Efficacy of a Novel Vectorized Antibody Targeting the C-terminal Domain of Tau, Using Systemic Dosing of a Blood Brain Barrier Penetrant AAV Capsid in Mouse Models of Tauopathies

Wencheng Liu, Maneesha Paranjpe, Jerrah Holth, Blaise Clarke, Jeffrey Thompson, Joe Clement, Elisabeth Knoll, Charlotte Chung, Adewale Adeluyi, Alex Powers, Vinodh Kurella, Dillon Kavanagh, Ishan Shah, Brian Ezell, Timothy Fiore, Kyle Grant, Jay Hou, Kelly Bales, Steve Paul, Todd Carter

Voyager Therapeutics Inc., 64 Sidney Street, Cambridge, MA 02139, USA

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SUMMARY

- We have vectorized Ab01 antibody and examined its efficacy in two tauopathy models.
- Vectorized Ab01 was well-tolerated at doses tested
- We observed robust efficacy in all the treatment groups in the hippocampal seeding model
- We observed significant efficacy in the P301S intrinsic model in vAb groups
- A trend of reduction on AT8 pathology was observed in the P301S intrinsic model treated with Ab01 by passive at the dose tested

INTRODUCTION

Anti-tau immunotherapy has become a promising therapy for Alzheimer's disease (AD) and tauopathies. With the hypothesis that tau pathology spreads via cell-to-cell transmission, including trans-synaptic propagation, success of anti-tau immunotherapy relies, in part, on the identification of efficacious antibodies and their delivery to affected or vulnerable brain regions with sufficient or enhanced exposure in the CNS. We have previously demonstrated broad distribution and expression of vectorized anti-tau antibodies in the mouse brain using a blood brain barrier penetrant capsid, VOY101, administered intravenously (IV). Several novel anti-tau antibodies that met the target profile of selectivity, functional inhibition and developability have been generated and are being evaluated *in vivo*. One of the antibodies discovered, antibody 1, exhibits strong affinity for PHF-tau, demonstrates specific binding to tau pathology on brain sections of AD and PSP patients, and potently prevents PHF seeding and propagation *in vitro* and *in vivo*. This antibody recognizes a phospho-specific epitope in the C-terminal region of tau and shows significant reduction of tau pathology in an AD-PHF induced P301S hippocampal seeding and propagation model. Furthermore, we have vectorized antibody 1 into an AAV expression vector with a BBB penetrant capsid and are evaluating it in two independent mouse models of tauopathy.

PROCEDURES

Paired helical filamentous tau (sarkosyl insoluble fraction enriched for PHF, abbreviated as ePHF) preparation: ePHF was isolated from cortices of Braak VI AD cases based on the protocol described *by Liu et al., J Neuroscience* 36, 12425, 2016.

Vector genome measurement: AAV vector genome levels were quantified via ddPCR and shown as per diploid genome number using the endogenous mouse transferrin receptor C gene (TFRC) for normalization.

Anti-tau antibody (Ab) measurement: Anti-tau Ab expression within the CNS was evaluated using a sandwich ELISA in which ePHF was used as a capture antigen and an anti-IgG antibody for detection. Anti-tau antibody distribution in CNS was evaluated by anti-IgG1 antibody-immunohistochemistry using DAB for detection (Brown) as described *by Liu et al., J Neuroscience* 36, 12425, 2016.

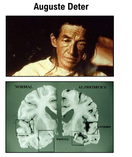
Detection of tau pathology: AT8 ELISA was used to detect tau pathology in the CNS of tauopathy models as described by Liu et al., *J. Neuroscience 36*, 12425, 2016.

Statistics: Statistics were performed using a one-way ANOVA-Tukey's multiple comparison test for all graphs except Ab01/passive graph in Figure 4C, which was done by student T test. Data is expressed as mean ± SEM. (*,**,*** and **** indicate statistical significance (p<0.05; 0.005, 0.0005 and 0.0001., respectively)

Figure 1. Alzheimer's Disease (AD): A Global Pandemic with Huge Need for Effective Therapies

AD is a progressive fatal neurodegenerative disease[1]:

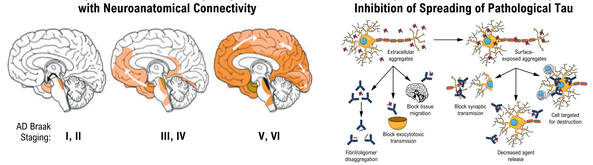
- 6.2 million AD patients in the US today
- Number of patients expected to grow rapidly as population of 65 and older continues to grow; >1 in 9 Americans 65 and older has AD
- In 2020 $257 billion spent by families for out-of-pocket AD care in the US
- >50 million patients globally; expected to double by 2050
- Pathology: amyloid plaques/neurofibrillary tangles with tau aggregates in the brain, neuronal loss, synaptic loss, brain atrophy, and inflammation



Source: (1) 2021 Alzheimer's Disease Facts and Figures

Current treatments limited to symptom management with modest impact

Figure 2. Spreading Mechanism Hypothesis May Provide Opportunity for Therapeutic Antibody Intervention for Alzheimer's Disease/Tauopathies



Adapted from Rault and Voisin-Chiret, *Eur J Med Chem*, 2017

Adapted from Marciniuk et. al, *Clin Dev Immunol*, 2013

Figure 3. 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

Potential Challenges Today

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

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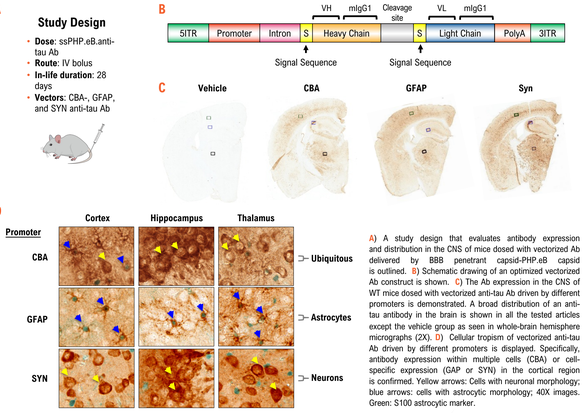
- **Modular Antibody Vectorization Cassettes** with cell-specific or general expression
- In-house PoC for **serum delivery through muscle** expression of full-length Abs
- Potential research in **drugging intracellular proteome** through vectorized nanobodies, degraders, and other innovative platforms

Figure 4. Vectorized Anti-tau Antibody Delivery Results in Promoter-driven, Cell-specific Expression in Mouse CNS

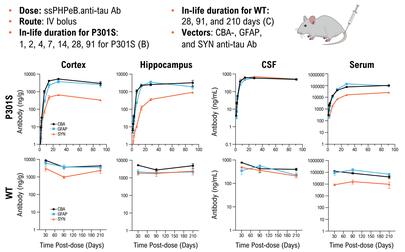


A) A study design that evaluates antibody expression and distribution in the CNS of mice dosed with vectorized Ab delivered by BBB penetrant capsid-PHP.eB capsid is outlined. B) Schematic drawing of an optimized vectorized Ab construct is shown. C) The Ab expression in the CNS of WT mice dosed with vectorized anti-tau Ab driven by different promoters is demonstrated. A broad distribution of an anti-tau antibody in the brain is shown in all the tested articles except the vehicle group as seen in whole-brain hemisphere micrographs (2X). D) Cellular tropism of vectorized anti-tau Ab driven by different promoters is displayed. Specifically, antibody expression within multiple cells (CBA) or cell-specific expression (GAP or SYN) in the cortical region is confirmed. Yellow arrows: Cells with neuronal morphology; blue arrows: cells with astrocytic morphology; 40X images. Green: S100 astrocytic marker.

Figure 5. IV Dosing with Full-Length Anti-Tau Ab Vectors Achieves Durable High Levels of Antibody Expression in Mouse CNS

Study Design

- **Dose:** ssPHPeB.anti-tau Ab
- **Route:** IV bolus
- **In-life duration for P301S:** 1, 2, 4, 7, 14, 28, 91 for P301S (B)
- **In-life duration for WT:** 28, 91, and 210 days (C)
- **Vectors:** CBA-, GFAP, and SYN anti-tau Ab



A) An experiment design that evaluates the kinetics of vAb expression and VG is outlined. A BBB penetrant capsid, PHP.eB was used to deliver Ab vector. CNS tissues, CSF, and serum were collected at various time points as listed for measuring antibody levels and VG at each time point. B) Durability of a vectorized Ab (vAb) expression driven by different promoters in CNS, CSF and serum of P301S mice is evaluated. The antibody expression can be detected as early as 2 days post-dose, approaches maximum levels at 7 days, and plateaus at 14-28 days following IV administration. Expression of a vectorized Ab can still be detected at a high level at 91 days post-dose. C) Extended time points of a vAb expression driven by different promoters in the CNS regions and fluid compartments is investigated. This is evaluated in WT mice as the P301S mouse strain we used can not survive > 6 months of age. As shown in the graph, Ab expression can still be detected in the CNS regions up to 210 days after dosing. Evaluation of Ab expression in the CNS regions and CSF of mice dosed with vectorized anti-tau at a one-year time point after dosing is still in-life.

Table 1. VYGR Anti-tau Antibody-Ab01: Biophysical Properties

Property	Criteria*	Ab01
Approximate Epitope	-	C terminal
Binding affinity to immunopurified PHF Tau		43.9 pM
Selectivity: iPHF:WT rec. Tau*	> 100-fold	>838*
Selectivity: ePHF:WT rec. Tau*	≥ 100-fold	>222*
IHC Fixed - Human AD Brain	positive	Positive
IHC Fixed - Human Ctl Brain	negative	Negative
IHC Fixed - Mouse P301S	-	Positive
IHC Fixed - Mouse Tau KO	-	Pos/Wea
IHC Fixed - Mouse WT	-	Weak
IHC Frozen - Human AD Brain	positive	Positive
IHC Frozen - Human Ctl Brain	negative	Negative
IHC Frozen - Mouse P301S		Positive
Inhibition of ePHF seeding in Biosensor Cells	≤ 20 nM IC_{50}	18.2
Low Polyspecificity (using BVP ELISA)	in range of comp. Abs	√
Solution and Colloidal Stability at >10 mg/mL	95% pure by SEC, no particulates	√

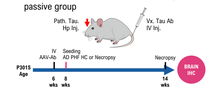
A summary of biophysical characteristics including selectivity, *in vitro* functional inhibition and developability of Ab01 is listed. Ab01 was vectorized using the construct shown in Figure 4 for efficacy studies in two tauopathy models. Antibody binding to immunopurified PHF (iPHF) or WT Tau was measured using Surface Plasmon Resonance (SPR) on Biacore 8K instrument.

* No binding on highest concentration tested.

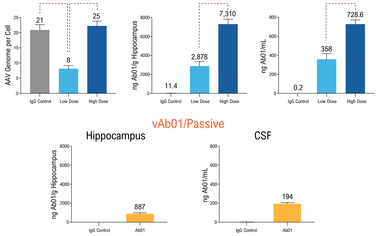
Figure 6. P301S Seeding Model Treated with Vectorized Ab01 Antibody

A Study Design

- **Vector:** PHPeB.CBA.Ab01
- ePHF is used to seed hippocampus
- **Route:** Vector IV dosed at age of 6 weeks
- **Live phase:** 8 weeks
- **2 doses:** Low (1e13 Vg/Kg) or High (3e13 VG/Kg)
- **Critical readout:** AT8 ELISA
- Ab is present in hippocampus when seeded
- Ab was dosed one week prior to seeding in passive group



B AAV Biodistribution and Ab01 Expression in the CNS Regions at Sacrifice



Treatment with vAb01 or with recombinant Ab01 administered passively demonstrated robust reduction of AT8 pathology in an ePHF seeding model. A) Ab01 was cloned as a full-length monoclonal antibody into a PHP-eB AAV vector using a ubiquitous promoter (CBA) and dosed intravenously (IV) two weeks prior to seeding. Two doses of vAb01 were used (low and High-dose). In an independent experiment, recombinant Ab01 was administered Intraperitoneally (I.P.) twice in the week prior to seeding. 5 additional doses were administered by I.P. weekly after seeding in this experiment. For seeding, we injected 2 µl of ePHF (75 ng) into CA1 of hippocampus of P301S mice (Allen et al., *J Neuroscience*, 2002), after which we allowed the pathology to develop for additional 6 weeks. Ipsilateral hippocampi were dissected, and their soluble and insoluble fraction were isolated for measuring antibody expression and tau pathology, respectively. B) AAV biodistribution and antibody expression of the vAb treatment groups are shown on the left. Ab expression from a passive group is used is shown on the right. Dose dependent increase of VG and Ab expression is observed in the CNS of animals treated with vAb01. C) Significant reduction of AT8 pathology is observed in all treatment groups compared to IgG control.

C Reduction of Tau Pathology in the Hippocampus

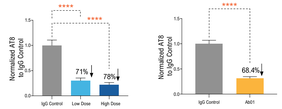


Table 2. Ab01 Expression and Efficacy: vAb vs Passive, Seeding Model

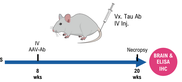
Group	HC Ab Level (ng/g Hippocampus)	CSF Ab Levels (ng/mL)	Reduction of AT8 Pathology
vAb01, low dose	2,878	358	71%****
vAb01, High dose	7,310	728	78%****
Passive, 40mg/kg	887	194	68.4%****

Comparison of Ab expression and efficacy for different treatment groups.

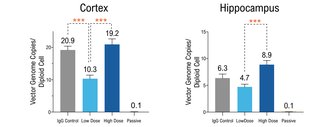
Figure 7. P301S Intrinsic Model Treated with vAb01

A Study Design

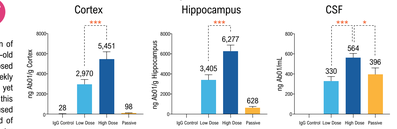
- **Vector**: PHPeB.CBA.Ab01
- **Route**: Vector IV dosed at age of 8 weeks
- **Live phase**: 13 weeks
- **2 doses**: Low (1e13 Vg/Kg) or High (3e13 VG/Kg)
- **Critical readout**: AT8 ELISA
- IgG control (High-dose)



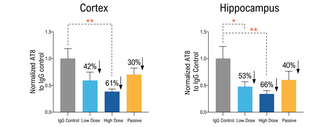
B AAV Biodistribution



C Ab01 Expression at Sacrifice



D Efficacy Measured by AT8 ELISA



Treatment with vAb01 demonstrated significant reduction of AT8 pathology in the intrinsic model. A) Eight-week-old P301S mice (Allen et al., *J Neuroscience*, 2002) were dosed with vAb01 by IV or recombinant Ab01 dosed by I.P. weekly for 13 weeks. Note that tau pathology is not developed yet in hippocampus and cortex at the age of 8 weeks in this model. Two doses (low or high-dose) of vAb01 were used in the treatment, which lasted for 13 weeks. At the end of study, hippocampus and cortex were isolated for biochemical analyses as described in Figure 4. B) AAV biodistribution in the cortex and the hippocampus for all groups is shown. Dose dependent increase of VG is observed in the vAb treated groups; C) Antibody expression in the CNS regions and CSF for all groups is demonstrated. Dose dependent expression is observed for the groups with vAb01 treatment; D) Compared to IgG control, significant reduction is observed in the examined CNS regions with vAb01 treatment. A trend of reduction on AT8 pathology was observed in the passive arm.

Table 3. Ab01 Expression and Efficacy: vAb vs Passive, Intrinsic Model

Group	Ab Level (ng/g Cortex)	Ab Level (ng/g Hippocampus)	CSF Ab Levels (ng/mL)	Reduction of Cortical AT8 Pathology	Reduction of Hippo. AT8 Pathology
vAb01 (low dose)	2,970	3405	330	42%	53%*
vAb01 (high dose)	5,451	6277	564	61%**	66%**
Passive, 40mg/kg	98	628	396	30%	40%

Comparison on Ab expression and efficacy for different treatment groups.

CONCLUSION

Substantial anti-tau antibody expression was achieved in the hippocampus, cortex and CSF of mice dosed with Vectorized anti-tau vectors, and showed robust efficacy in P301S tauopathy hippocampal seeding and intrinsic models. Expression of the antibody is sustained at high levels up to 7 months in CNS regions after dosing, regardless which promoter is used. This gene therapy-based approach has potential advantages over traditional passive immunization, including 1) continuous expression of antibody in the central nervous system (CNS) after a single gene therapy administration compared to repetitive administrations of high dose of antibody by passive immunotherapy; 2) increased CNS exposure of tau antibody relative to passive immunotherapy; and 3) the potential to target intracellular tau aggregates which are less effectively accessed by passively delivered antibody. These results add to accumulating evidence that systemic dosing of a vectorized anti-tau antibody using a BBB-penetrant AAV capsid results in reduced tau pathology and may represent a new single-dose therapeutic strategy for treating various tauopathies.