#P0643

Selection of an Anti-tau Antibody Candidate Targeting Pathological Tau for the Treatment of Alzheimer's Disease

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INTRODUCTION

The current hypothesis for the progression of tau pathology in Alzheimer's disease (AD) is that neuron-to-neuron transmission of pathologic tau, including especially trans-synaptic propagation, plays a major role. Our goal is to identify a selective, potent and efficacious anti-tau antibody clinical candidate that blocks pathologic tau spreading *in vivo* for the treatment of AD.

Immunization of mice with AD patient-derived PHF-tau (paired helical filamentous tau) as the immunogen yielded 113 anti-tau antibody hits with significant binding to PHF-tau and an absence of detectable binding to wild type recombinant tau. These antibodies were characterized and prioritized based on affinity, biophysical characteristics, efficacy in animal models of tau spreading and differentiation from clinically ineffective anti-tau antibodies.

Four anti-tau antibodies were selected (Ab01, Ab03, Ab04 and Human Ab5) with novel sequences and epitopes that fit our target profile based on selectivity, functional inhibition *in vitro* and *in vivo*, and developability. Ab01, Ab03 and Ab04 are murine antibodies that target the same C-terminal epitope, whereas Human Ab5 targets the mid-domain of tau. Among these four antibodies, Ab01 demonstrated superior efficacy in the mouse seeding model and has been humanized.

We plan to leverage the Ab01 antibody for a passive immunotherapy for AD. The clinical candidate has been chosen based on selectivity for pathological tau, potency, functional inhibition *in vitro* and developability.

OVERVIEW

Figure 1. Voyager Anti-Tau Antibody Discovery

GOAL: Identify anti-tau antibodies that block tau-seed mediated propagation of pathology

APPROACH:

Focus on antibodies that target pathological tau species

Generate a diverse starting pool of anti-tau Abs

Immunization Campaigns

- Host animals: WT mouse, Tau-KO mouse for murine Ab's; human mouse for human Abs
- Immunogens: Paired Helical Filamentous tau (ePHF or sarkosyl insoluble fraction) from AD brain

Screen and prioritize anti-tau Abs based on:

- Biochemical selectivity for pathological tau
- IHC selectivity for AD/PSP vs WT human brain
- Functional inhibition of PHF seeding *in vitro* and *in vivo*
- Developability based on low polyspecificity and lack of aggregation

Characterize additional attributes

- Sequence
- Epitope
- Western blot binding to AD, PSP vs WT human brain

Mouse immunization strategy and candidates' selection profiles for identifying efficacious anti-tau candidates.

STATUS:

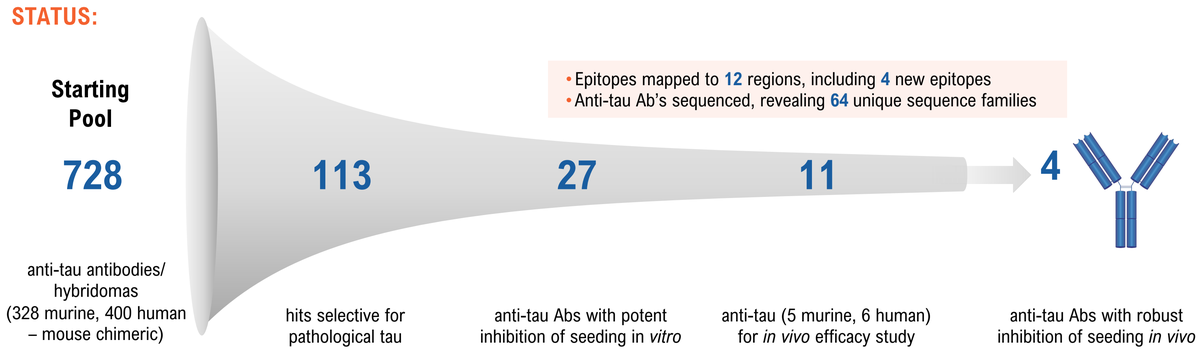
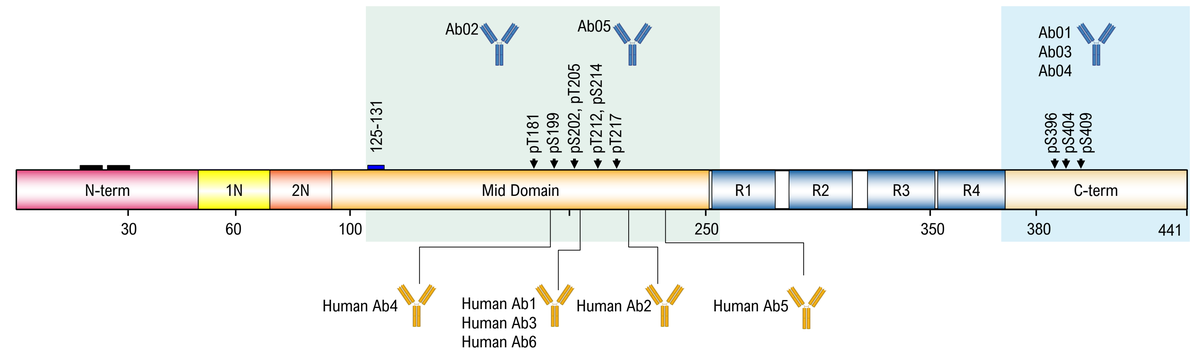


Figure 2. Voyager Anti-tau Antibodies Target Various Epitopes Throughout Full-length Tau



The 11 anti-tau Abs candidates, that were selected for *in vivo* efficacy studies, target diverse locations within full-length tau including 8 in mid-domain and 3 in C-terminus.

Table 1. Biophysical Properties of Voyager Anti-tau Antibodies

		MURINE ABS					HUMAN ABS					
Property	**Criteria[a]**	**Ab01**	**Ab02**	**Ab03**	**Ab04**	**Ab05**	**Human Ab1**	**Human Ab2**	**Human Ab3**	**Human Ab4**	**Human Ab5**	**Human Ab6**
Affinity (Biacore)	nM	0.044	0.3	0.058	0.12	<4.4	0.6	11	0.4	0.2	6	0.3
Selectivity: ePHF:WT rec. Tau*	≥ 100-fold	>222*	>415*	>140*	>123*	Not selective**	>23*	>59*	>40*	>137*	Not selective**	>181*
IHC Fixed – Human AD Brain	positive	Positive	Positive	Positive	Positive	Positive	Weak	Positive	Positive	Positive	Positive	Positive	Positive
IHC Fixed – Human Ctl Brain	negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative (Nu)	Negative	Negative	Negative
IHC Fixed – Human PSP	-	Positive	Positive (Wk)	Positive	Positive	Positive	Positive	Positive	Positive	Positive	Wk Positive	Positive (Nu)
IHC-Fixed – Human Ctl Brain	-	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative (Nu)	Negative	Negative	Negative
IHC Frozen – Human AD Brain	-	Positive	Positive	Positive	Positive	Positive	Positive	Positive	Positive	Positive	Wk Positive	Positive
IHC Frozen – Human PSP	positive	Positive	Positive	Positive	Positive	Positive	Positive	wk Positive	Positive	Positive	Wk Positive	Positive
IHC Frozen – Human Ctl Brain	negative	Negative	Negative	Negative	Negative	weak	Negative	Negative	Negative (Nu)	Negative	Negative	Negative
Inhibition of ePHF seeding in Biosensor Cells	≤ 20 nM IC_{50}	18.2	4.8	16.5	18.6	3.0	36.3	9.39	8.29	18.1	36	12.5
Low Polyspecificity (using BVP ELISA)	in range of comp. Abs	√	√	√	√	√	√	√	√	√	√	√
Solution and Colloidal Stability at >10 mg/mL	95% pure by SEC, no particulates	√ b	√ b	√ b	√ b	√ b	√ b	√ b	√ b	√ b	√ b	√ b

* No binding to highest concentration tested; ** not selective, 1:1 binding ; [a]: dash indicates not a criteria ; b: tested at 1 mg/mL

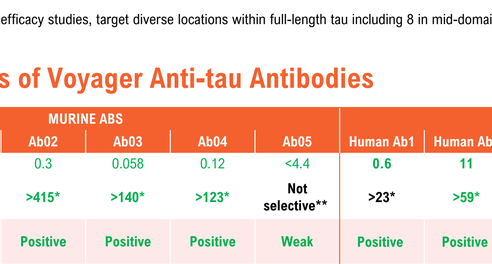
RESULTS

Figure 3. AD-PHF Seeding Model in P301S Transgenic Mouse

A **P301S Hippocampal Seeding Model**

- FTD Mutant tau transgenic animals
- AD-derived PHF seeding in hippocampus
- Experiment terminated +6 weeks
- Readout: pathological tau as assessed by AT8 ELISA (relative AT8 signal)

B **Hippocampus**



Inj: side ipsilateral to injection; Con: contralateral side

C **Hippocampus, Vehicle Inj.** / **Hippocampus, ePHF Inj.**

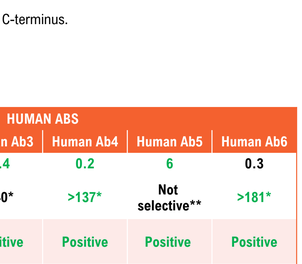


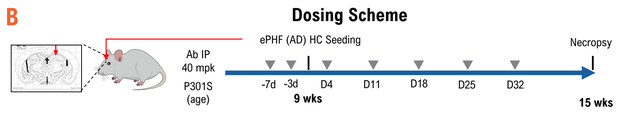
Figure 4. Reduction of p-Tau in P301S Mouse Seeding Model by Murine Antibody Candidates

A

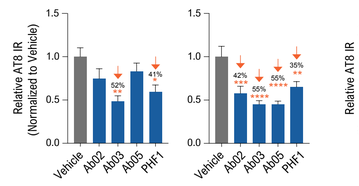
PROPERTY	CRITERIA	Ab01	Ab02	Ab03	Ab04	Ab05	PHF1
Affinity to iPHF (Biacore)	nM	0.044	0.3	0.058	0.12	<4.4	0.16
Affinity to iPHF (Octet)	nM	0.26	0.15	4.01	2.75	0.55	0.65
Affinity to WT rec. Tau (Octet)	nM	>218*	>218*	>218*	>218*	>218*	>218*
Inhibition of seeding in biosensor Cells with ePHF	≤ 20 nM IC_{50}	18.2*	4.8	16.5*	18.6*	3.0	5.7

*No binding at highest concentration tested

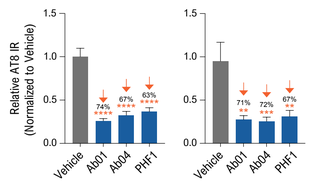
B **Dosing Scheme**



C **Hippocampus** — Ipsilateral / Contralateral



D **Hippocampus** — Ipsilateral / Contralateral



The top 5 murine Ab candidates (Ab01-Ab05) were evaluated in the P301S mouse seeding model at 40 mpk IP (7 doses in total), which was the maximum anti-tau Ab dose used in several published similar studies of other anti-tau Ab's (A, B). All 5 murine Abs exhibited lowering of p-tau in the hippocampus ipsilateral and/or contralateral to ePHF hippocampal (HC) seeding (C, D). Ab01 was selected for humanization based on its superior efficacy in lowering p-tau *in vivo*. Statistical significance was evaluated with a one-way ANOVA with Tukey's multiple comparisons post-hoc test; *, **, ***, and **** indicate $p < 0.05$, 0.005, 0.0005 and 0.0001, respectively, compared to the vehicle group. Data are shown as the group mean ± SEM.

Table 2. Summary of Voyager Anti-tau Abs - Activity/Efficacy

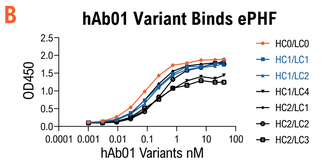
ANTIBODY	SPECIES	AFFINITY BY BIACORE (nM)	IPSILATERAL EFFICACY (REDUCTION OF AT8 IR VS VEHICLE OR IgG CONTROL)	CONTRALATERAL EFFICACY (REDUCTION OF AT8 IR VS VEHICLE)
Ab01	Mouse	<0.04	74% or 64-70%*	71%*
Ab02	Mouse	0.3	no	42%*
Ab03	Mouse	<0.06	52%*	55%*
Ab04	Mouse	0.12	67%*	72%*
Ab05	Mouse	<5	no	55%*
Human Ab1	Human	0.6	33%	TBD
Human Ab2	Human	11	no	no
Human Ab3	Human	0.36	no	no
Human Ab4	Human	0.24	no	no
Human Ab5	Human		53%*	TBD
Human Ab6	Human	0.3	TBD	TBD
PHF1	Mouse	0.16	41-63%*	35-67%*

Figure 5. Affinity of Humanized Ab01 (hAb01) Variants to Immuno-purified PHF and Selectivity of hAb01 Variants for Enriched PHF vs WT Tau

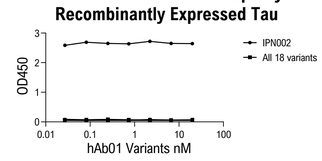
A

		AFFINITY TO IPHF (K_D, pM)	AFFINITY TO PTAU PEPTIDE (pM)	EPHF BINDING (EC_{50} nM)	SELECTIVITY (WT 20 nM/ EPHF EC_{50})
mouse Ab01				0.037	
HC0/LC0		**16.2**		**0.083**	
1	HC1/LC1	29.3	38.6	0.15	>133
2	HC1/LC2	39.1	39.8	0.16	>124.8
4	HC2/LC1	33.4	39.8	0.133	>149.9
8	HC3/LC1	49.6	53.3	0.168	>119
9	HC3/LC2	43.4	40.8	0.085	>232.9
11	HC4/LC1	32.5	45.7	0.095	>210.3
12	HC4/LC2	44.0	76.3	0.1	>198.4
13	HC4/LC3	30.4	43.6	0.14	>118.8
15	HC5/LC1	24.9	40.3	0.164	>113.8
16	HC5/LC2	35.3	36.1	0.181	>122
17	HC5/LC3	24.8	29.4	0.191	>128.5

B **hAb01 Variant Binds ePHF**



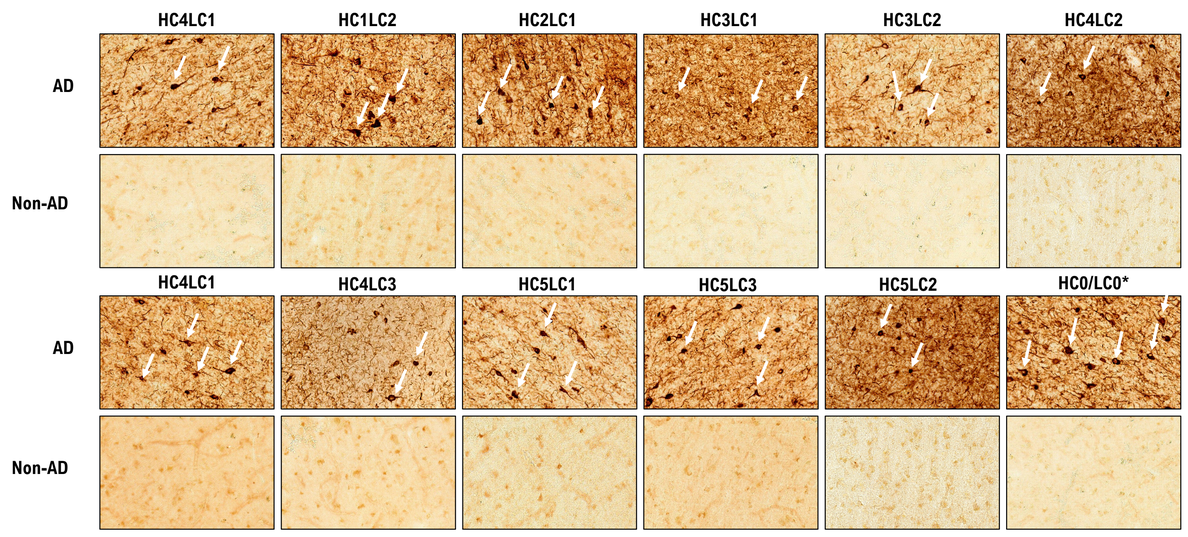
C **hAb01 Variant Binds Non-Phosphorylated Recombinantly Expressed Tau**



IPN002 was used as positive control; no binding up to 20 nM

Eleven of 18 humanized Ab01 variants were the focus of further work based on selectivity and affinity. A) iPHF and pTau peptide affinity measurements by Biacore. Binding affinity was measured using Surface Plasmon Resonance (SPR) on Biacore 8K instrument. For iPHF binding, iPHF was directly immobilized on CM5 sensor chip by amine coupling at 199 RU density. For pTau binding, 1 µg/ml biotinylated pTau peptide was captured on Biotin CAP chip via CAPture reagent to achieve 5-10 RU levels. Antibody was injected using Single Cycle Kinetics (SCK) mode with association and dissociation times of 5 and 10 min, respectively at a concentration range of 0.78 to 2.5 nM. The sensorgrams were fitted to 1:1 binding model in the Biacore Evaluation software to determine kinetic rate constants and affinity values. HC0/LC0: mouse variable/human constant chimeric served as a control (**red**). B,C) Examples of affinities measured by ePHF or WT tau ELISA. Serial 3- fold dilutions of each antibody variant were prepared starting from 66 nM for a total of 12 antibody dilutions. These dilutions were exposed to a plate coated with ePHF or WT tau for a direct ELISA as described in Liu, et al (2016). For each variant, OD450 readings were plotted against the corresponding antibody concentrations. The EC_{50} was then determined by non-linear regression using Graphpad Prism.

Figure 6. All Top 11 Humanized Ab01 Variants Selectively Immunostain Tangles in AD Cortex



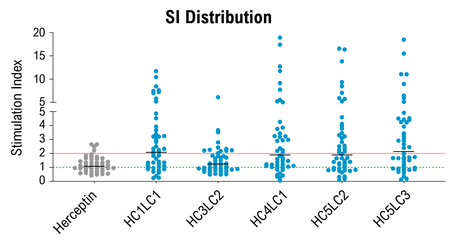
All 11 hAb01 variants demonstrated specific binding to neuronal tau pathology (white arrows) with minimal staining of non-AD cortex. *HCO/LCO: mouse variable/human constant chimeric control. 20X magnification.

Table 3. Summary of *in Vitro*, Biochemical and Biophysical Characteristics for Top 11 Humanized Ab01 Variants

PROPERTY	mAb01	HC0LC0	HC1LC1	HC1LC2	HC2LC1	HC3LC1	HC3LC2	HC4LC1	HC4LC2	HC4LC3	HC5LC1	HC5LC2	HC5LC3
Affinity to iPHF (Biacore, pM)	16.5	16.2	29.3	39.1	33.4	49.6	43.4	32.5	44	30.4	24.9	35.3	24.8
Affinity to pS422 tau peptide (pM)			38.6	39.8	39.8	53.3	40.8	45.7	76.3	43.6	40.3	36.1	29.4
Affiinity to ePHF EC_{50} (nM)		0.08	0.15	0.16	0.13	0.17	0.085	0.095	0.1	0.14	0.16	0.18	0.19
Selectivity: ePHF:WT rec. Tau[a]		>240	>133	>124.8	>149.9	>119	>232.9	>210.3	>198.4	>118.8	>113.8	>122	>128.5
IHC Fixed - Human AD Brain	Positive	Positive	Positive	Positive	Positive	Positive	Positive	Positive	Positive	Positive	Positive	Positive	Positive
IHC Fixed - Human Ctl Brain	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative
Western Frozen - Human AD Brain	Positive	Positive	Positive	Positive	Positive	Positive	Positive	Positive	Positive	Positive	Positive	Positive	Positive
Western Frozen - Human Ctl Brain	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative
Inhibition of ePHF seeding in Biosensor Cells	18.2		78.1	17.6	49.8	17.6	32.2	58.1	49.8	34.5	24.1	37.9	43.8
Low Polyspecificity (using BVP ELISA)		3.9	1.8	1.6	6	1.6	1.7	2.2	1.8	3.2	1.6	1.4	2.7
PTM liability			0.505	0.505	0.505	0.505	0.505	0.505	0.505	0.505	0.505	0.505	0.505
Predict aggregation		Inc.	Low	Inc.	Inc.	Inc.	Inc.	Inc.	Inc.	Low	Low	Low	Low

[a]: no binding at highest concentration tested; Inc.: inconclusive; gold boxes indicate top 5 humanized Ab01 variants selected for further developability work

Figure 7. T-Cell Immunogenicity Assay (CD4+ Cell Proliferation)



ANTIGEN	# OF DONORS	% OF DONORS
KLH	50	100
Herceptin	4	8
Voyager_1 (HC1LC1)	27	54
Voyager_9 (HC3LC2)	12	24
Voyager_11 (HC4LC1)	23	46
Voyager_16 (HC5LC2)	24	48
Voyager_17 (HC5LC3)	25	50

Relative immunogenicity risk for 5 humanized Ab01 variants was assessed by the CD4+ T-cell proliferation assay. Antibodies were incubated with PBMC cells from 50 healthy donors representative of the global population based on HLA-DRB1 expression and cell proliferation was measured by flow cytometry. Two assay read outs: 1. Stimulation Index (SI): ratio of # of proliferating T cells of sample over blank. SI ≥ 2.0 is considered positive response. 2. Percentage of responding donors.

CONCLUSIONS

- Diverse starting pool of proprietary Abs targeting pathological tau from human AD brain were generated and screened
- Four Abs (Ab01, Ab03, Ab04 and Human Ab5) with novel sequences and epitopes in the mid-domain or C-terminus of tau, were selected that fit the target profile based on selectivity, functional inhibition *in vitro* and *in vivo*, and developability
- Ab01 demonstrated superior efficacy *in vivo* in the P301S mouse seeding model in reducing pathological tau, and was chosen for humanization
- Humanization of Ab01 has been completed and Voyager_9 (HC3LC2) has been selected as the clinical candidate VY-TAU01