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JULY 27-31 > TORONTO, CANADA, AND ONLINE

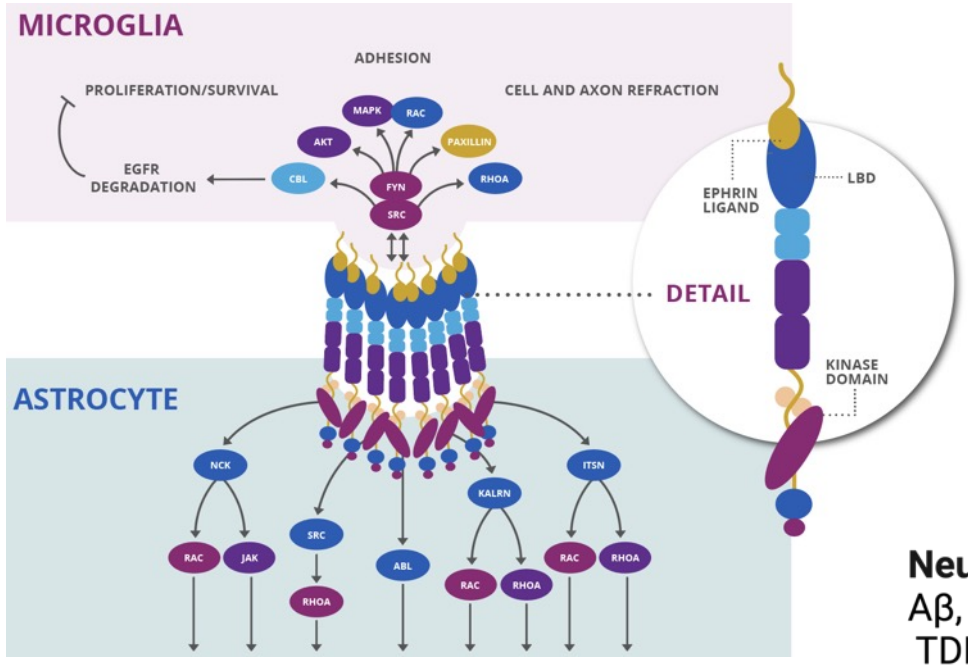
Novel Small Molecule EPHB3 Inhibitors to Treat Neurodegenerative Disease by Targeting Astrocyte-Mediated Disease Mechanisms

Evan Lebois, PhD

Disclosures

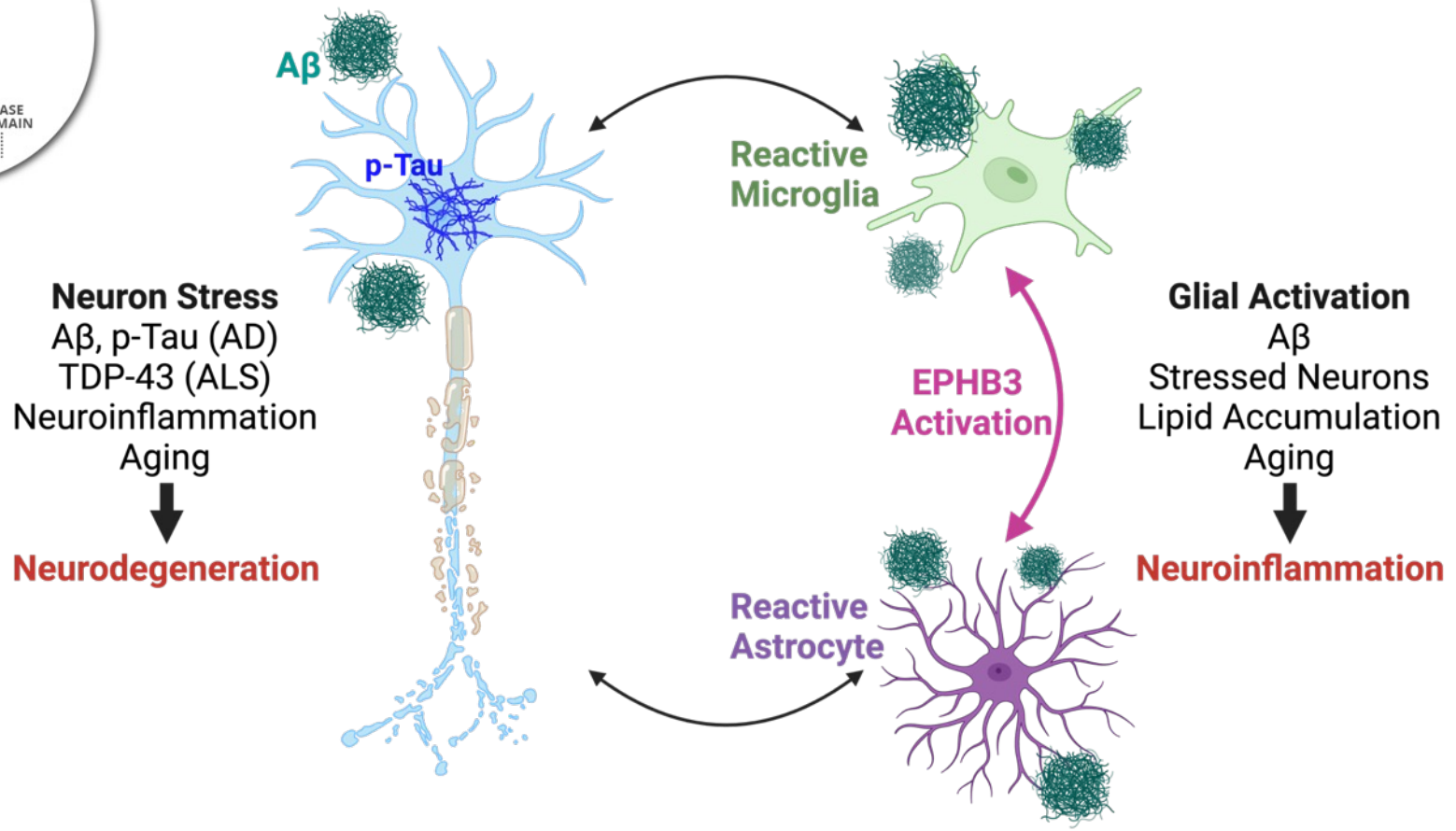
- Employee of Violet Therapeutics with stock options

EPHB3 is an astrocyte receptor tyrosine kinase that facilitates interactions with microglia to drive neuroinflammation



Classical role: neuronal development and axon guidance

New role: regulator of astrocyte-microglial interactions



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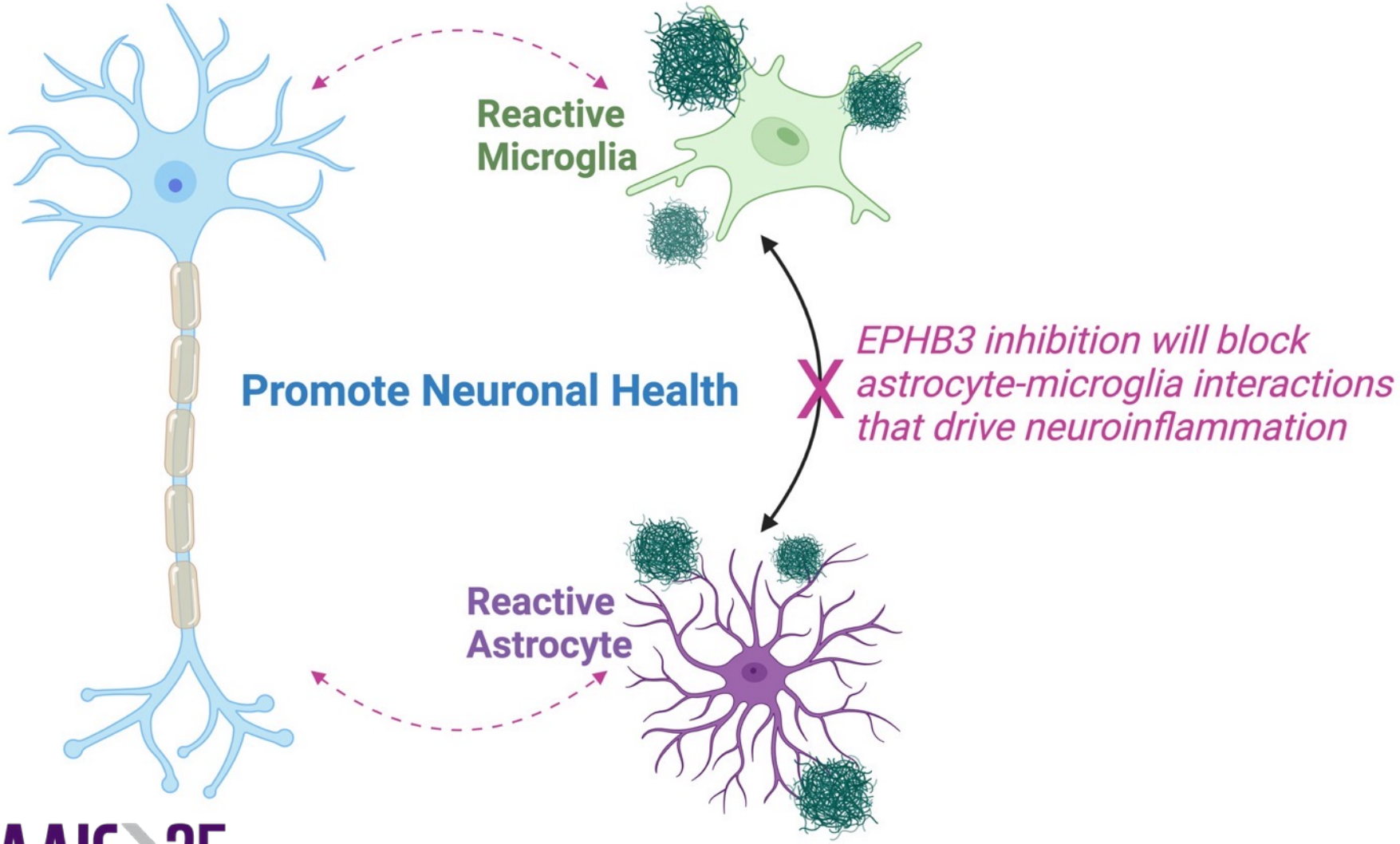
The views and opinions expressed do not necessarily reflect the Alzheimer's Association.

Clark, IC, Gutiérrez-Vázquez, C, Wheeler, MA...Quintana, FJ. 2021. Science. 372(6540): eabf1230.

Therapeutic Hypothesis: EPHB3 inhibition will promote neuron health by blocking astrocyte-microglia interactions that drive neuroinflammation



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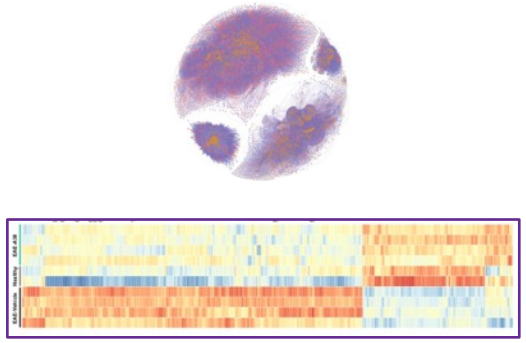
Construction of EPHB3 astrocyte activation score and in silico mapping to human disease and mouse model datasets

Target ID

RABID-seq identified EPHB3 activation in astrocytes as neuroinflammation driver in EAE

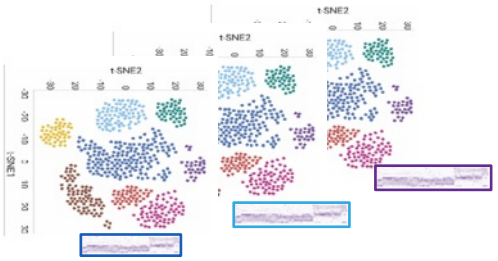
Target signature

162 transcriptional changes that occur in Astrocytes when EPHB3 is activated



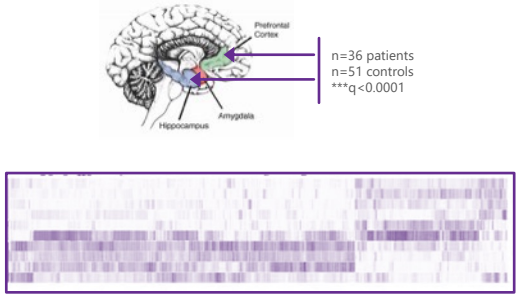
Mine human and mouse data sets

Bulk and scRNA-seq datasets are mined for target signature



Indication and mouse model selection

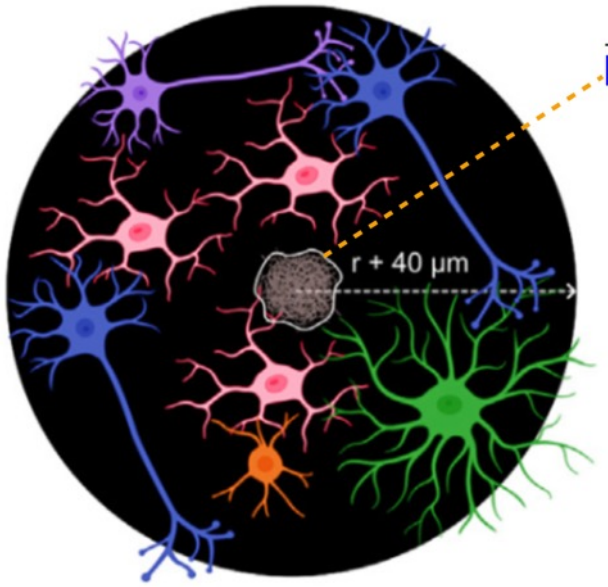
Target signature is found in human disease and mouse model data sets

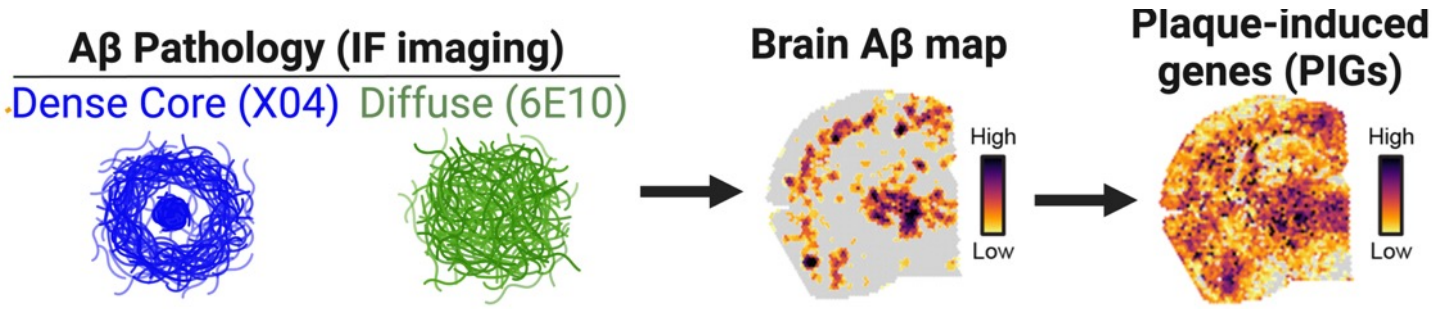


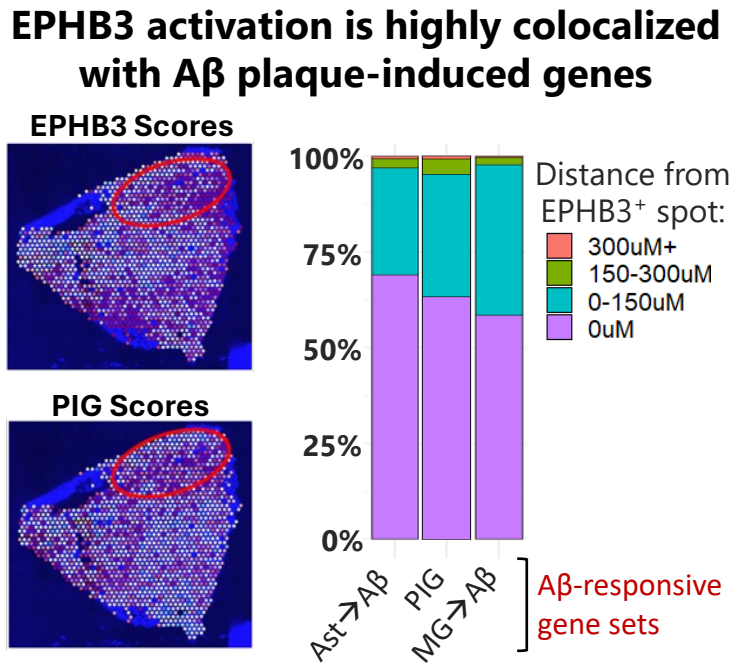
Aim = map EPHB3 activation to:

1. human disease data sets for indication relevance
2. in vivo mouse model data sets for therapeutic development

EPHB3 activation is highly co-localized and correlated with Aβ plaque-induced gene (PIG) response in AD, revealed by spatial transcriptomics







Key Takeaways:

- Astrocyte and MG gene expression altered around Aβ plaques
- This drives neuroinflammation
- EPHB3 activation happens in the same place

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Miyoshi, E, … Swarup, V. 2024. Nature Genetics. 56: 2704 – 2717.
Mallach, A, … De Strooper, B. 2024. Cell Reports. 43(6): 114216.

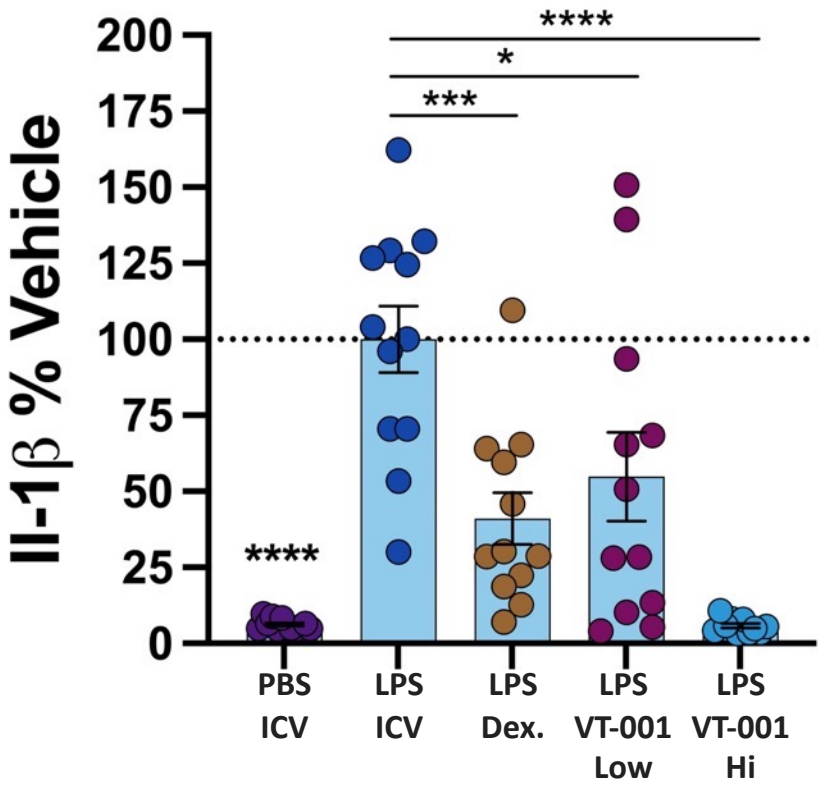
Highly selective, brain penetrant tool compound VT-001 shows robust pharmacology and in vivo efficacy in ICV LPS assay

Drug-like small molecule with excellent selectivity

	Selected assay/ Target	VT-001
Properties	CNS MPO[1]	5
	Kinetic solubility (7.4, uM)	192
	MW, cLogD	<410, <1
	PPB % unbound (m, r, d, c, h)	64, 71, 65, 61, 51
Biochemical	EphB3 (IC50, uM)	0.048
	Carna NanoBRET (196 kinases)	1/240 (EPHB3)
Kpuu	Rat	40%
In vitro tox	CYPs	< 50% @ 10uM
	hERG	37% @ 10uM
	SafetyScreen44	1/44 (3 µM AChE)

1. CNS MPO: combination of cLogp/ cLogD/ MW/ TPSA/ HBD/ pKa
2. ER – extraction ratio. Predicted Clp/hepatic blood flow; in vivo Clp/ hepatic blood flow

Highly efficacious in acute neuroinflammation assay: LPS



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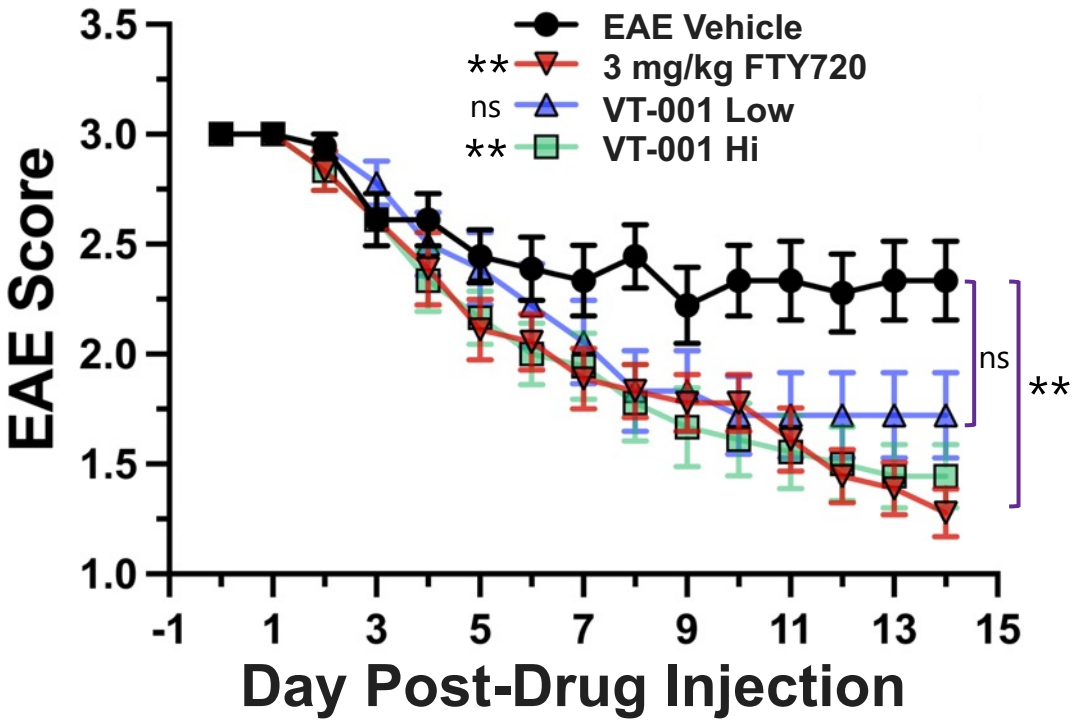
VT-001 significantly rescues clinical EAE score deficits in mice

Previously at ADPD 2025

Dosing initiated at peak EAE



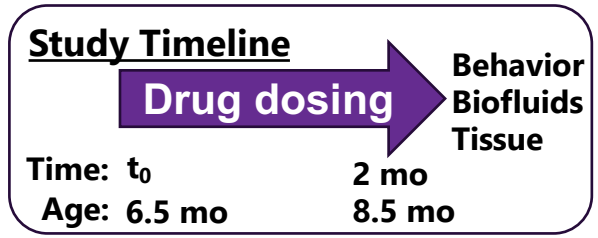
scRNA-seq in total spinal cord showed in astrocytes:

- ✓ VT-001 decreased EPHB3 activation scores, pro-inflammatory, and reactive astrocyte gene expression signatures

Global proteomics in total spinal cord showed:

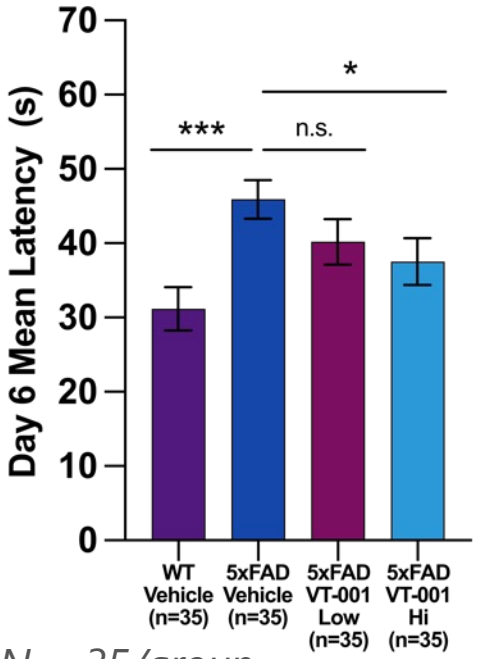
- ✓ VT-001 significantly upregulated synaptic signaling pathways and suppressed immune pathways
- ✓ Restored neuronal health and decreased inflammation

VT-001 rescues cognitive deficits and Aβ plaque-induced gene (PIG) expression signature in cortex of 5xFAD mice (2 mo)

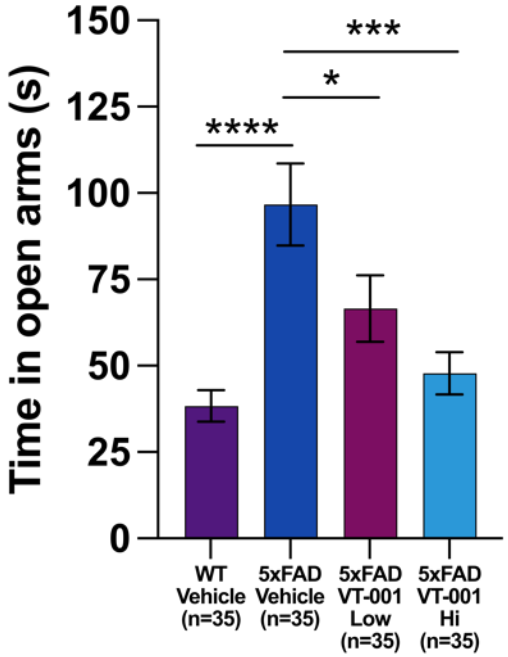


Behavioral efficacy

Morris water maze



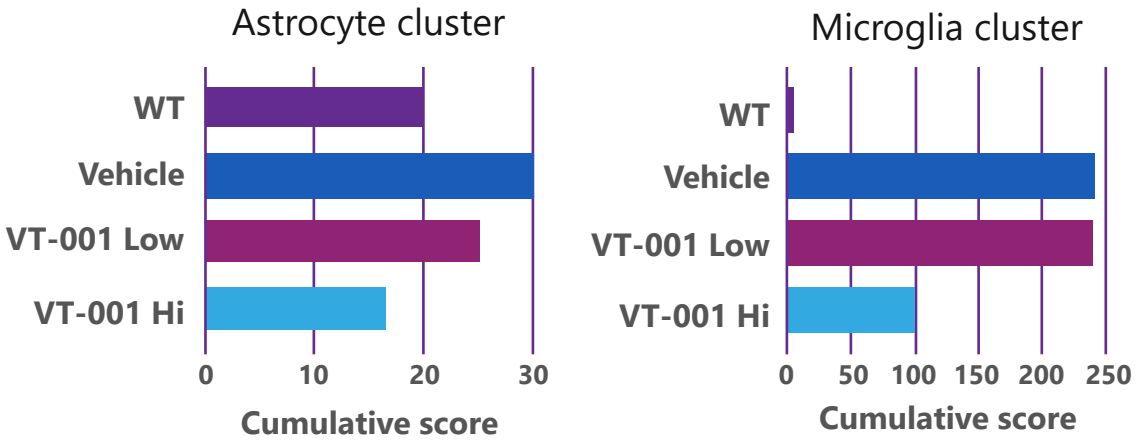
Elevated plus maze



N = 35/group

Reduced astrocyte and microglia Aβ plaque-induced gene expression in cortex (snRNA-seq)

Aβ plaque induced gene (PIG) response



Astrocytes in 5xFAD mice have **increased plaque inflammatory response**

- Inflammation local to Aβ plaques damaging to neurons that represents pathogenic astrocyte-microglia crosstalk

VT-001 attenuates astrocyte and microglia inflammatory response

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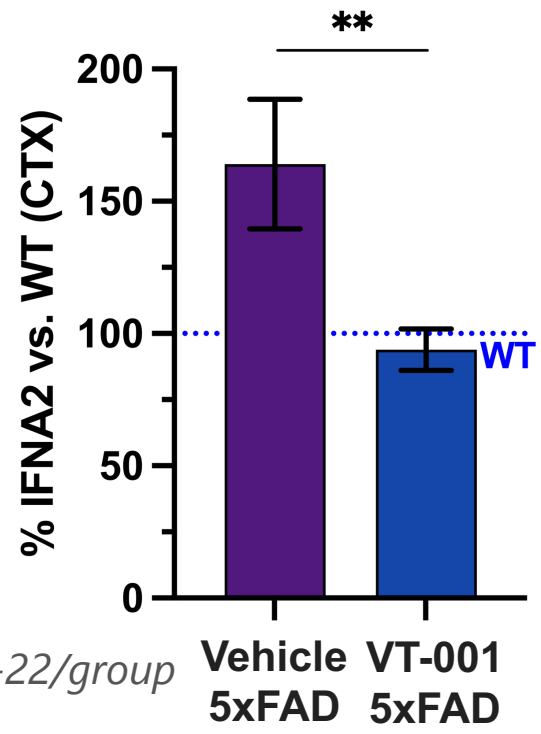
Mallach, A, … De Strooper, B. 2024. Cell Reports. 43(6): 114216.

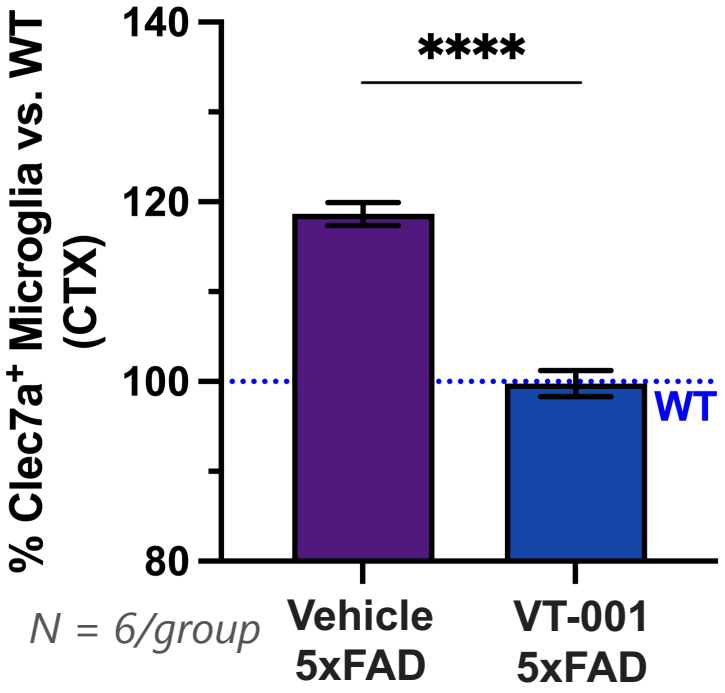
VT-001 blocks type I IFN inflammation in 5xFAD cortex, together with associated microglia and astrocyte gene signatures, linked to AD synapse loss

Type I IFN Inflammation (NULISA)



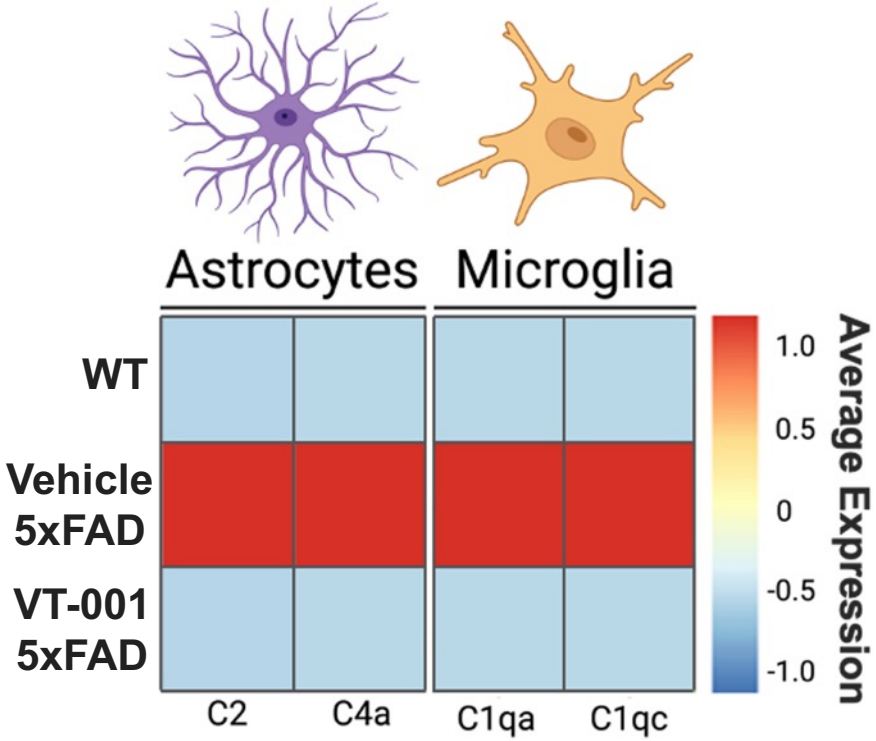
N = 20-22/group

Clec7a⁺ Microglia (snRNA-seq)



N = 6/group

Complement Component Expression (snRNA-seq)



VT-001 decreases inflammatory mechanisms in brain linked to AD synapse loss

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Johnson, ECB, … Seyfried, NT. 2022. Nature Neuroscience. 25: 213 – 225.

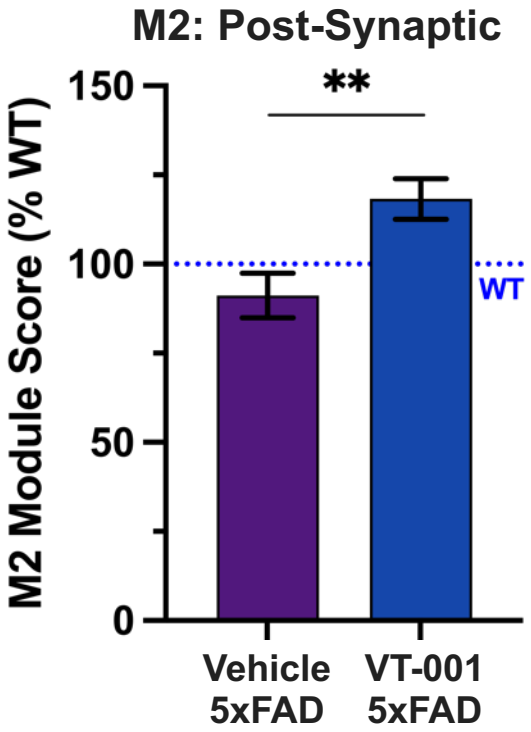
VT-001 rescues AD CSF synapse protein signatures in 5xFAD CSF

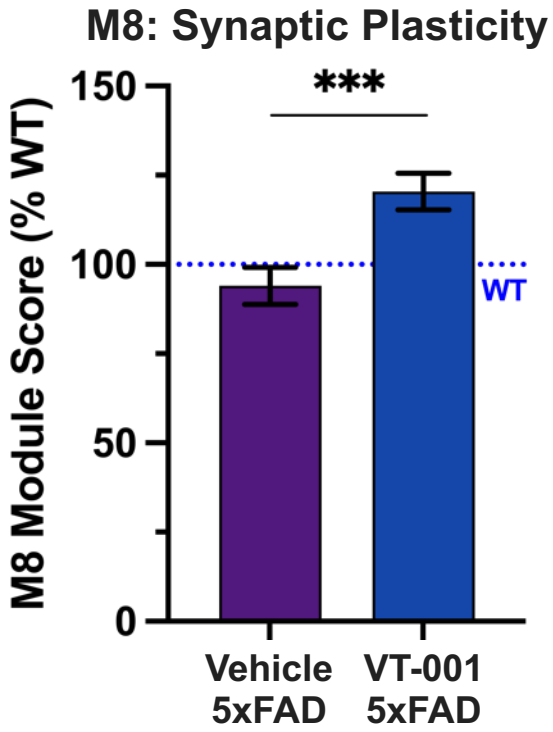
CSF

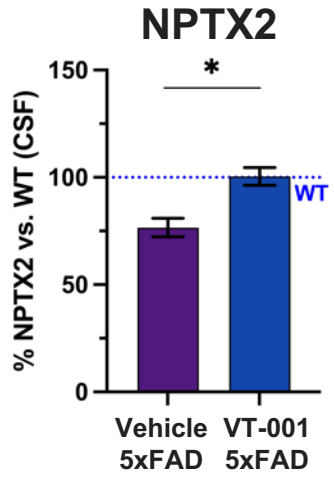
Unbiased proteomics

N = 35/group

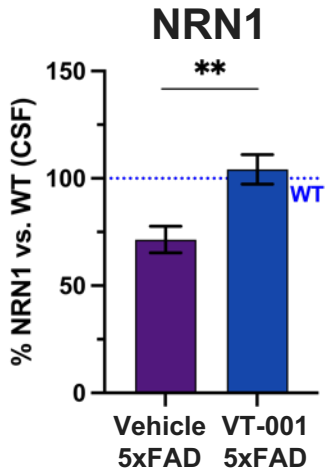
AD CSF Synapse Protein Signatures







Top AD protein decreased with cognitive impairment



Top AD resilience protein

VT-001 significantly rescues the levels of key human AD synaptic proteins in 5xFAD CSF: efficacy biomarkers

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CSF: Bangs, MC, … Seyfried, NT. 2025. bioRxiv. doi: 10.1101/2025.03.14.643332

VT-001 rescues type I interferon inflammation and synaptic biomarkers in 5xFAD plasma

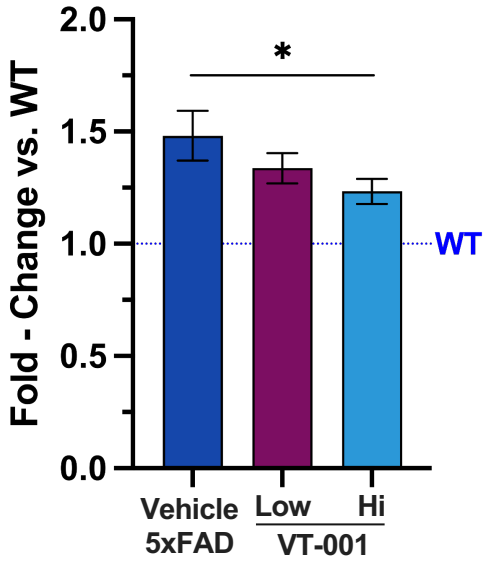
Plasma

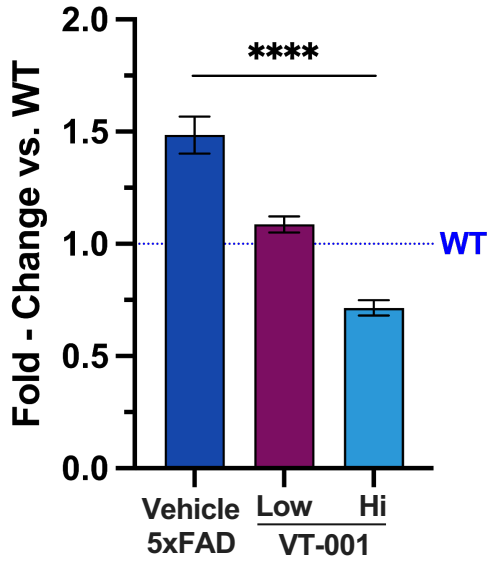
Targeted proteomics (NULISA CNS120)

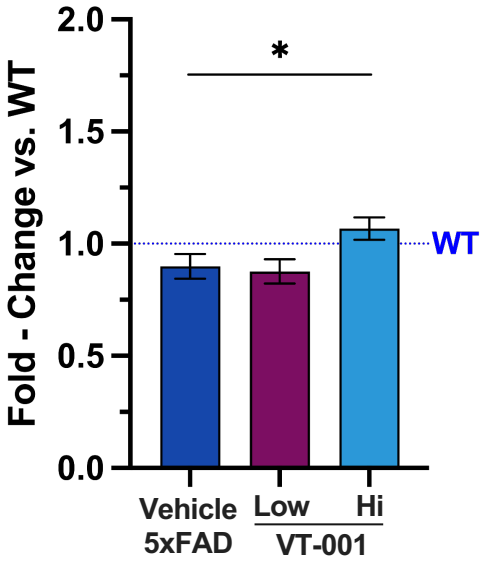
N = 20-22/group

Decreased by VT-001

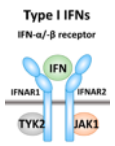
Increased by VT-001



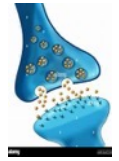




Astrocyte



Type I interferon



Synapse

VT-001 decreases inflammatory mechanisms linked to synapse loss and rescues synapse markers in plasma

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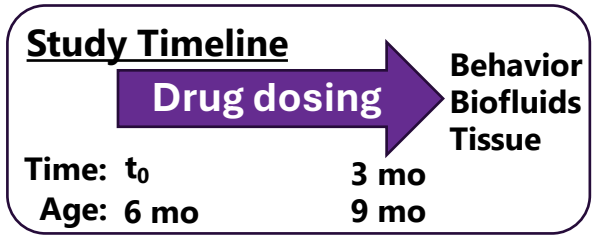
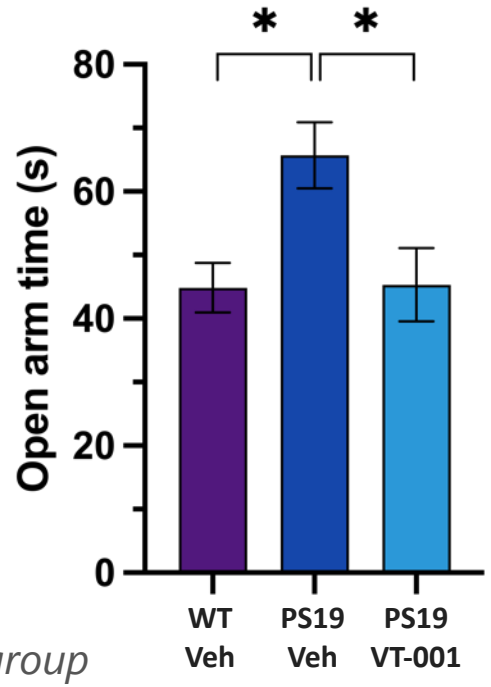
VT-001 is highly efficacious in PS19 tauopathy mice (3 mo)

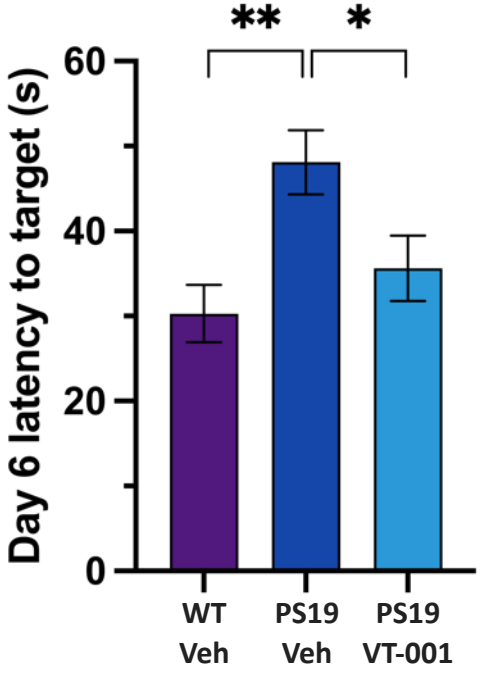


Elevated Plus Maze (EPM)

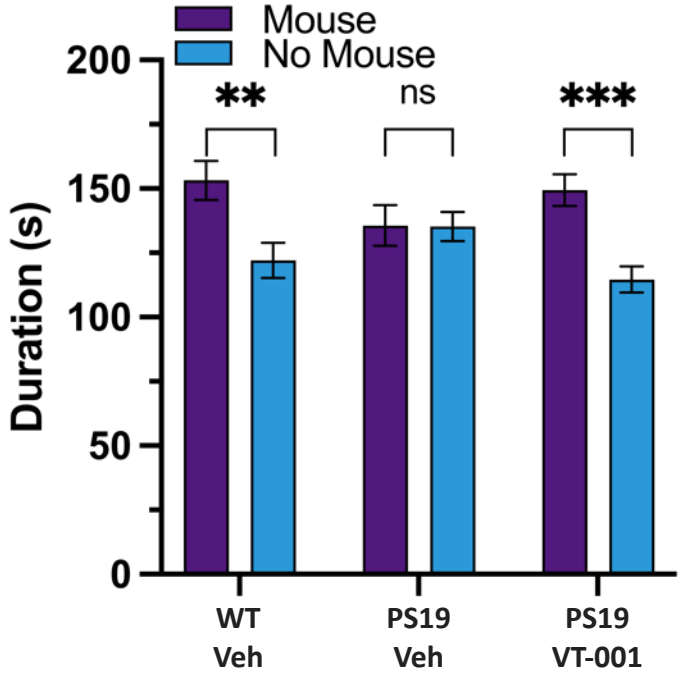


N = 35/group

Morris Water Maze (MWM)



Social Preference Test (SPT)



VT-001 prevents cognitive deficits in PS19 tauopathy mice in multiple orthogonal behavior assays (3 months dosing)

Conclusions and next steps

Overall Conclusion: VT-001 is highly efficacious in vivo in LPS, EAE, 5xFAD, and PS19 models

- ✓ In 5xFAD mice VT-001 significantly:
  - rescues cognitive deficits in MWM and EPM
  - reduces Aβ PIG expression in cortex astrocytes and microglia (snRNA-seq)
  - blocks type I IFN inflammation-associated mechanisms at protein and RNA levels:
    - Blocks type I IFN levels in cortex (NULISA)
    - Blocks Clec7a+ microglial state in cortex: known driver of type I IFN inflammation and AD synapse loss (snRNA-seq)
    - blocks complement component expression by astrocytes and microglia (snRNA-seq)
  - rescues known human AD synaptic biomarkers strongly linked to cognitive impairment (CSF, plasma proteomics)
- ✓ In PS19 mice VT-001 significantly rescues cognitive deficits in MWM, EPM, and SPT

Next Steps

1. Neurohistology in 5xFAD and PS19 models to quantify synapse density
2. Brain proteomics in 5xFAD, also brain + CSF proteomics in PS19 to identify VT-001 biomarkers
3. Spatial transcriptomics to establish VT-001 MOA local to Aβ and tau pathology

Thank you

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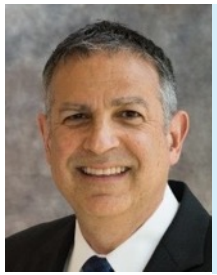

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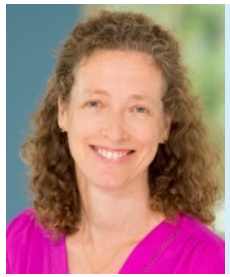

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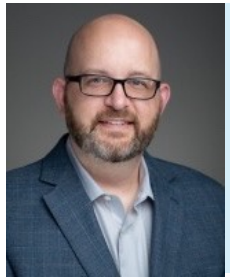

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