# UTILIZATION OF FEATURES OF THE EARLY DISEASE PHENOTYPES IN A ZEBRAFISH MODEL OF ALS FOR PHARMACOLOGICAL SCREENING TO DETECT SMALL MOLECULE NEUROPROTECTIVE COMPOUNDS.

**Tennore M.Ramesh, UK**
**Grant: € 30 000**

ALS/MND is an adult onset motor neuron degenerative disease with a lifetime risk of ~1/1000. Approximately 80% of cases are fatal within five years of diagnosis. There is no cure and the only approved drug Riluzole, has a minor effect on disease progression and survival. A familial form of ALS caused by mutations in the SOD1 gene produces disease that is indistinguishable from the sporadic form of ALS. Despite the availability of mouse models of MND, they are not suitable for performing large drug screens due to costs, space and time required. Screening of 2000 drugs in mouse model would require over 20 years (@100 drugs/ year) and involve huge sums of money. Despite research over two decades, the exact mechanism of mutant Sod1 toxicity in ALS is unknown. The Sod1 mutation has served as a way to model ALS in drosophila, mice and rats. Despite the usefulness of these models, each has their drawbacks. The mouse models are highly inbred, and some lines express very high levels of mutant protein, which may not accurately reflect the human condition. The Drosophila model shows motor neuron loss but does not display progressive weakness or premature death. The availability of animal models that allow for medium throughput drug screens is very critical in rapidly identifying disease modifying agents for ALS and also provides tools to better understand the mechanism of neurotoxicity.

The zebrafish model of ALS serves as an ideal translational model for such purposes, permitting researchers to undertake large screens that are difficult, if not impossible, in larger vertebrates such as mice. The transparency of embryos allows us to visualize the internal organs at a single cell level using fluorescent markers (Fig-1) and a freely swimming larva emerges within 72 hours post fertilization (hpf).

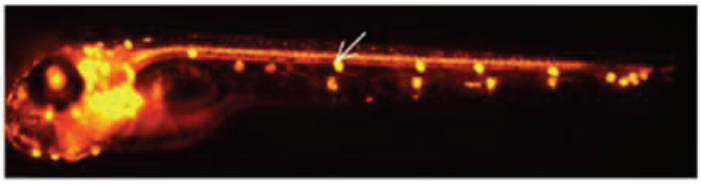

**Fig-1: Mutant Sod1 induces cellular stress:** G93ROs10 larvae show induction of Hsp70-DsRed in the brain, spinal cord and neuromasts (arrow)

We can visualize and perform complex electrophysiology on single motor neurons and other spinal neurons cells in living animals. We can analyze cell fate, axonal outgrowth, synapses and study motor behaviour.

Many neurodegenerative diseases are identified by the accumulation of misfolded proteins that are detrimental to neuronal connectivity and plasticity, and trigger cell death pathways. These aggregates may be comprised of oligomeric complexes of non-native secondary structures that demonstrate poor solubility in aqueous or detergent solvent. Amyotrophic lateral sclerosis is another neurodegenerative disease that fall within this category of ‘‘protein conformational diseases’’ because of the emergence of protein aggregation in the spinal cord. One of the first reactions of the cells to misfolded proteins is the induction of the heatshock response that allow molecular chaperones to try refolding the damaged proteins, though this is not always feasible and productive . Nevertheless, the molecular chaperone response provides a good readout of the cellular pathophysiology. Oxidative stress and excitotoxicity can channel their toxicity through protein modification that leads to protein aggregation.

Our zebrafish model of ALS serves as an ideal translational model for drug screens due to the early embryonic phenotypic readout of the assay. We have already generated a transgenic zebrafish over expressing mutant forms of Sod1 that displayed the characteristic hallmark features of ALS. Upon identifying the transgenic lines, we found that the fish carrying the Sod1 mutations turned on the heat shock response independent of heat shock (referred to as Sod1mut Hsp70 induction). This suggests that fish containing mutant Sod1 are exhibiting chronic cellular stress that may affect neuronal physiology, and eventually lead to their demise in ALS. We propose to use this chronic mutSod1 hsp70 response as a read-out of mutant Sod1 toxicity. In this proposal we plan to fully characterize the Sod1mut Hsp70 induction and the heatshock induced changes in endogenous Hsp70 induction within the spinal cord of Sod1 mutants.

Our transgenic zebrafish will provide suitable read-outs for drug screening and will allow to perform

- a drug modifier screen based on mutant Sod1 specific induction of Hsp70 promoter linked Dsred expression to identify drugs that alter mutant Sod1 toxicity..
- a secondary validation screen to detect and quantify changes in endogenous Hsp70, based on increased sensitivity of mutant Sod1 transgenics to heatshock stress after heatshocking.

We plan to use this unique readout of cellular stress to initially screen a drug library that include a large number of already approved drugs. If any of these show promise, there is the potential for rapid translation into clinical trials in MND patients.

## TEAM

**Tennore M.Ramesh** is a well rounded scientist with widespread experience in academia, corporate and non-profit world. He has over 14 years of in vivo experience in functional genomics, pharmacology, and toxicology of drug development. He founded the research program at ALS Therapy Development Institute, Cambridge, USA and served as its Chief Scientific Officer from inception in 2000 until 2003. He

developed the first adult onset neurodegeneration model in Zebrafish. The Department of Neuroscience is based in the University of Sheffield School of Medicine and Biomedical Sciences and collaborates with the MRC (Medical Research Council). Centre for Developmental and Biomedical Genetics. A new state of the art research center dedicated for MND research headed by Prof. Pamela Shaw, a world leader in MND research, includes a drug screening laboratory at the Sheffield Institute for Translational Neurosciences (SITraN) and is scheduled for completion in summer 2010. The overall goal of both centres is to translate basic science into new therapies for neurodegenerative diseases. This is achieved by combining and integrating the strengths of scientists and clinicians with expertise in molecular/cellular biology, pre-clinical animal models, and clinical neuroscience.

Dr. Tennore M. Ramesh,
DVM, PhD, Principal Investigator

Ms. Natasha Redhead,
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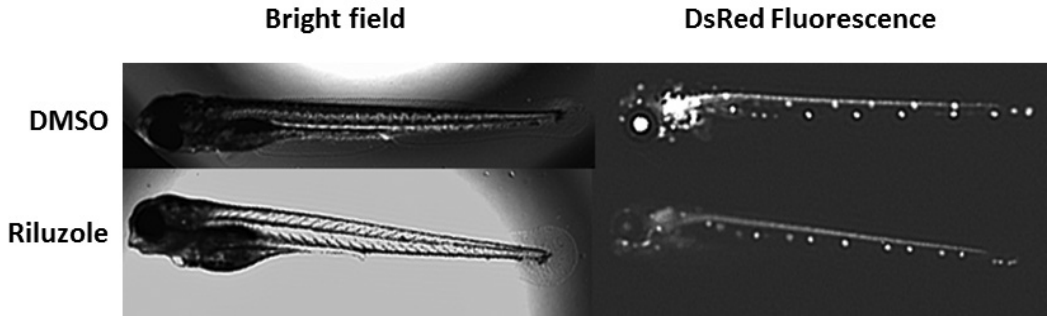
# Utilization of features of the early disease phenotypes in a zebrafish model of ALS for pharmacological screening to detect small molecule neuroprotective compounds.

**Call for projects 2010**
**Grant:** 30 000 €
**Project Duration:** 1 year

**Investigator:** Tennore Ramesh, University of Sheffield, United Kingdom

**Updated results - December 2012**

We have successfully completed the aims that we set out to in the grant application to have a fully validated the assay for drug screening using the mutant sod1 zebrafish model of ALS. Towards this we have developed, optimised and validated our screening protocol with a positive control, which is riluzole. We took the initial assay which involved testing compounds in batches of 25 embryos that were not genotyped and grown on petri dish and converted into a 96 well plate format, 1 embryo/well, and genotyped embryonic assay. This greatly increased the throughput of the screen and also allowed reduced wastage of precious drugs used in screening. We validated the screen using riluzole as our positive control (Figure-1) and showed that the assay can accurately pick 92% of any positive compounds that show activity with 100 % reliability. This demonstrated that the assay is sensitive and reliable to perform drug screening.




Additionally we have made great progress in understanding the early processes that lead to ALS symptoms and discovered an important role played by relay neurons, that communicate with motor neurons in ALS. Specifically we identified that the earliest event in ALS in the sod1 zebrafish model involves neuronal stress in relay neurons that apply brakes on motor neurons. This is followed by stress in motor neurons and degeneration of connection with the muscle (Figure-2). Riluzole, the approved drug for use in ALS, modulates neuronal stress in interneurons, indicating a novel mechanism of riluzole action. The findings of this study are accepted in the journal “Annals of Neurology”.

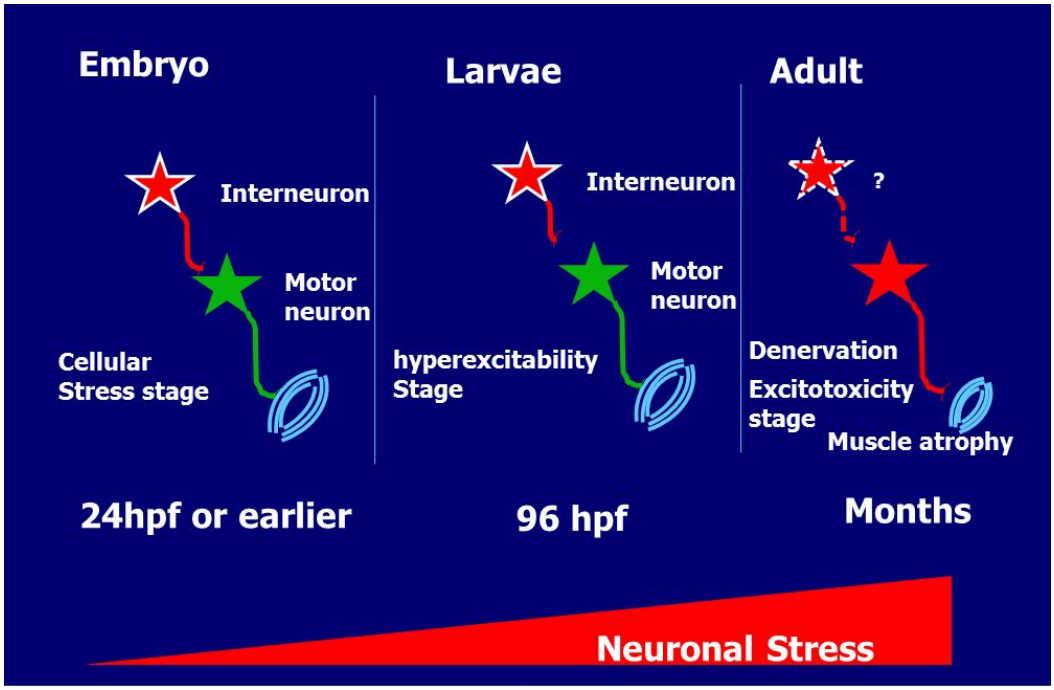



**Figure-2: Chronic neuronal stress may play an important role in predisposing motor neurons to injury.** In Embryonic and larval stages the brakes that regulate motor neurons (Inhibitory interneurons) are stressed and fail, potentially causing motor neuron hyperexcitability. In adults, the hyperexcitability leads to stress in the motor neurons due to excitotoxicity and lead them to fail and cause denervation and muscle atrophy.