Discrimination of Anti-tau Antibodies Targeting Different tau Epitopes by a P301S Mouse Hippocampal Seeding Model of Tauopathy

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INTRODUCTION

Objectives

VY7523 is a recombinant humanized IgG4 monoclonal version of murine Ab-01 designed to inhibit the spread of pathological tau in Alzheimer's disease (AD). Ab-01 targets an epitope in the C-terminus of tau, binds to pathological tau (p-tau) with high affinity and selectivity over wild-type tau, and blocks paired helical filaments (PHF) seed-induced tau aggregates in the P301S mouse hippocampal seeding model.

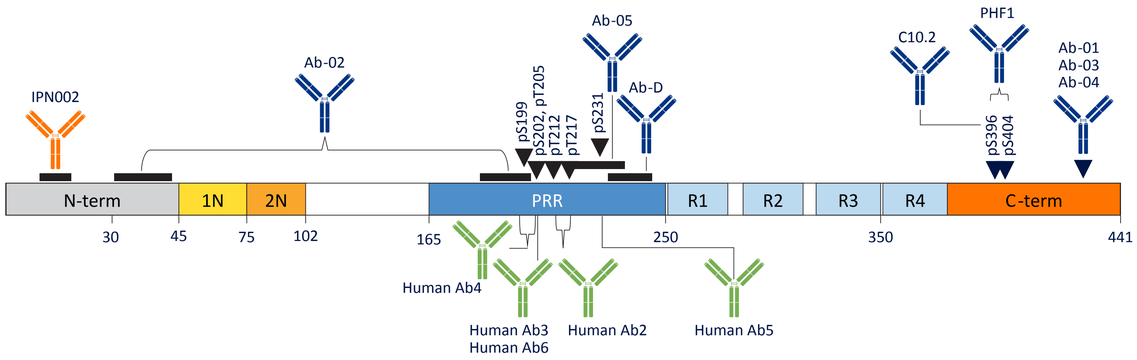
Here, we compared Ab-01 to murine surrogates of antibodies in clinical development targeting different tau epitopes for reduction of p-tau levels in this seeding model, including murine surrogates of anti-tau antibodies that failed to meet primary endpoints in the clinic.

Observations

Our P301S mouse seeding model, where the predominant driver of tau pathology is seeding and propagation of human p-tau following hippocampal injection of enriched PHF from AD brain, was able to discriminate 14 different antibodies targeting 11 different epitopes distributed across tau. Notably, a murine surrogate of an anti-tau antibody targeting an N-terminal epitope that failed to meet primary efficacy endpoints in clinical trials were ineffective in reducing pathological tau in this model, whereas those targeting the proline-rich region and C-terminal epitopes, including Ab-01, the murine surrogate of our antibody in the clinic, were highly effective. Furthermore, we show with multiple methods that PHF from AD brain, Braak stages II to VI, including that used in our P301S mouse seeding model, contain substantial levels of the C-terminal epitope of Ab-01, in contrast to other reports. These results suggest that our P301S mouse hippocampal seeding model may serve as a negative predictor for clinical outcomes of anti-tau antibodies, and indicate that anti-tau antibodies targeting the proline-rich region or C-terminus inhibit seeding and spread of pathological tau, relevant to treating human AD.

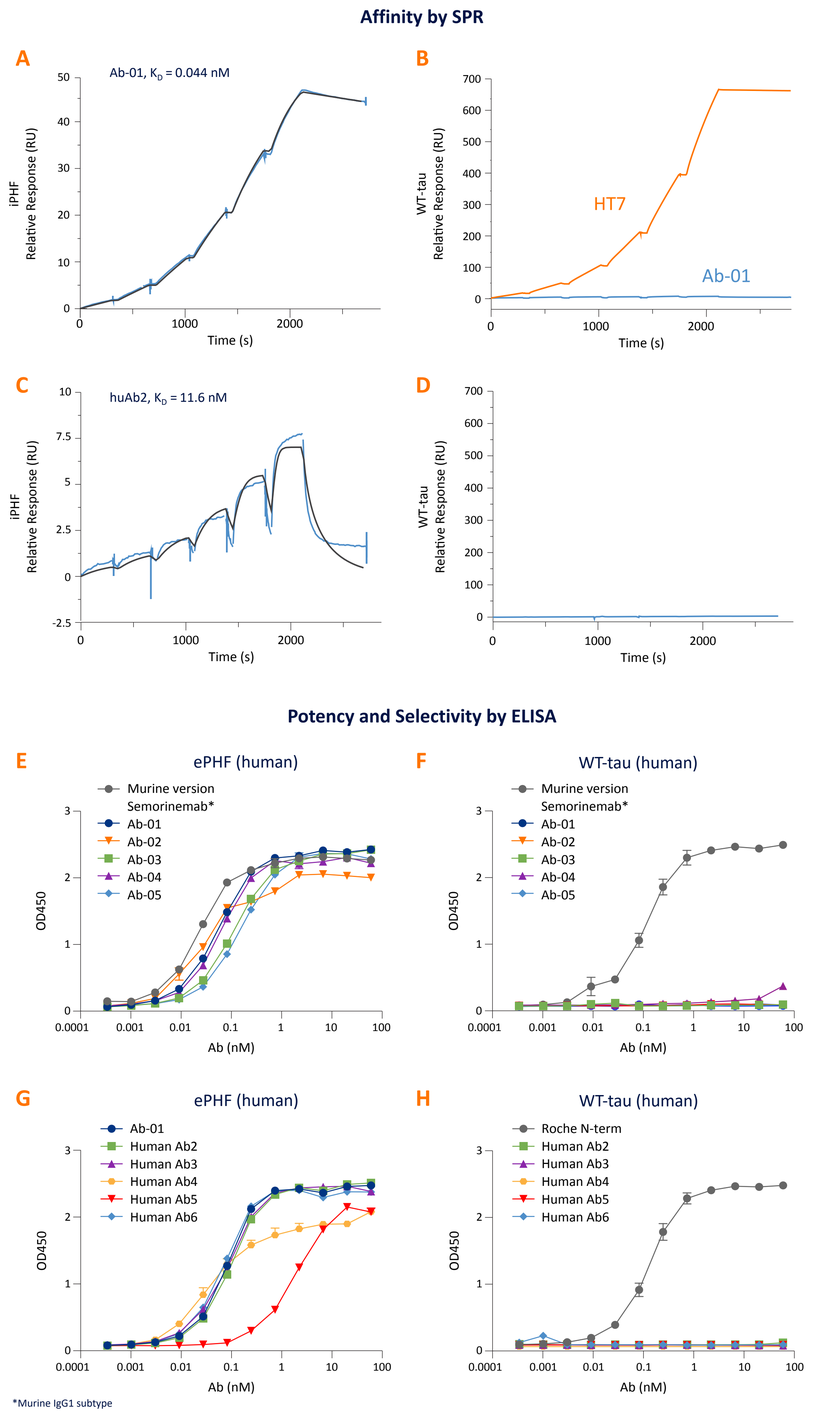
RESULTS

Figure 1. Epitopes of Ten Novel Anti-tau Antibodies Map to Seven Locations and Include Six New Epitopes



The 14 anti-tau Abs candidates, that were selected for *in vivo* efficacy studies, target diverse locations within full-length tau including 7 in proline rich domain (PRR). 5 in C-terminus, 1 in N-terminus, and 1 in both N-terminus and PRR (conformational target).

Figure 2. Binding Affinities and Potencies Show Selectivity of the Voyager Anti-tau Antibodies for PHF Over WT tau



*Murine IgG1 subtype

Antibody	K_D (pM)	k_a (1/ms)	k_d (1/s)
VYGR's Murine Abs			
Ab-01	43.9	1.70E+06	7.09E-05
Ab-02	297	2.92E+06	7.87E-04
Ab-03	57.8	5.22E+05	3.00E-05
Ab-04	125	5.89E+05	7.32E-05
Ab-05	2330	4.84E+07	1.13E-01
VYGR's Human Abs			
huAb2	11650	1.82E+06	2.14E-02
huAb3	356	1.04E+08	3.69E-02
huAb4	238	5.45E+06	1.30E-03
huAb5	**	**	**
huAb6	293	3.84E+06	1.13E-03
Previously Described Murine Abs			
IPN002	6.7	9.83E+05	6.60E-06
Ab-D	48.3	1.89E+06	9.11E-05
C10.2	*	*	*
PHF1	59	1.63E+06	9.61E-05

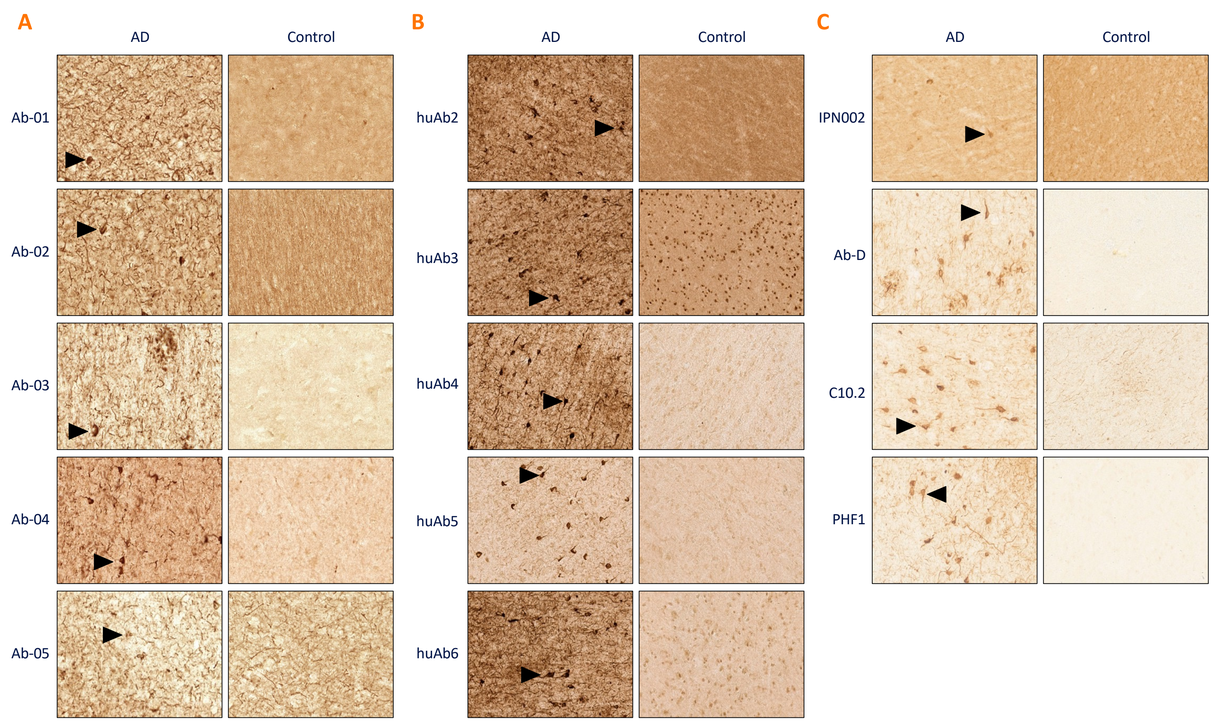
* poor fit to binding model
** no detectable binding at highest concentration of 25 nM

Antibody	ePHF Binding Potency EC_{50} (pM)	WT-tau Binding Potency EC_{50} (pM)
VYGR's Murine Abs		
Ab-01	55	*
Ab-02	29	**
Ab-03	115	**
Ab-04	59	**
Ab-05	150	**
VYGR's Human Abs		
huAb2	95	**
huAb3	81	**
huAb4	41	**
huAb5	1800	**
huAb6	64	**
Previously Described Murine Abs		
IPN002	41	21
Ab-D	47	11
C10.2	26	3600
PHF1	36	11730

* no detectable binding at highest concentration of 66 nM
** no detectable binding at highest concentration of 60 nM

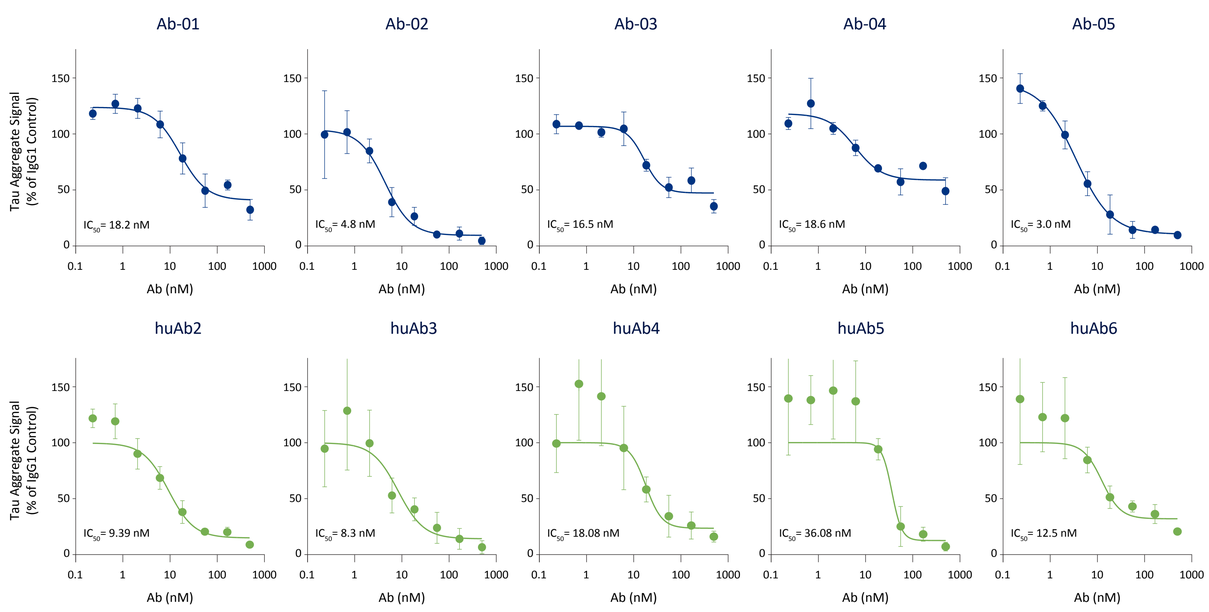
Binding affinity: Voyager (VYGR) anti-tau antibodies bind selectively to immunopurified PHF (iPHF) tau with high affinity but not to wild-type (WT) tau. Antibody binding to iPHF or WT Tau was measured using Surface Plasmon Resonance (SPR) on a Biacore 8K instrument. Sensorgrams are shown for Ab-01 (**A** and **B**) and huAb2 (**C** and **D**) which demonstrated high affinity binding to iPHF (**A** and **C**) but did not bind to WT tau (**B** and **D**) up to 100 mM, the maximum feasible concentration tested. Affinities (K_Ds) for binding to iPHF were calculated from k_{on} and k_{off} for each antibody, as shown in the table on the right. Binding potency and selectivity: Voyager (VYGR) anti-tau antibodies demonstrated potent and selective binding to ePHF (**E** and **G**) but not human WT tau (**F** and **H**), as assessed by ELISA. Binding potencies (EC_{50}s) were calculated using non-linear regression analysis with a variable slope four-parameter curve fit (GraphPad Prism) and are shown in the table on the right. The affinity, potency and selectivity of binding were also evaluated for murine versions of 4 previously described antibodies (IPN002, Ab-D, C10.2 and PHF1). IPN002 and Ab-D demonstrated high affinity of binding to both PHF and WT tau, whereas C10.2 and PHF1 showed high affinity to PHF but weak binding affinity and potency to WT tau.

Figure 3. Immunostaining of AD and Non-AD Cortex Demonstrates Selectivity of the Voyager Anti-tau Antibodies for Binding tau Tangles



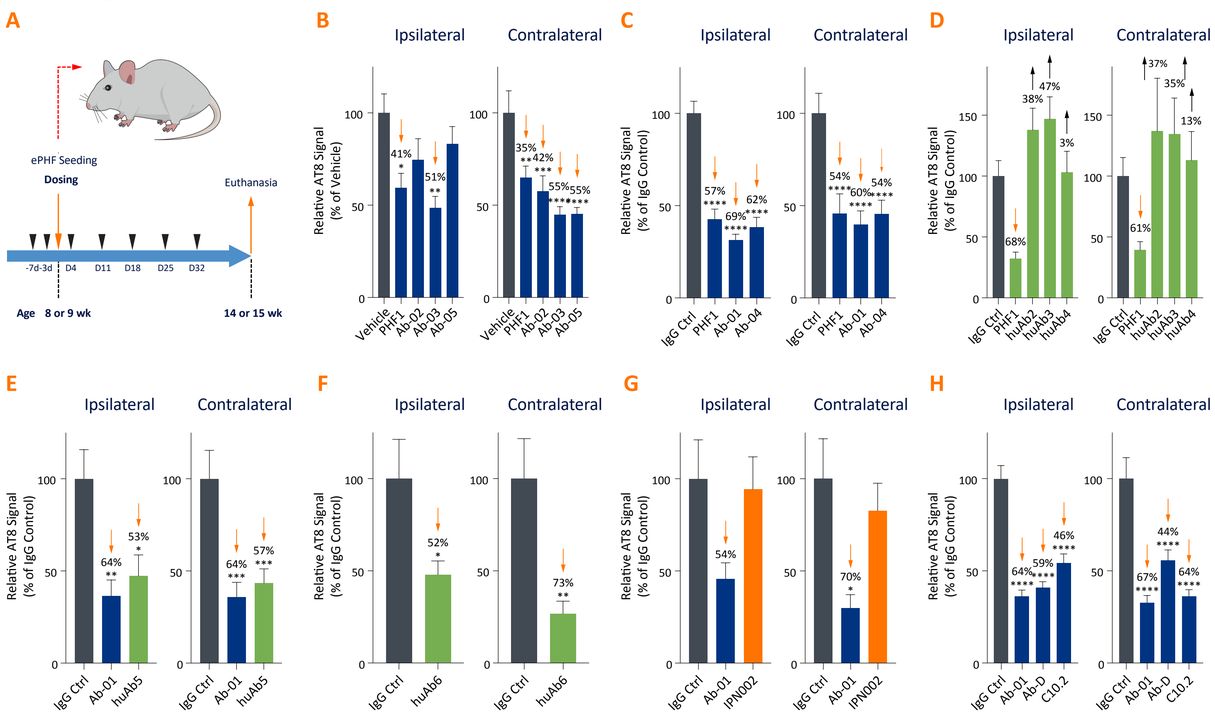
The Voyager antibodies (**A**, **B**) bind specifically to tau pathology on fixed cortical sections from AD but not non-AD brain. Additionally, IPN002, Ab-D, C10.2 and PHF1 also predominantly bind to tau pathology on the sections of AD cases but not non-AD brain (**C**). Cortical sections were purchased from Banner Sun Health Research Institute, Sun City, AZ.

Figure 4. Inhibition of Seeding *in vitro* by the Voyager Anti-tau Antibodies



The Voyager anti-tau antibodies prevent PHF seeding *in vitro* in a dose-dependent manner. Tau RD P301S FRET biosensor cells were cultured and transfected with PHF that had been immunodepleted with an anti-tau antibody at a concentration of 0.2, 0.7, 2.1, 6.2, 18.5, 55.6, 166.7, or 500 nM or with isotype control IgG1 antibody at a concentration of 500 nM. After 48 hours, the cells were fixed, stained, and imaged. Tau spot signals were obtained by measuring the number of YFP FRET positive spots. To obtain the relative % tau spot, tau spot signals with a given anti-tau immunodepletion were normalized to the average signal with isotype control IgG1 antibody depletion at 500 nM. The IC_{50} from the curve fit is shown in each graph.

Figure 5. Anti-tau Antibodies Targeting the C-terminus or Proline-rich Region, but not N-terminus, are Efficacious in Reducing P-tau in the P301S Mouse Hippocampal Seeding Model



Efficacy of 14 murine anti-tau antibodies were examined in the P301S mouse hippocampal seeding model. (**A**) To test efficacy of a given antibody, IP injections were initiated one week before surgery (2 doses). Five additional doses were administered weekly after surgery. At six weeks post-surgery, animals were euthanized, and hippocampi of each animal were isolated for AT8 ELISA to measure levels of p-tau. Levels of p-tau in each treatment group were normalized to the vehicle (**B**) or IgG control group (C-H). There was a significant reduction of p-tau in both ipsi- and contra-lateral hippocampi after treatment with Ab-01 (**C**), Ab-03 (**B**), Ab-04 (**C**), huAb5 (**E**), huAb6 (**F**), C10.2 (**H**), Ab-D (**H**) and PHF1 (**B-D**), relative to the vehicle or IgG control group. Ab-01, Ab-03, Ab-04, PHF1 and C10.2 target the C-terminus, whereas huAb5, huAb6 and Ab-D target the PRR domains. Ab-02, which targets both the N-terminus and PRR domain, significantly reduced p-tau in the contralateral hippocampus only. Note that PHF1 was used as a positive control (**B-D**).

CONCLUSIONS

- Murine surrogate of VY7523 demonstrates strong efficacy in the P301S seeding model.
- A subset of antibodies targeting PRR-domain/C-terminus show similar efficacy in the seeding model.
- Our P301S mouse hippocampal seeding model may serve as a negative predictor for clinical outcomes of anti-tau antibodies.
- Antibodies targeting the PRR-domain and C-terminus of tau, including Ab-01, the murine surrogate of VY7523, and murine surrogates of other antibodies in clinical development, have similar efficacy in reducing p-tau levels in this model.