Validating the efficacy of a potent DYRK1a inhibitor (DYR533) in the 3xTg mouse model of Alzheimer's Disease

Samantha Bartholomew[1, 2] Wendy Winslow[1], Yeng Shaw[3], Samantha Rokey[4], Christopher Foley[4], Christopher Hulme[3, 4], Travis Dunckley[1, 5], Ramon Velazquez[1, 2, 5]

[1]Neurodegenerative Disease Research Center, Arizona State Univ., Tempe, AZ; [2]School of Life Sciences, Arizona State Univ., Tempe, AZ; [3]Division of Drug Discovery and Development, Dept. of Pharmacology & Toxicology, [4]Dept. of Chemistry and Biochemistry, College of Sciences, The Univ. of Arizona, Tucson, AZ; [5]Arizona Alzheimer's Consortium, Phoenix, AZ

Background Information

Alzheimer's Disease

- Alzheimer's Disease (AD) is a neurodegenerative disorder characterized by amyloid-β (Aβ) plaques and neurofibrillary tau tangles (NFTs) leading to inflammation and cell death in the brain.
- AD currently has no effective therapies to ameliorate the pathological progression and associated cognitive deficits.
- 1 in 3 seniors dies every year with AD or another kind of dementia.
- It's estimated that by 2050 ~14 million Americans will be diagnosed with AD (*2022 Alzheimer's Disease Facts and Figures*).

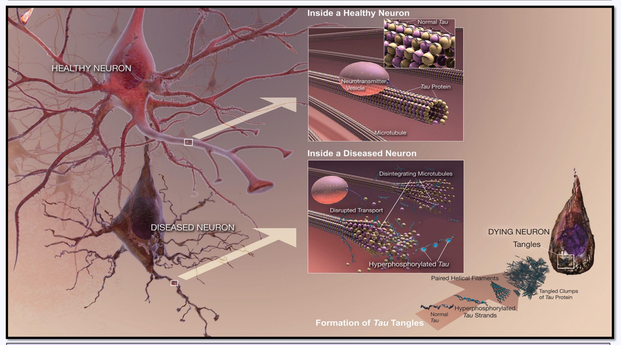

Figure 1: Depiction of tau in a healthy neuron vs. the disintegration of microtubules (loss-of-function) leading to the aggregation into paired helical filaments and NFTs (gain-of-function) in diseased neurons.

- The tau protein plays an important role in stabilizing microtubules within neurons, allowing for adequate protein transport along the axon (Mandelkow, et al. 2012).
- Kinases can phosphorylate tau which can lead to its removal from the microtubules and contribute to aberrant protein accumulation (Mandelkow, et al. 2012).
- In AD and a class of disorders known as the tauopathies, aberrant post-translational modifications to the tau protein contributes to pathological tau aggregation and destabilization of microtubules.
- Among these kinases is the dual-specificity tyrosine phosphorylation-regulated kinase 1a (Dyrk1a), which directly phosphorylates tau at multiple serine and threonine sites (Ryoo, et al. 2007).
- Human postmortem brain tissue from AD patients show upregulation of DYRK1a (Velazquez et al., 2019).

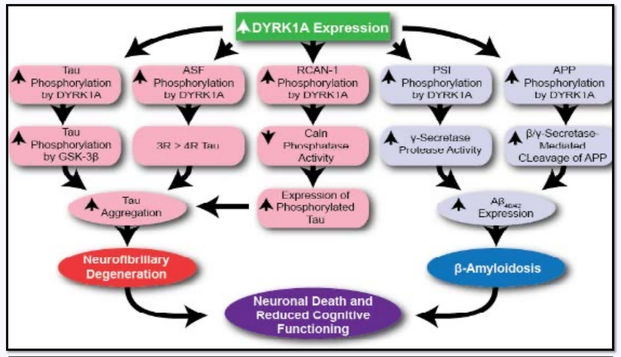

Figure 2: Various mechanistic pathways by which DYRK1a contributes to Alzheimer's Disease (T. Dunckley, 2020).

- DYRK1a can also phosphorylate the amyloid precursor protein (APP) at threonine 668, regulating amyloid precursor intracellular domain (AICD) translocation into the nucleus, contributing to neurodegeneration (Chang et al., 2006).
- We have shown that pharmacological inhibition of a novel inhibitor (DYR219) reduces both tau aggregation and Aβ pathology in the hippocampus and cortex of the 3xTg-mouse model of AD (Velazquez et al., 2019; Branca et al., 2017).
- **Here, we used an optimized version of DYR219, called DYR533, to further study the effects of DYRK1a inhibition on AD-like pathology and inflammatory molecules in the 3xTg-AD mouse model of AD.**

DYR533	DYR219
• **4-hour half-life** • **100% oral bioavailability in mice** • **S(35) score of 0.03 in Kinome scan** • **Brain to plasma ration of 0.32**	• **15-minute half-life** • **0% oral bioavailability** • **S(35) score of 0.19 in Kinome scan**

Table 1: A comparison between the DYRK1a inhibitor (DYR533) used in this current study vs. the DYRK1a inhibitor (DYR219) used in previous studies.

The Dyrk1a kinase directly phosphorylates tau at multiple serine and threonine residues.

Methods

3xTg-AD Mouse Model

Human mutations	Behavior phenotype	Aβ plaques	Tau pathology
APP^{Sw} $PSEN1^{M146V}$ $MAPT^{P301L}$	Cognitive Impairment at 6 months of age.	Aβ plaques begin at 6 months, widespread by 12 months.	Neurofibrillary tangles widespread by 12 mo.

Figure 3: The 3xTg-AD mouse model is currently one of the most widely used mouse model for AD research to study both Aβ and tau pathology. Female 3xTg-AD mice show consistent pathology, while males show inconsistent pathology. Thus, only female mice are being used for this project.

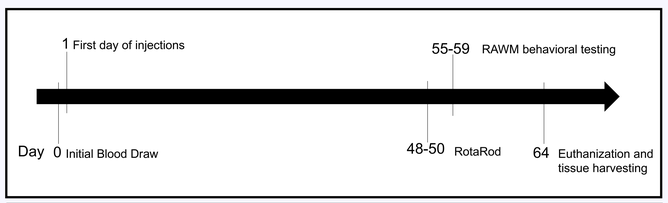

Figure 4: Timeline of experiment from initial blood draws to euthanization and tissue harvesting.

Dose	3xTg-AD	NonTg
Vehicle	15	14
1.0 mg/kg	15	14
2.5 mg/kg	15	14
5.0 mg/kg	15	14

Table 2: Female non-transgenic (NonTg) and 3xTg-AD mice were randomly assigned to one of four dosage groups. Mice begin dosage regimen at ~7 months of age for 64 days. Mice were then perfused to remove blood from the brain and brain tissue underwent neuropathological assessment.

VLoND

Scan here to learn more about this project and ongoing research from the Velazquez lab.

References

1. Velazquez R, Meechoovet B, Ow A, Foley C, Shaw A, Smith B, Oddo S, Hulme C, Dunckley T. Chronic Dyrk1 Inhibition Delays the Onset of AD-Like Pathology in 3xTg-AD Mice. Mol Neurobiol. 2019 Dec;56(12):8364-8375. doi: 10.1007/s12035-019-01684-9. Epub 2019 Jun 25. PMID: 31240602.
2. Branca, C., Shaw, D.M., Belfiore, R., Gokhale, V., Shaw, A.Y., Foley, C., Smith, B., Hulme, C., Dunckley, T., Meechoovet, B., Caccamo, A. and Oddo, S. (2017), Dyrk1 inhibition improves Alzheimer's disease-like pathology. Aging Cell, 16: 1146-1154. https://doi.org/10.1111/acel.12648
3. Wegiel, J., Gong, C. X., & Hwang, Y. W. (2011). The role of DYRK1A in neurodegenerative diseases. The FEBS journal, 278(2), 236–245. https://doi.org/10.1111/j.1742-4658.2010.07955.x
4. Mandelkow EM, Mandelkow E. Biochemistry and cell biology of tau protein in neurofibrillary degeneration. Cold Spring Harb Perspect Med. 2012 Jul;2(7):a006247. doi: 10.1101/cshperspect.a006247. PMID: 22762014; PMCID: PMC3385935.
5. Ryoo SR, Jeong HK, Radnaabazar C, Yoo JJ, Cho HJ, Lee HW, Kim IS, Cheon YH, Ahn YS, Chung SH, Song WJ (2007) DYRK1A-mediated hyperphosphorylation of Tau. A functional link between Down syndrome and Alzheimer disease. J. Biol. Chem. 282, 34850–34857.
6. Alzheimer's Association. 2022 Alzheimer's Disease Facts and Figures. Alzheimers Dement 2022;18.

Results

DYRK1a levels in the hippocampus (Hp) and cortex (Ctx) are reduced with DYR533

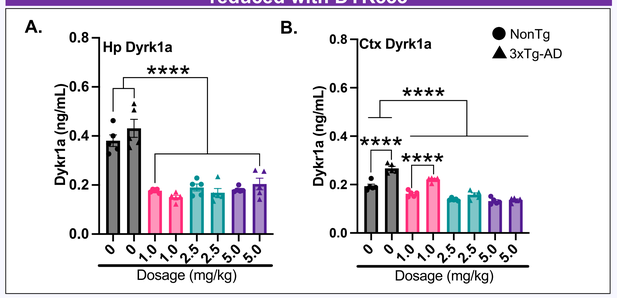

Figure 3: A. DYR533 significantly reduces the levels of Dyrk1a in the hippocampus (Hp) across all groups compared to vehicle (0 mg/kg) groups. **B.** In the cortex (Ctx), Dyrk1a is significantly elevated in 3xTg-AD mice compared to NonTg mice. DYR533 significantly reduces levels of Dyrk1a. **** $p < 0.0001$.

Cortical soluble Amyloid-β_{42} levels are reduced with DYR533

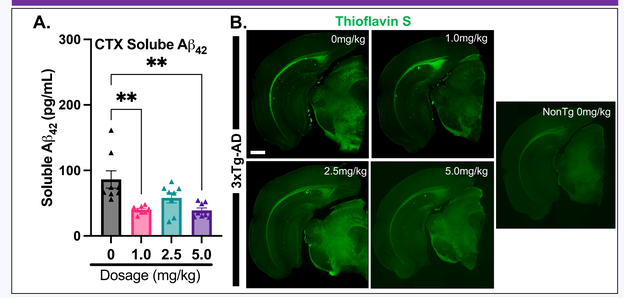

Figure 4: A. DYR533 significantly reduces the levels of Ctx soluble Aβ42 in 3xTg-AD mice. No other significant effects were detected in soluble or insoluble Aβ40 and 42 within the Hp and Ctx (graphs not shown). **B.** Photomicrographs depicting thioflavin S staining among the 3xTg-AD treatment groups. Scale bar = 1000μm. ** $p < 0.01$.

Early phosphorylated (p) tau at threonine 217 is reduced in the Cortex of DYR533 treated mice

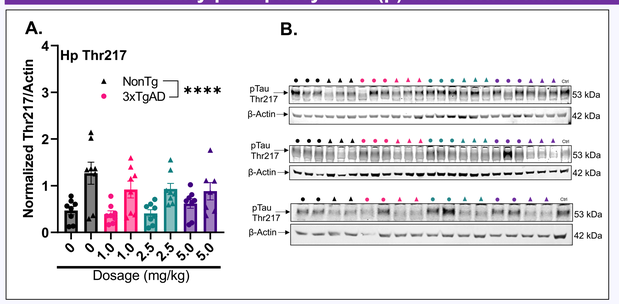

Figure 5: A. 3xTg-AD mice show significantly increased levels of Hp phosphorylated (p) tau at threonine (Thr) 217 compared to the NonTg mice. **B.** Western blot of pTau at Thr217 and loading control β-Actin. **** $p < 0.0001$. "Ctrl" represents a between gel control of pooled Hp samples.

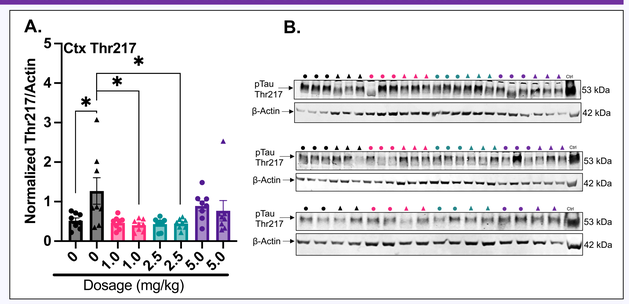

Figure 6: A. 3xTg-AD mice show significantly elevated Ctx pTau Thr217 compared to NonTg mice. DYR533 significantly reduces pTau Thr217 in the 1.0 and 2.5 mg/kg groups. **B.** Western blot of pTau at Thr217 and loading control β-Actin. * $p < 0.05$. "Ctrl" represents a between gel control of pooled Ctx samples.

Soluble and insoluble fractions of ptau at serine 396 is reduced in the hippocampus and cortex of DYR533 treated mice

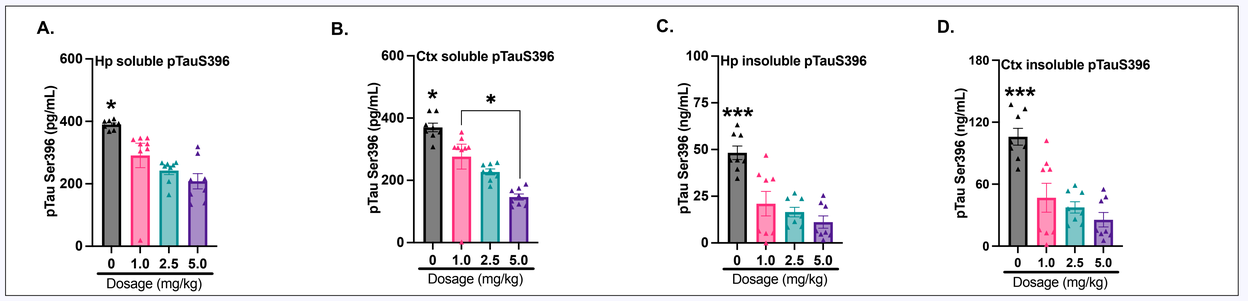

Figure 7: A. DYR533 significantly reduces the levels of Hp soluble pTau at Serine (S) 396 in 3xTg-AD mice. **B.** DYR533 significantly reduces Ctx soluble pTauS396 in 3xTg-AD mice. Notably, the 5.0mg/kg group exhibits significantly lower levels of pTauS396 compared to the 1.0mg/kg group. **C-D.** DYR533 significantly reduces both Hp and Ctx insoluble levels of pTauS396 in all dosed groups compared to the vehicle (0mg/kg) groups. * $p < 0.05$, *** $p < 0.001$.

DYR533 reduces pro-inflammatory cytokine Tumour Necrosis Factor alpha (TNF α) in blood, hippocampus and cortical samples

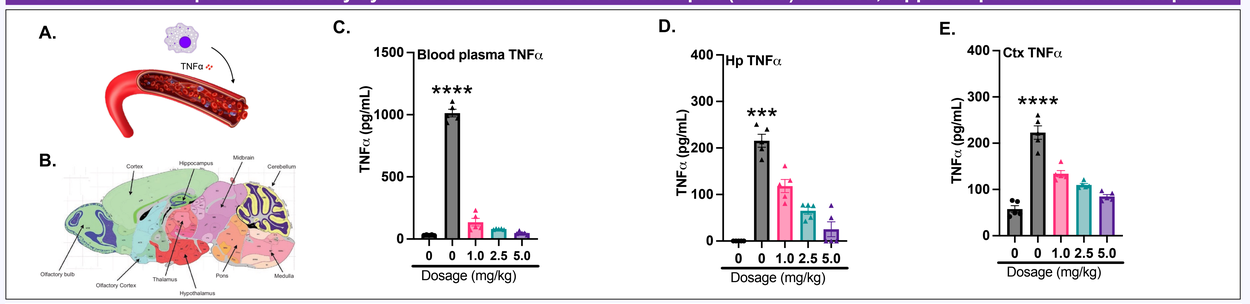

Figure 8: A, B. Plasma from blood was extracted to measure tumorous necrosis factor α, a pro-inflammatory cytokine. Hippocampal (Hp) and cortex (Ctx) brain homogenates were obtained to measure TNFα. **C.** 3xTg-AD mice show significantly elevated plasma TNFα compared to NonTg 0mg/kg (control) mice and DYR533 reduced levels in 3xTg-AD mice to that of NonTg levels. **D, E.** 3xTg-AD mice show significantly elevated Hp and Ctx TNFα compared to NonTg control mice and DYR533 reduced levels in 3xTg-AD mice to that on NonTg levels in both brain regions.

Conclusion

- Our results show that DYR533 reduces DYRK1a, cortical $A\beta_{42}$, ptau at threonine 217, serine396, and TNF-α in the 3xTg-AD mouse model of Alzheimer's Disease.
- These results may help springboard clinical testing of DYR533 as a therapeutic for AD and related tauopathies.