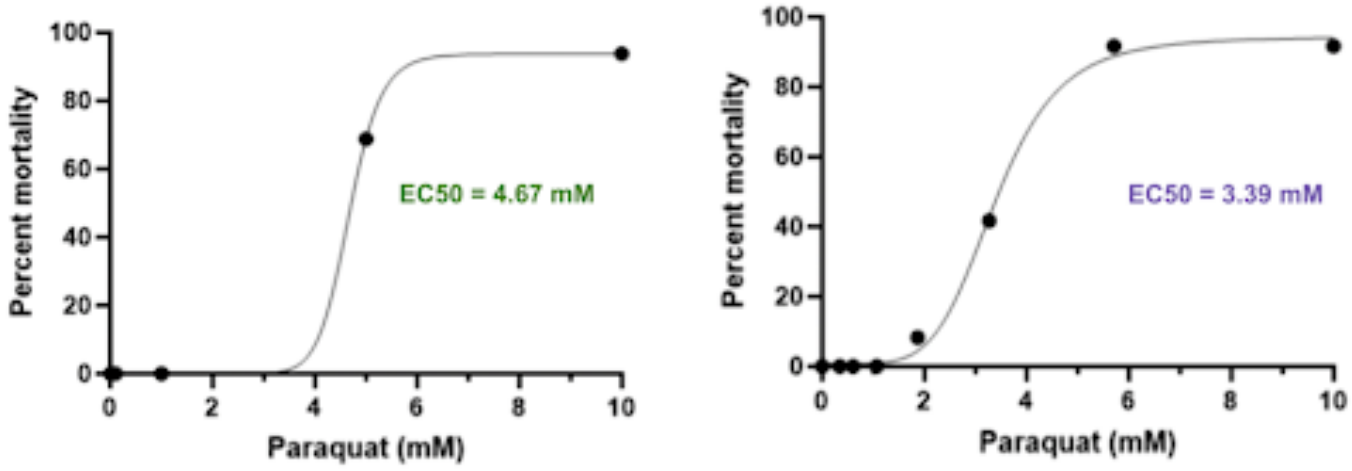

Figure 1. Two tests (1 & 3) measuring the EC50 of PQT at 5 dpf after two days of treatment.

After establishing the effective concentration of paraquat (EC50) that causes 50% mortality in larval zebrafish [Figure 1], we wanted to assess the effect of the Massachusetts biopharma's Compound X on rescuing zebrafish' survival and locomotive abilities.

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Within a week of our initial meeting with Massachusetts biopharma, we administered Compound X to a subset of zebrafish - the control was untreated, and the positive control group received a serotonin reuptake inhibitor. Then, all of the zebrafish were exposed to the PQT toxin. Morphological improvements were observed in the fish treated with Compound X and the positive control, such as decreased spinal curvature [Image 1]. Data also showed increased survival for the treated fish [Figure 2].

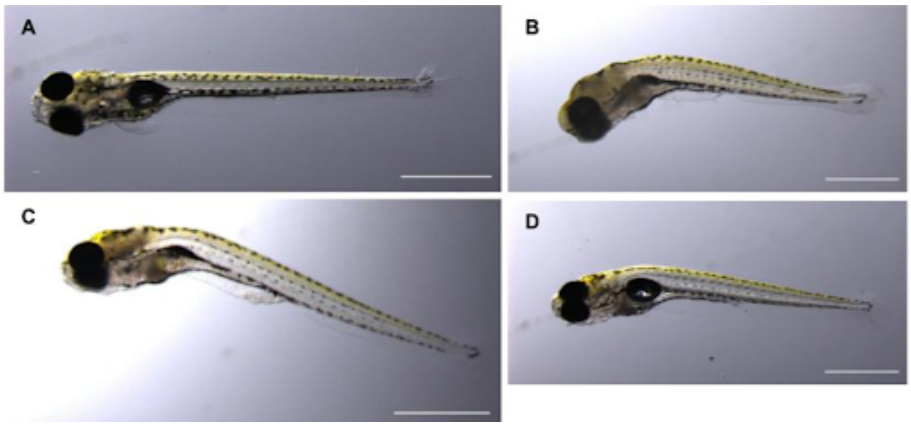

Image 1. Images showing spinal curvature in 7 dpf fish exposed to (A) 0.1% DMSO only, (B) 0.1% DMSO and 3 mM PQT, (C) 3 µM Compound X and 3 mM PQT, or (D) 0.3 µM positive control, a serotonin reuptake inhibitor, and 3 mM PQT (Test 5). Scale bar is 500 µm.

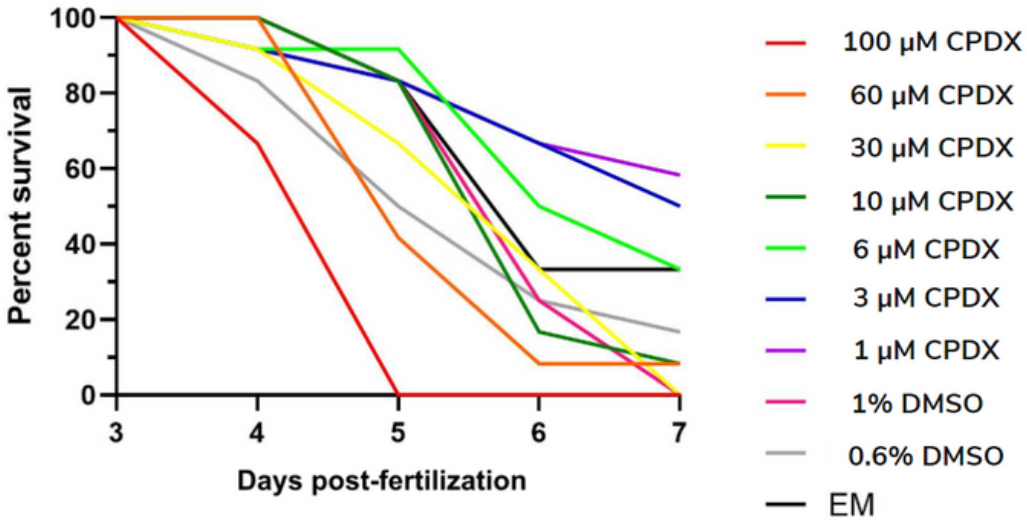

Figure 2. Compound X modestly increased survival by 7dpf in fish exposed to PQT.

To understand the molecular effect of Compound X on the response to PQT toxin-induced stress, our expert team performed RNA extraction and submitted samples for RNA-seq. RNA-seq analysis allows us to reveal global gene expression. In whole animal models such as zebrafish, this analysis gives us valuable insight into the mechanisms of action behind processes such as oxidative stress. One of the Massachusetts biopharma' goals with this project was to identify the pathways involved in oxidative stress. This information allowed them to identify target genes for further therapeutic interventions.

InVivo Biosystems was able to provide the Massachusetts biopharma with the data they needed, showing that their compound triggered a response in inflammatory pathways and produced a change in expression of 109 genes [Figure 3]. In our report, we identified and listed the top 22 genes that could act as targets for future drug therapies to help them narrow their search.

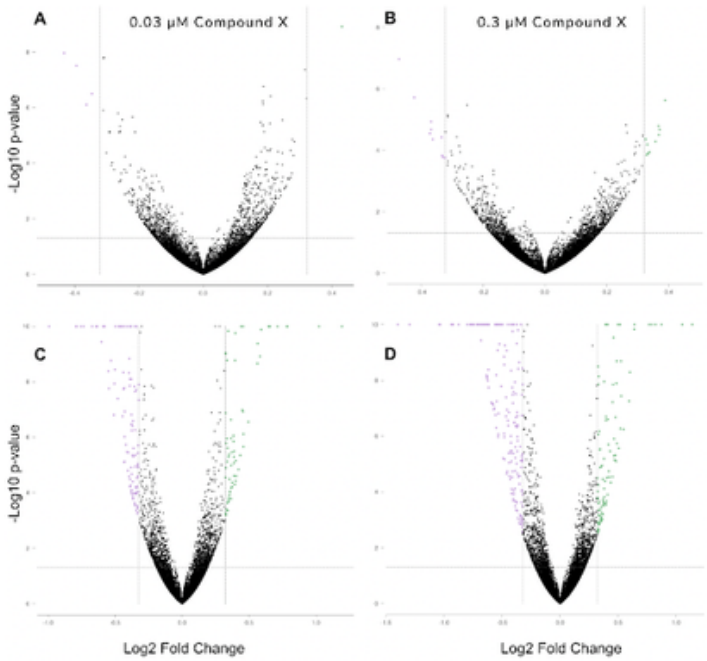

Figure 3. Volcano plot of all differentially expressed genes for Compound X, high and low concentration comparison groups.

The Massachusetts biopharma has already been able to benefit from the data on their compound and secured more investment to continue the drug development process. Thanks to our collaboration, the team at the Massachusetts biopharma feels confident in the choice to utilize a zebrafish model. They credit the fast turnaround time to their ability to quickly move through the grant application process and focus on what they see as the most important part of their company: the science of drug development.

> We think that [using a] multi-model approach is the way to do it. And now, with these findings, the door is open to further studying our compound as a drug candidate, as well as better understanding the role of oxidative stress in neurodegeneration.
>
> -Director of the Massachusetts biopharma company

Our Zebrafish CRO Services:

Therapeutic Testing

Determine if a lead compound ameliorates disease phenotypes or affect important metabolites

Genetic Target Validation

Determine if genes are involved in disease processes and make good targets for further development

Library Screening

Medium throughput screen of compound libraries and biologics (up to BSL2)

ADME-Tox

Tissue preparation for ADME analysis

General and tissue specific toxicity

References:

Congressional Budget Office (2021). Research and Development in the Pharmaceutical Industry. https://www.cbo.gov/publication/57126

Kim, G. H., Kim, J. E., Rhie, S. J., & Yoon, S. (2015). The Role of Oxidative Stress in Neurodegenerative Diseases. *Experimental neurobiology*, *24*(4), 325–340. https://doi.org/10.5607/en.2015.24.4.325

About InVivo Biosystems

Founded in Eugene, Oregon in 2011, InVivo Biosystems is working to accelerate deep in-vivo insights into human biology and enable researchers to develop and deliver solutions that improve human health. An expert in CRISPR genome editing, InVivo Biosystems provides a unique capability for creating custom genome edited zebrafish and *C. elegans* that enable therapeutic research on genetic models of aging, developmental, and neurodegenerative disease, uncovering potential cures. The company's *in vivo* analytical testing platforms and technologies provide faster, cost-effective investigations that focus on proof-of-principle experiments for rapid go/no go decision making so that biopharma and nutraceutical companies around the world can better understand aging and aging related diseases and explore potential treatments.

All our projects include on-call project status updates, as well as regularly scheduled communication. We also provide on-call consulting and interpretation with our Ph.D. level, subject-matter experts.

What we do:

- Deliver scientific data on test results in less than 5 months.
- Produce the best outcome measures for anti-aging products.
- Provide information about mechanisms of action (MoA).
- Support your Marketing and IP claims with real science.

Contact us to start a conversation about how our services can support your innovation.

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