Pharmacological Restoration of Visual Function in a Blind Zebrafish Mutant Following Histone Deacetylase Inhibitor (HDACi) Treatment.

**Background:** Controversially, histone deacetylase (HDAC) inhibitors are in clinical trial for the treatment of inherited retinal degenerations. Previous studies report that patients suffering from Retinitis Pigmentosa (RP) show improved visual field and acuity following treatment with the HDAC inhibitor valproic acid (VPA) (Clemson, Tzekov et al. 2011). However, other studies disagree with these findings (van Schooneveld, van den Born et al. 2011) and (Bhalla, Joshi et al. 2013). Thus, we sought to determine if treatment with HDACi rescued visual function and retinal morphology in a blind zebrafish **dying-on-edge** (**dye**) mutant identified from a forward genetics screen.

**Methods:** Visual function was assessed by optokinetic response (OKR) (Brockerhoff 2006) and visual motor response (VMR) (Deeti et al 2014) assays. Cone photoreceptor outer segment (OS) morphology, ciliary marginal zone (CMZ) apoptosis and cone photoreceptor outer segment (OS) length were assessed by light microscopy. Larvae were drug treated with HDACi (1 µM TSA, 6 µM Scriptaid, 10 µM MC1568 and 10 µM MS275) from 3-5 dpf at 28.5 °C. An unbiased shotgun proteomic analysis of TSA treated **dye** larval eyes was carried out by LC-MS/MS with minor modifications. The dataset was analysed by Ingenuity pathway analysis (IPA) software to identify affected signalling pathways. Inhibition of TrkB signalling was acheived via co-treatment with ANA-12, an antagonist of the TrkB receptor. Expression levels of proteins involved in Bdnf/TrkB signaling (Bdnf, TrkB, and pAkt) was analysed by western blot.

**Results:** The **dye** mutant has a 98% reduction in OKR, 85% reduction in average MAX ON VMR and 79% reduction in average MAX OFF VMR compared to unaffected siblings. **dye** mutants display several defects in retinal morphology including increased cell death in the ciliary marginal zone (CMZ) and brain indicated by pyknotic nuclei. The photoreceptor outer segments (OS) are decreased in length and lack even distribution and upright orientation. HDACi treatment of **dye** mutants results in improved OKR (42.8 fold increase) and MAX ON VMR (3.1 fold increase). TSA treatment rescued gross morphological defects, reduced CMZ cell death by 80% and increased photoreceptor OS length. Proteomic analysis identified significantly differentially expressed proteins in response to treatment, including proteins involved in phototransduction and neuroprotection. Pathway analysis identified Bdnf/TrkB signaling as an upstream regulator of these changes. TSA + ANA-12 co-treatment reduces visual function rescue (69% and 85% maximum reduction in OKR and VMR respectively) and blocks Bdnf/TrkB signaling via attenuation of Akt activation (62% decrease) in the **dye** mutant.

**Conclusions:** HDAC inhibition is effective in restoring visual function and rescuing morphological defects in a zebrafish model of retinal degeneration.


