Efficacy of a Vectorized Anti-Tau Antibody Using Systemic Dosing of a Blood Brain Barrier Penetrant AAV Capsid in Mouse Models of Tauopathies

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Abstract No. 105

Voyager THERAPEUTICS

Disclosure

- Wencheng Liu is a full-time employee of Voyager Therapeutics, Cambridge, MA, USA

Antibody Vectorization:
Monoclonal Antibody Delivery via AAV Gene Therapy

Vectorized Antibodies

Targeted antibody therapies have been revolutionary solutions for medicine (e.g., oncology, inflammation), but similar efforts in neurological indications (e.g., AD, tauopathies, synucleinopathies) have been met with significant challenges

Challenges Today

- **Delivery to CNS** with passive immunotherapy is **very limited** (i.e., 0.1% of Abs pass through BBB)
- Inability to target the **intracellular proteome**
- Delivery of **antibody fragments** remains a challenge due to **PK liabilities**
- Potential for unspecific or **toxic off-target effects**
- Prohibitive **manufacturing volumes & costs**

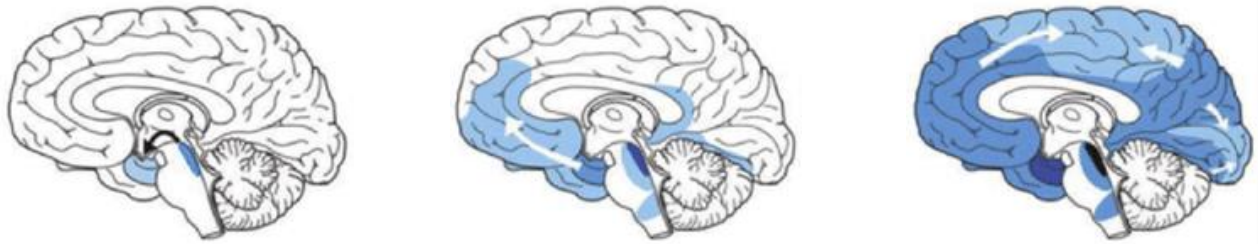
Voyager's Solutions

- **Modular Antibody Vectorization Cassettes** with cell-specific or general expression
- In-house PoC for **serum delivery through muscle** expression of full-length Abs
- Active research in **drugging intracellular proteome** through vectorized nanobodies, degraders, and other innovative platforms

Spreading Mechanism Hypothesis May Provide Opportunity for Therapeutic Antibody Intervention – Tauopathies/AD

Distribution of Tau Pathology by Stage

Correlates with Neuroanatomical Connectivity



AD Braak Staging:	I, II	III, IV	V, VI

Tauopathies: Progressive neurodegenerative diseases defined by the presence of tau aggregates in the brain

- Alzheimer's Disease
- Frontotemporal Dementia (includes MAPT mutations)
- Progressive Supranuclear Palsy
- Corticobasal Syndrome

Current Treatments Are Symptomatic-only

- Abnormal tau accumulation is a hallmark of AD, FTD, PSP, CBS (including neurofibrillary tangles in AD)
- Tau pathology closely correlates with disease progression and cognitive decline in AD
- Imaging agents are now available to visualize pathological proteinopathies

Significant **limitations to passive immunization**, including:

- Modest (20-40%) reductions in tau pathology in animal models
- Requirement for high doses of antibody to be administered weekly or monthly
- Very low levels of antibody achieved in the brain that may limit efficacy
- Limited intraneuronal antibody exposure
- Antibody fragments cannot be used due to pharmacokinetic half-life liabilities

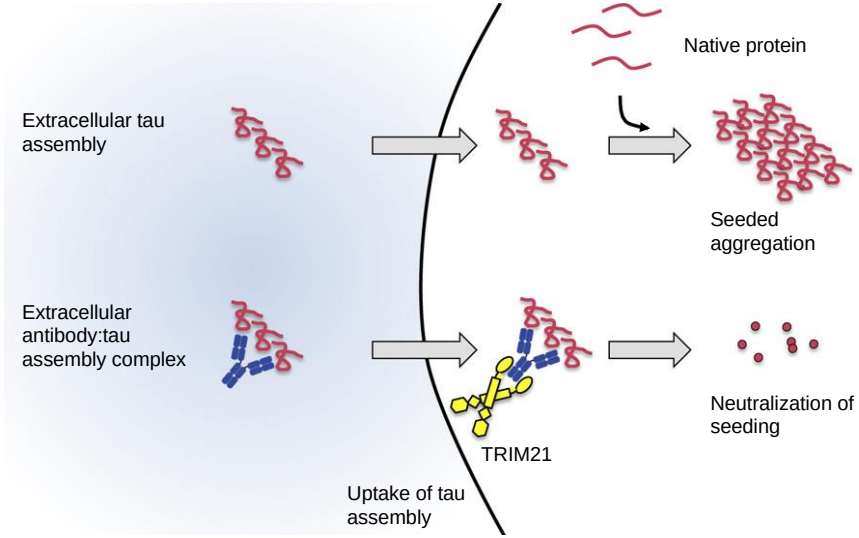
Adapted from Jouanne M, Rault S, Voisin-Chiret AS. *Eur J Med Chem.* 2017 Oct 20;139:153-167.

Two Potential Pathogenic Mechanisms

Cell Autonomous

Intracellular Therapeutic Activity

- Can deliver non-secreted intrabodies with either cell-type specific or pan-cell type capsids/promoters
- Voyager exploring use of intrabody efforts are early stage

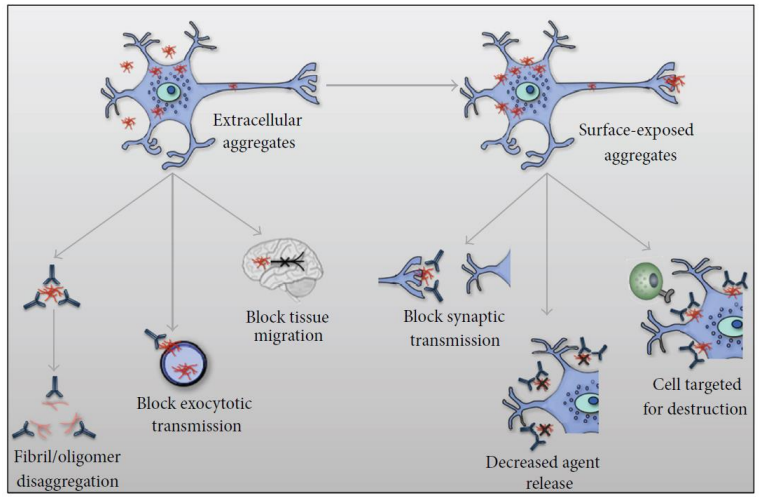


Adapted from McEwan WA, et al. *Proc Natl Acad Sci USA.* 2017 Jan 17;114(3):574-579.

Cell Non-Autonomous

Extracellular Therapeutic Activity

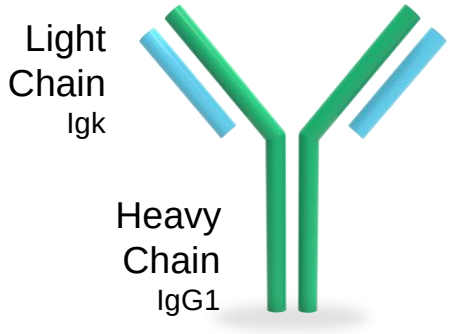
- Can deliver as secreted antibody
- Tau and alpha-synuclein programs represent basic PoC for rodent delivery and antibody expression in CNS
- Could include both prion-like spread as well as other extracellular cell-to-cell mechanisms

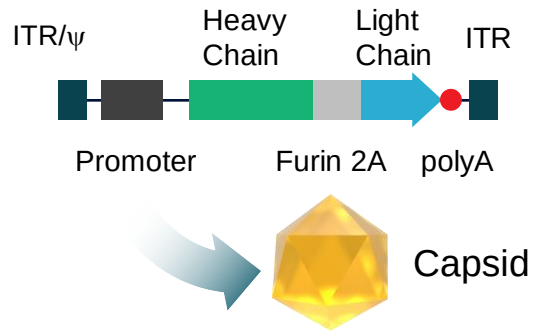


Adapted from Marciniuk K, Ryan Taschuk R, Napper S. *Clin. Develop. Immunol.* 2013, Id. 473706

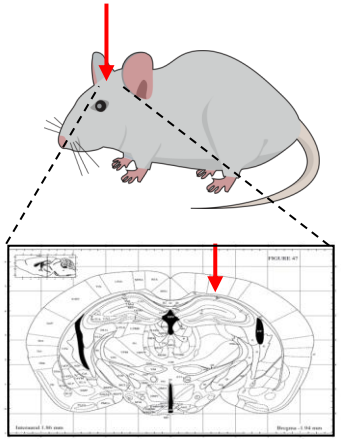
AAVrh.10PHF1 Treatment Reduces Insoluble p-Tau in the Hippocampus of P301S Mice

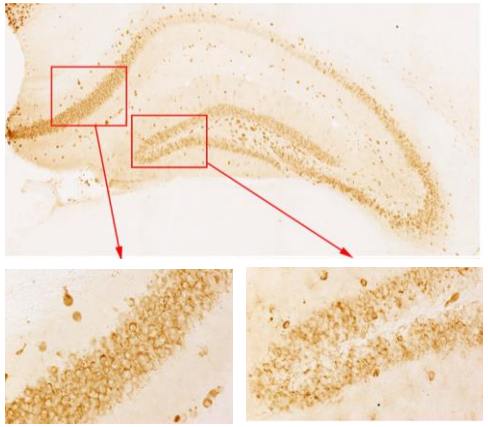
Vectorized PHF1 Antibody



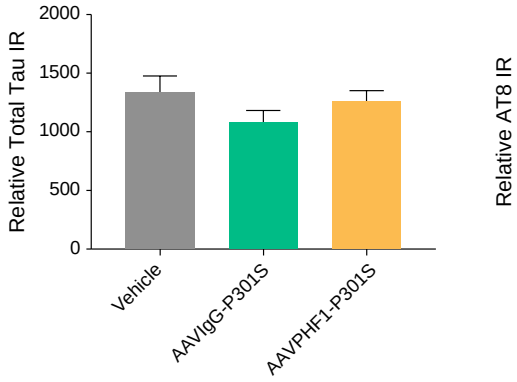


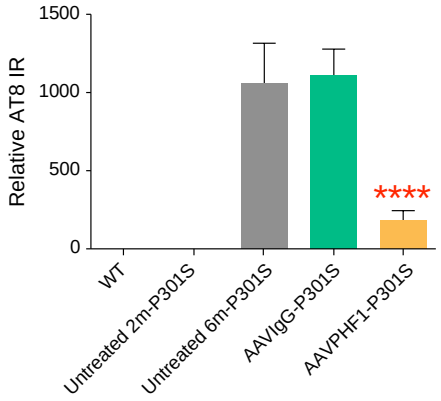
PHF1 Distribution in Hippocampus

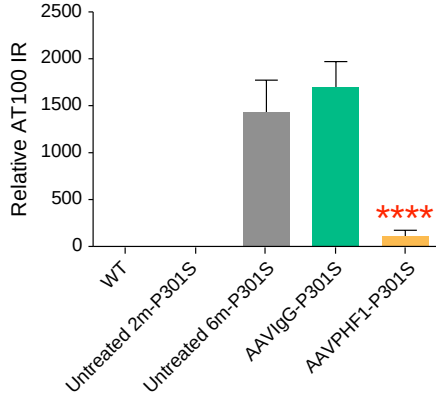




Efficacy Against Insoluble PHF





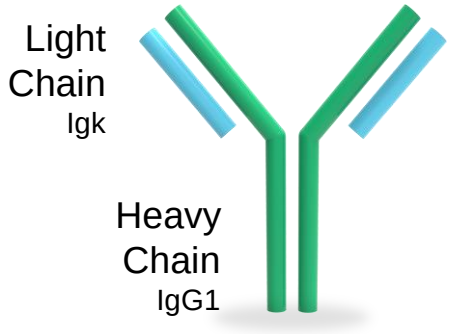


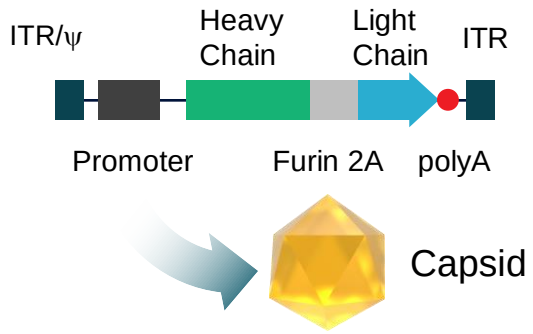
Total Tau Levels

Insoluble PHF Levels

Optimization of Vectorized Antibodies

Vectorized Antibody Example





Promoters Evaluated

Promoter	Expected Expression
CAG	Ubiquitous
CBA	Ubiquitous
GFAP	Astrocyte Specific
Synapsin	Neuron Specific

Components for Optimization

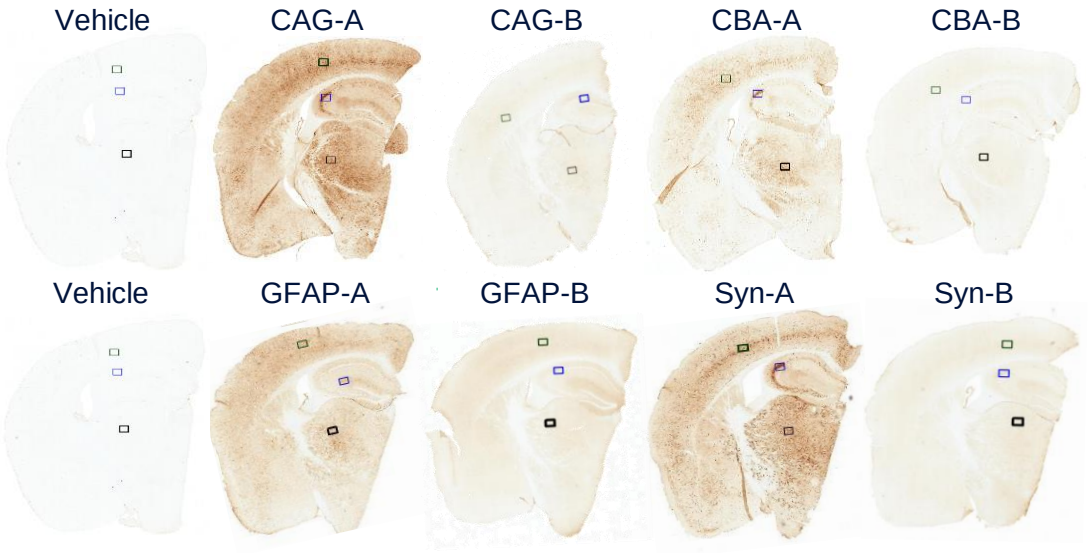
- Intron
- Signal peptide sequences
- Ab H- and L-chain order
- Ab H- and L-chain codons
- 2A or IRES site
- Cleavage site
- Poly-A
- Stuffer sequence (if needed)

Vectorized Anti-Tau Antibody Delivery Results in Promoter-Driven, Cell-Specific Expression in Mouse CNS

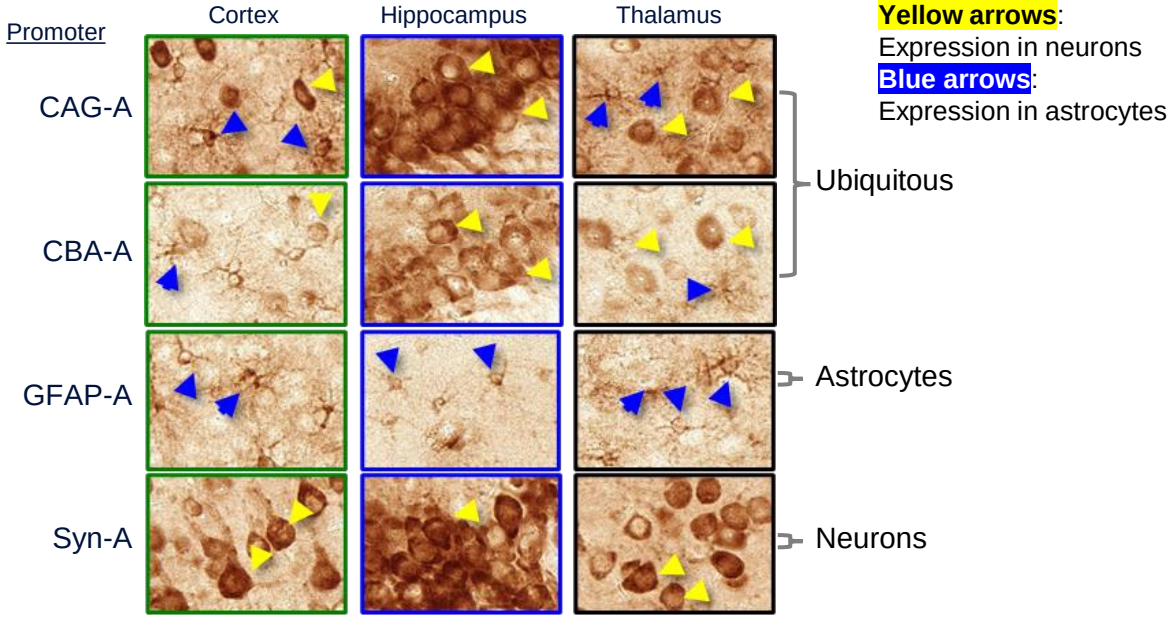
Experiment Design

- Dose: 1.4E13 vg/kg, ssAAV101.anti-tau Ab
- Route: IV bolus
- In-life duration: 4 weeks
- (Anti-IgG IHC for anti-tau Ab expression)

Low magnification (2X) of brain hemispheres from mice dosed with different promoters/constructs showing stronger staining with Construct A



Higher Magnification (40X) showing cell-specific expression in multiple brain regions



Source: Wencheng Liu, Oral Presentation, ASGCT 2019

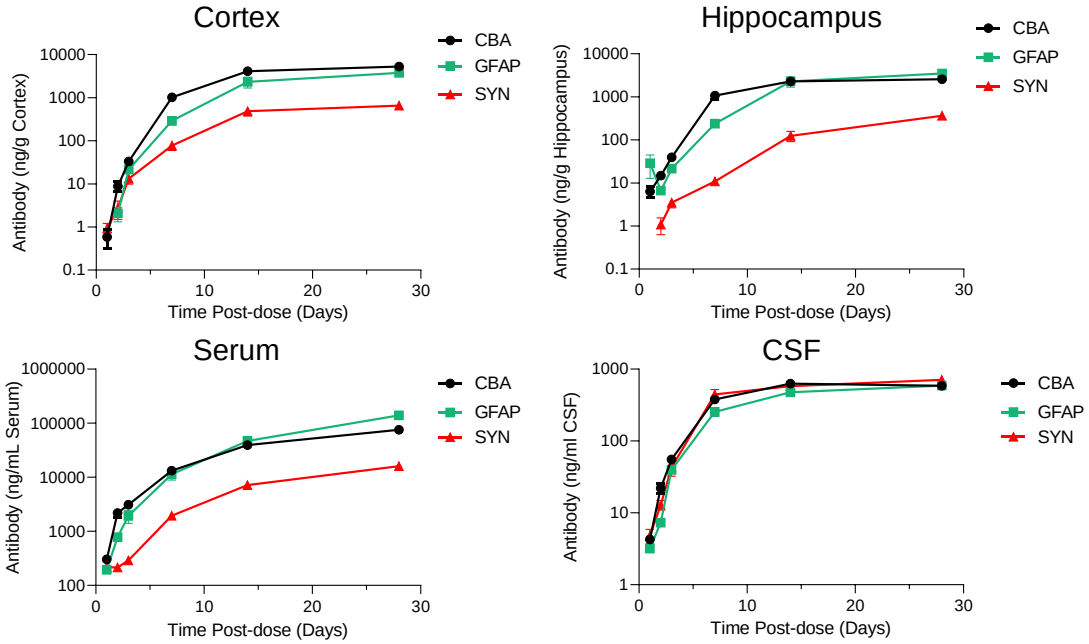
IV Dosing with Full-Length Anti-Tau Ab Vectors Achieves High Levels of the Antibody Expression in Mouse CNS

Experiment Design

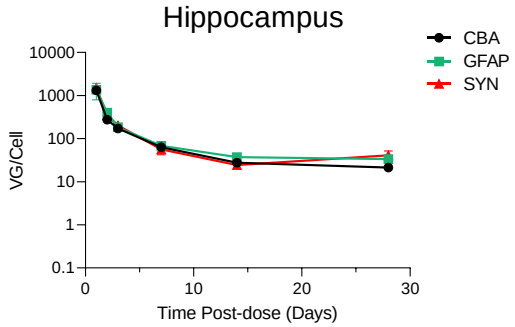
- Dose: 1.4E13 vg/kg, ssAAV101.anti-tau Ab
- Route: IV bolus
- In-life duration: 1, 2, 3, 7, 14 and 28 days
- Vectors: CBA-, GFAP, and SYN anti-tau Ab

Results

Kinetics of Antibody Expression

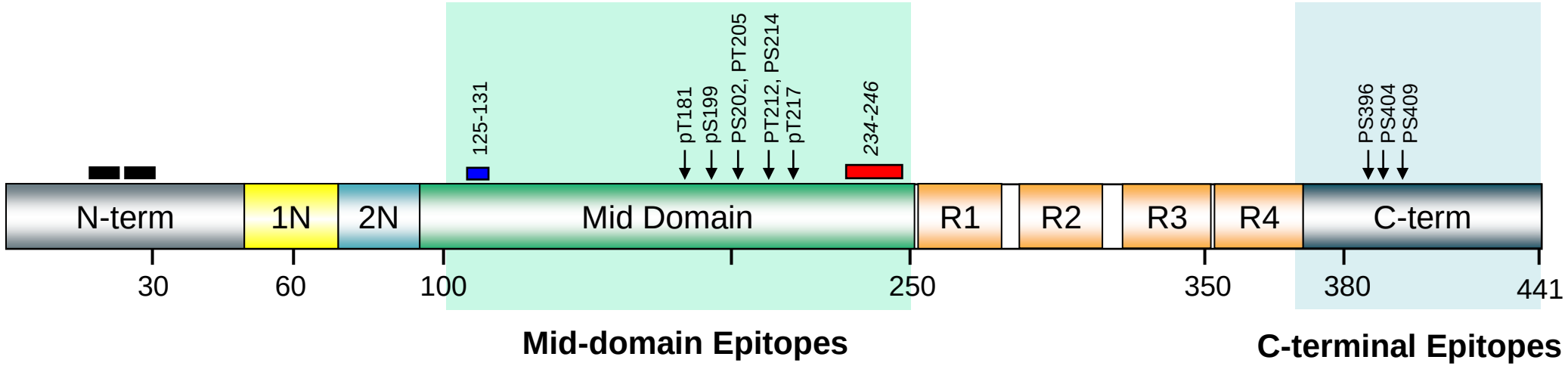


Kinetics of VG in Hippocampus



- Antibody expression detected as early as 2 days post-dose, approaches maximum levels at 7 days, and plateaus at 14-28 days following IV administration
- Concentrations of antibody stabilized at 2750 ng/g tissue (CBA and GFAP promoters) and 450 ng/g tissue (SYN promoter); AAV biodistribution stabilized at 30 vg/cell in hippocampus

Evaluation of Vectorized Tau Antibodies in Mouse Models of Tauopathies

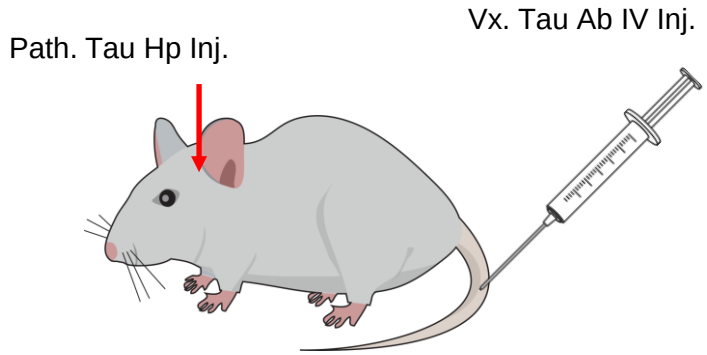


- Tau is an abundant soluble cytoplasmic protein that binds to microtubules to promote microtubule stability and function
- In AD and other tauopathies, tau aggregates become hyperphosphorylated and form insoluble NFTs.
- Passive immunization targeting tau has emerged as one of the more promising approaches for reducing or preventing tau pathology
  - N-terminal epitopes: have failed to show beneficial effects on patients in several clinical trials with PSP (AbbVie and Biogen) and AD (Roche)
  - Mid-domain epitopes: have emerged as one of major targeted domains (Biogen, J&J, UCB/Genentech/Roche, AD/PD 2021)
- Vectorized anti-tau antibodies targeting two different phosphorylated tau domains: Mid domain and C-terminal
- Used mouse BBB-penetrant capsid to deliver vectorized antibodies to evaluate efficacy in animal models

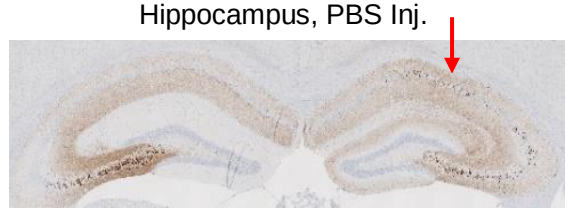
Mouse Tauopathy Models for Efficacy Studies

P301S Hippocampal Seeding Model

- FTD Mutant Tau TG Animals
- Seeding into Hippocampus
- Vector IV dosed -2 weeks (anti-seeding)
- Experiment terminated +6 weeks
- Critical Readout: AT100 stained cells in Ipsilateral Hippocampus (contra also examined)

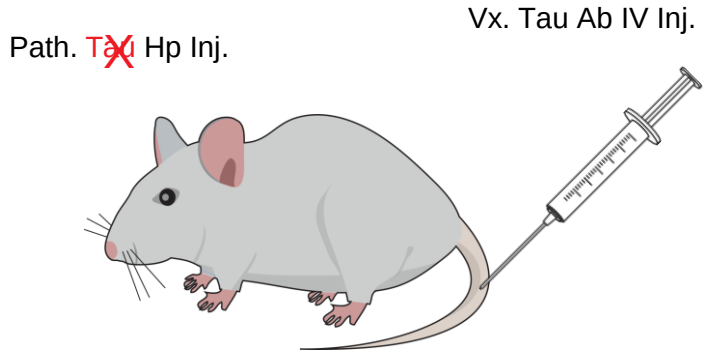


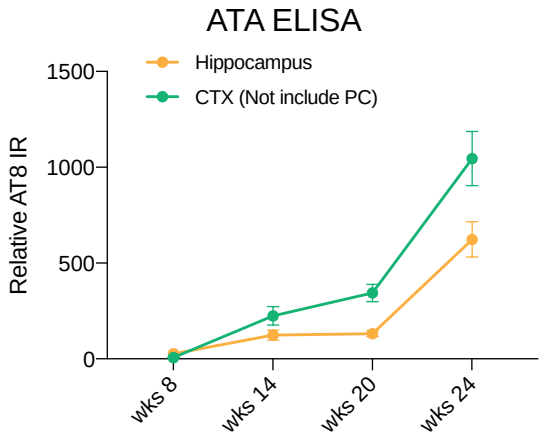

Hippocampus, PBS Inj.


Hippocampus, AD Tau Seed Inj.

P301S Intrinsic Model

- FTD Mutant Tau TG Animals
- No seeding
- Vector IV dosed at age weeks
- Experiment terminated +3 weeks
- Critical Readout: AT8 ELISA or AT100 stained cells in Hippocampus





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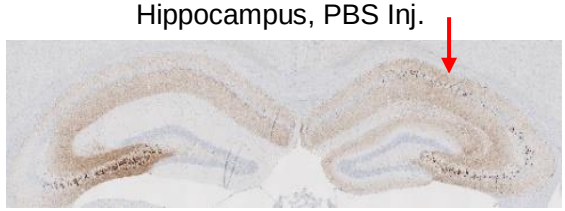
Hippocampus, PBS Inj.



Hippocampus, AD Tau Seed Inj.

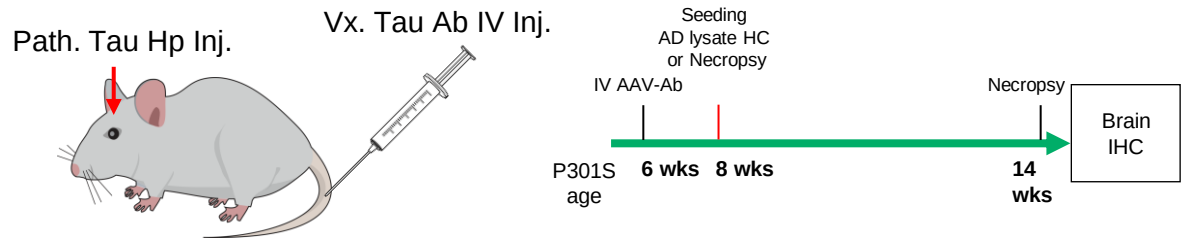
P301S Intrinsic Model

- FTD Mutant Tau TG Animals
- No seeding
- Vector IV dosed at age weeks
- Experiment terminated +3 weeks
- Critical Readout: AT8 ELISA or AT100 stained cells in Hippocampus

Vectorized Mid-domain Ab Demonstrates Dose-Dependent Expression and Efficacy

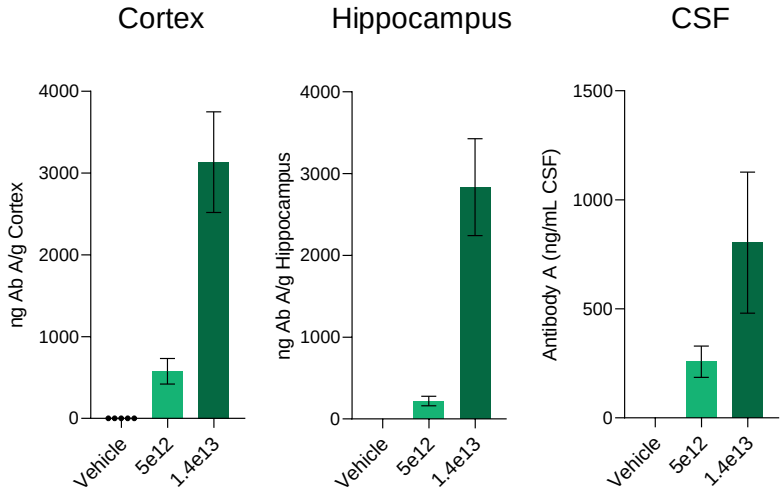
P301S Seeding Model Experiment Design

- Vector: VOY101.CBA.Mid-domain Ab
- Route: Vector IV dosed at age of 6 weeks
- Live phase: 8 weeks
- 2 doses: 5e12 or 1.4e13 Vg/Kg
- Critical readout: AT100 IHC

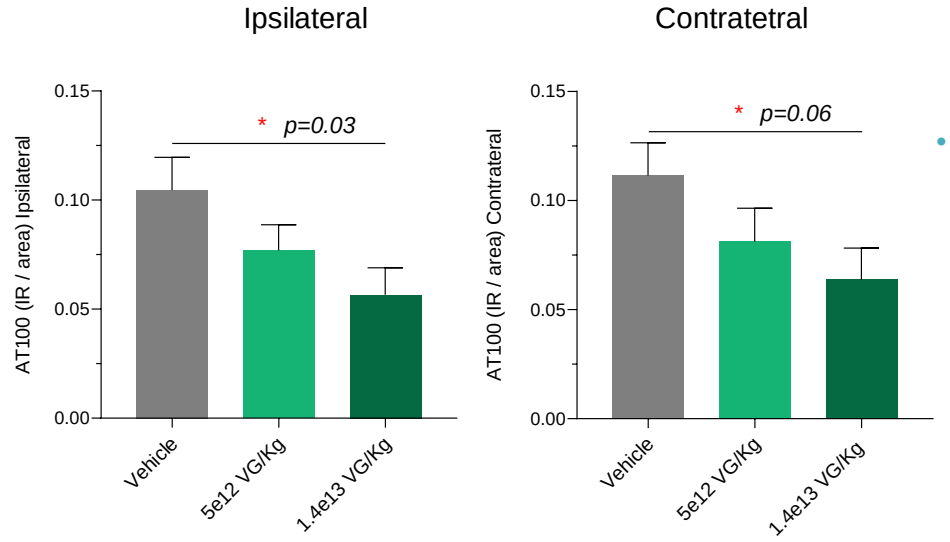


Results

Mid-domain Ab Level at Seeding



Efficacy Measured by AT100 IR

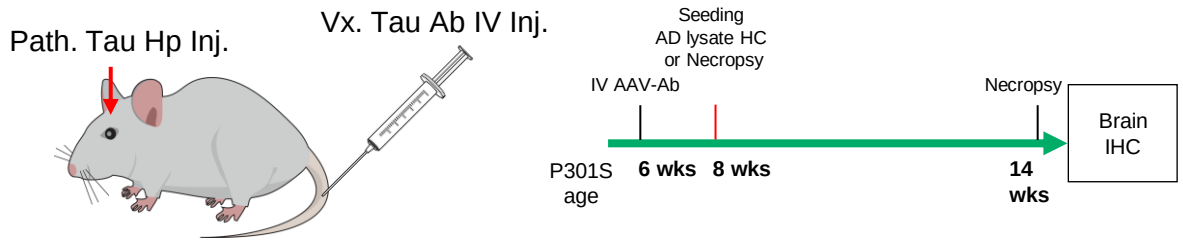


- Expression of Mid-domain Ab can be detected, in dose dependent manner, at the time of seeding and termination
- 46% significant reduction of AT100 positive area is observed in the ipsilateral hippocampus of seeding model that were treated with 1.4e13 Vg/kg of vectorized mid-domain Ab by CBA Promoter

Dose-Dependent Expression and High-Dose Efficacy Using Vectorized C-Terminal Ab

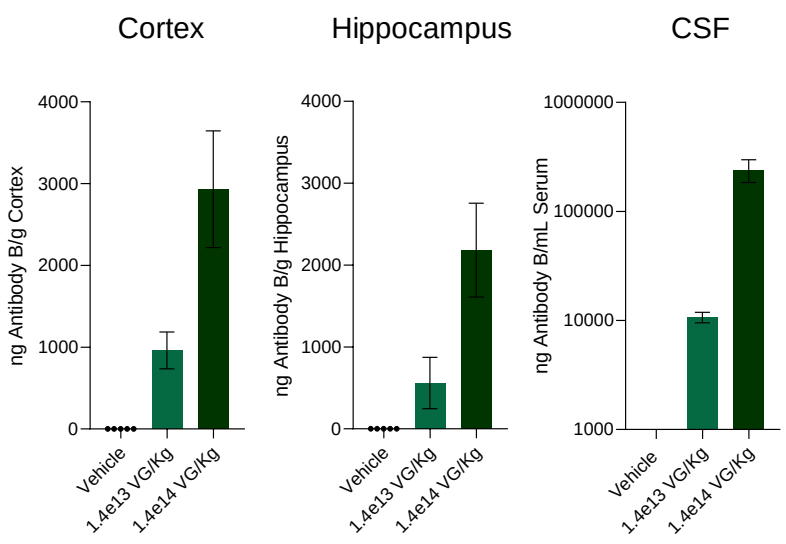
P301S Seeding Model Experiment Design

- Vector: VOY101.CBA.Mid-domain Ab
- Route: Vector IV dosed at age of 6 weeks
- Live phase: 8 weeks
- 2 doses: 1.4e13 or 1.4e14 Vg/Kg
- Critical readout: AT100 IHC

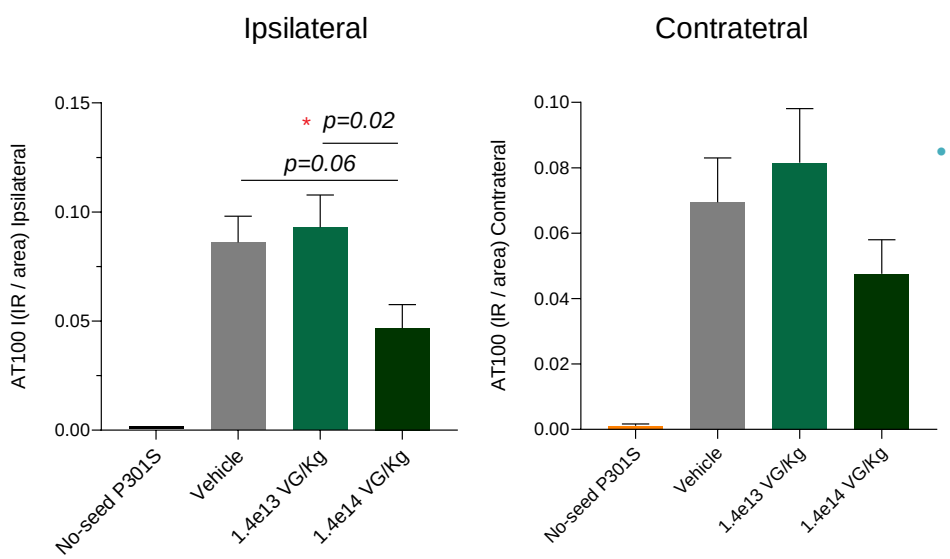


Results

C-term Ab Level at Seeding



Efficacy Measured by AT100 IR



- Expression of C-term. Ab can be detected, in dose dependent manner, at the time of seeding and termination
- 46% reduction of AT100 positive area is observed in the seeding model that were treated with 1.4e14 Vg/kg of vectorized C-term. Ab driven by CBA promoter

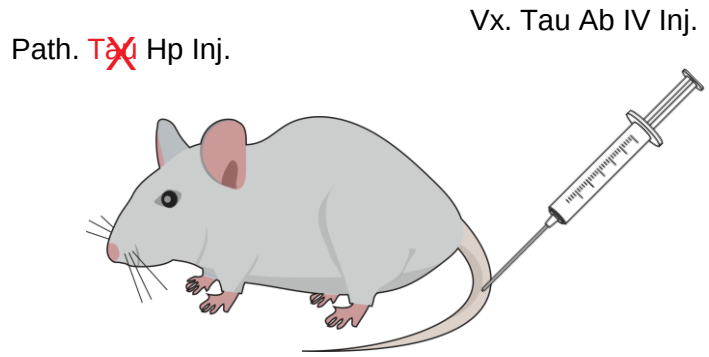
Mouse Tauopathy Models for Efficacy Studies

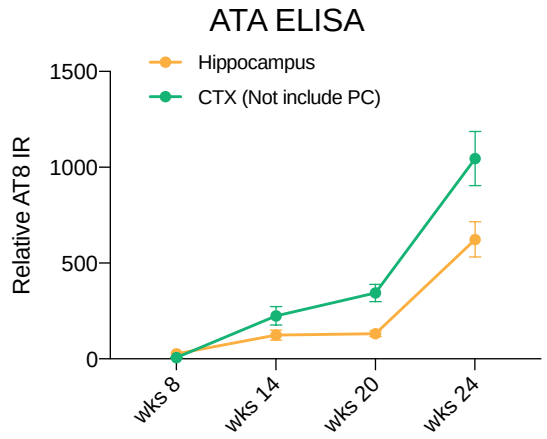
P301S Hippocampal Seeding Model

- FTD Mutant Tau TG Animals
- Seeding into Hippocampus
- Vector IV dosed -2 weeks (anti-seeding)
- Experiment terminated +6 weeks
- Critical Readout: AT100 stained cells in Ipsilateral Hippocampus (contra also examined)

P301S Intrinsic Model

- FTD Mutant Tau TG Animals
- No seeding
- Vector IV dosed at age weeks
- Experiment terminated +3 weeks
- Critical Readout: AT8 ELISA or AT100 stained cells in Hippocampus

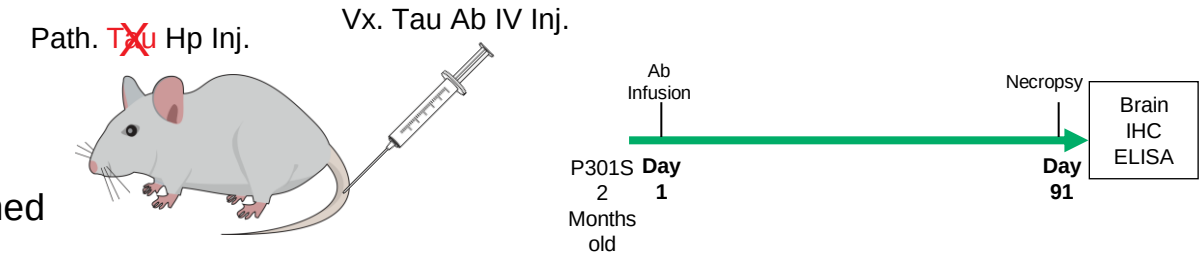




Dose-Dependent Expression and High-Dose Efficacy Using Vectorized C-Terminal Ab

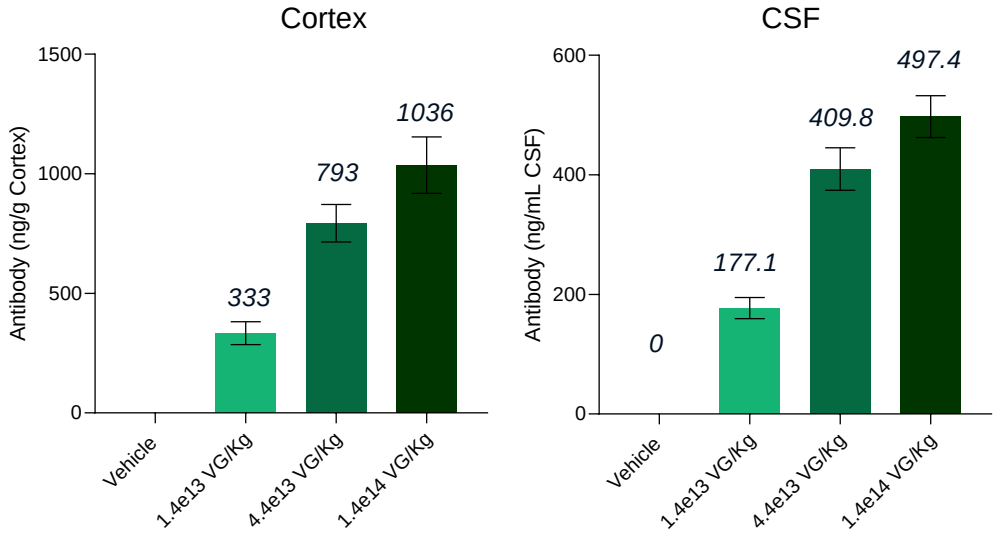
P301S Intrinsic Model Experiment Design

- No seeding
- Vector: VOY101.CBA.C-term. Ab
- Route: Vector IV dosed at age of 8 weeks
- Live phase: 13 weeks
- 3 doses: 1.4e13, 4.4e13, or 1.4e14 Vg/Kg
- Critical Readout: AT8 ELISA or AT100 stained cells in Hippocampus and Cortex

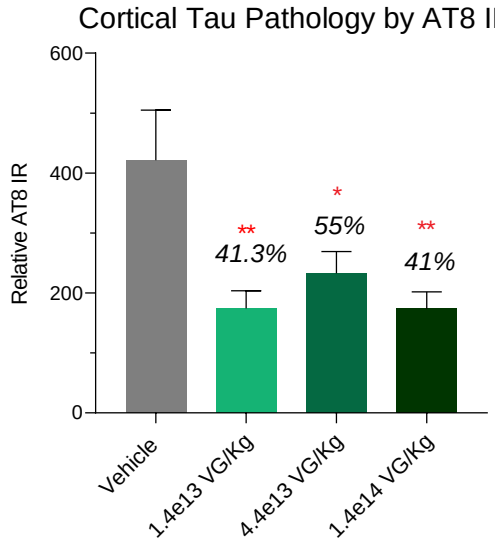


Results

C-term Ab Level at Termination



Efficacy Measured by AT8 ELISA



- Significant reduction of AT8 IR at cortex of all three doses
- Numbers in blue on the top of each column on the graph A and B represent antibody levels in cortex or CSF, respectively
- The % of AT8 IR of treatment groups relative to vehicle control is shown on the top of each column in graph C

Vectorized Antibodies Demonstrate Efficacy in Multiple Animal Tauopathy Models

- AAV vectorized antibodies can delivery high levels of antibody to the CNS

- Both Tau Mid-domain and C-terminal antibodies show efficacy in vectorized studies when driven by a ubiquitous CBA promoter:
  - P301S Seeding model: Significant reduction in pathological tau following seeding observed with both Mid- and C-terminal domain antibodies
  - P301S Intrinsic model: Significant reduction in pathological tau observed with C-terminal domain antibody. Mid-domain analyses in progress

- Future Investigations
  - Evaluation of Mid-domain antibody in Intrinsic model
  - Evaluation of antibodies in efficacy models using GFAP or SYN promoters to drive cell-specific expression

- Multiplexing antibody delivery?
  - Potential to vectorized multiple fragment antibodies in a single AAV vector
  - Could targeting multiple epitopes increase efficacy?

Acknowledgements

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Vinodh Kurella
Dillon Kavanagh
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Kelly Bales
Todd Carter