ANTI-TAU ANTIBODIES

1. REFERENCE TO A SEQUENCE LISTING

[0000] This application includes a Sequence Listing submitted electronically as an xml file named ABV21639USO1_ST26.xml, created on February 14, 2025, with a size of 19,848 bytes. The Sequence Listing is incorporated herein by reference.

2. BACKGROUND

[0001] Tau is a neuronal microtubule-associated protein found predominantly in axons which functions to promote tubulin polymerization and stabilize microtubules. The physiological functions of tau are highly regulated by a range of posttranslational modifications, including phosphorylation, acetylation, glycosylation, isomerization, nitration, SUMOylation, and ubiquitination. The alteration of these modifications can affect tau functions and potentially lead to pathological conditions. The deposition of abnormal tau protein in the brain is associated with a group of neurodegenerative disorders known collectively as "tauopathies," which includes Alzheimer's disease.

[0002] Alzheimer's disease represents the most common cause of dementia in the elderly population; in 2020, approximately 50 million people worldwide suffered from dementia, with 60 to 70% of dementia cases attributed to AD. By 2050, the number of dementia cases is expected to increase to more than 150 million worldwide. Recent evidence has shown that the onset of AD may begin years before any signs of the disease become noticeable; by the time the patient notices cognitive impairment, the cascade of events leading to full AD may be irreversible without a disease-modifying therapy.

[0003] Hyperphosphorylated tau (pathological tau) is a major component of neurofibrillary tangles (NFTs), one of the characteristic pathological features of Alzheimer's disease (AD). Pathologically, AD is defined by the extracellular accumulation of Aβ plaques, intracellular accumulation of tau, neuroinflammation, neuronal and synaptic loss, and gross brain atrophy. Tau deposits in the brain correlate with memory decline, confirming the importance of tau pathology in AD. *See* Braak et al., "Neuropathological staging of Alzheimer-related changes," Acta Neuropathol. 1991; 82:239-59; Nelson et al., "Correlation of Alzheimer

Disease Neuropathologic changes with Cognitive Status: A Review of the Literature," J. Neuropathol. Exp. Neurol. 2012; 72:362-81.

[0004] In Alzheimer's disease, tau pathology spreads from one area of the brain to another in a stereotypical pattern along a neural network in a "prion-like" manner. NFTs appear in the entorhinal cortex and spread to anatomically connected regions across the entire cerebral cortex via the hippocampal areas. *See* Braak, supra. The progression of cognitive impairment correlates well with the tau pathology, as assessed by hallmarks such as aggregation or hyperphosphorylation. *See* Braak et al., "Staging of Alzheimer disease-associated neurofibrillary pathology using paraffin sections and immunohistochemistry," Acta Neuropathol. 2006; 112:389-404; Braak et al., "Neuropathological staging of Alzheimer-related changes," Acta Neuropathol. 1991;82:239-59.

[0005] Anti-tau antibodies and vaccines have been evaluated in preclinical studies, and at least eleven anti-tau antibodies have reached the clinic for the treatment of Alzheimer's disease. *See, e.g.*, Guo et al., "Tau-targeting therapy in Alzheimer's disease: critical advances and future opportunities", Ageing Neur Dis 2022;2:11; Panza et al., "The challenges of anti-tau therapeutics in Alzheimer's Disease" Nature Reviews Neurology 18, 577-78 (2022); Jadhav et al., "A Walk Through Tau Therapeutic Strategies," Acta Neuropathologica Communications, 22 (2019). However, no anti-tau therapies have been approved. A phase 2 study of the monoclonal anti-tau antibody semorinemab found that semorinemab did not slow the rate of cerebral tau accumulation or clinical decline in patients with prodromal to mild Alzheimer's disease. Teng et al., "Safety and Efficacy in Individuals with Prodromal to Mild Alzheimer's Disease: A Randomized Clinical Trial," JAMA Neurol. 2022; 79(8):758-767. Gosuranemab, another monoclonal antibody targeting the N-terminal region of tau, did not result in clinical benefit in participants with mild cognitive impairment due to AD or mild AD. Shulman et al., "Top-line results from TANGO, a phase 2 study of gosuranemab in participants with mild cognitive impairment due to Alzheimer's disease and mild Alzheimer's disease," J Prev Alzheimers Dis. 2021;8 (Suppl 1):S65. The anti-tau antibody zagotenemab was discontinued after its failure in a phase II study in Alzheimer's disease. *See* Muller, "Anti-tau antibody failures stack up," Nature Reviews Drug Discovery 20, 888 (2021).

[0006] There remains a need for novel anti-tau antibodies for use in treatment of neurodegenerative diseases like Alzheimer's Disease.

3. SUMMARY

[0007] The present disclosure provides antibodies that specifically bind to human tau, in particular antibodies that selectively bind to hyperphosphorylated tau T231 (pT231).

[0008] In embodiments, the present disclosure provides a full-length anti-tau antibody comprising a heavy chain variable region comprising a CDR-H1, a CDR-H2, and a CDR-H3, and a light chain variable region comprising a CDR-L1, a CDR-L2, and a CDR-L3, and wherein:

vH CDR-H1 has the amino acid sequence set forth as SEQ ID NO: 3;

vH CDR-H2 has the amino acid sequence set forth as SEQ ID NO: 4;

vH CDR-H3 has the amino acid sequence set forth as SEQ ID NO: 5;

vL CDR-L1 has the amino acid sequence set forth as SEQ ID NO: 8;

vL CDR-L2 has the amino acid sequence set forth as SEQ ID NO: 9; and

vL CDR-L3 has the amino acid sequence set forth as SEQ ID NO: 10.

[0009] In embodiments, the full-length anti-tau antibody comprises a heavy chain variable region having the amino acid sequence set forth as SEQ ID NO: 2 and a light chain variable region having the amino acid sequence set forth as SEQ ID NO: 7.

[0010] In embodiments, the present disclosure provides a full-length humanized monoclonal IgG_1 anti-tau antibody comprising two heavy chains and two light chains, wherein said heavy chains each comprise the amino acid sequence set forth as SEQ ID NO: 1, and said light chains each comprise the amino acid sequence set forth as SEQ ID NO: 6.

[0011] In embodiments, the present disclosure provides a full-length humanized monoclonal IgG_1 anti-tau antibody comprising two heavy chains and two light chains, wherein said heavy chains each comprise the amino acid sequence set forth as SEQ ID NO: 16, and said light chains each comprise the amino acid sequence set forth as SEQ ID NO: 6.

[0012] In embodiments, the present disclosure provides a full-length humanized monoclonal IgG_1 anti-tau antibody comprising two heavy chains and two light chains, wherein said heavy chains each comprise the amino acid sequence set forth as SEQ ID NO: 1 or SEQ ID NO: 16, and said light chains each comprise the amino acid sequence set forth as SEQ ID NO: 6.

[0013] Also provided herein are compositions comprising an anti-tau antibody of the present disclosure and a pharmaceutically acceptable carrier.

[0014] In embodiments, the present disclosure provides a method of treating Alzheimer's Disease, the method comprising administering an antibody according to the present disclosure to a patient in need thereof.

[0015] In embodiments, the present disclosure provides a method of treating Alzheimer's Disease, the method comprising administering a composition comprising an antibody of the present disclosure and a pharmaceutically acceptable carrier to a patient in need thereof.

4. BRIEF DESCRIPTION OF THE DRAWINGS

[0016] Figure 1 illustrates the *in vitro* specificity of the anti-tau antibody AbA for phosphorylated pT231 peptide.

[0017] Figure 2 illustrates the *in vitro* binding of the anti-tau antibody AbA to both phospho T231 tau and tau aggregates derived from the brain tissue of AD patients in a dose-dependent manner.

[0018] Figure 3 illustrates the *in vivo* tau seeding model according to the present disclosure.

[0019] Figure 4 provides a summary of the protocol for the *in vivo* tau seeding model and illustrates the effect of an anti-tau antibody of the present disclosure on hippocampal seeding in the rTG4510 mouse seeding model.

[0020] Figure 5A provides a schematic of an *in vivo* tau propagation model for Tau pathology.

[0021] Figure 5B shows photomicrographs of aggregated tau (AT100 IR) containing neurons in the first and second synapse brain regions following seeding in the olfactory bulb in the tau propagation assay according to the present disclosure. Following seeding with AD lysate, AT100 IR containing neurons increases in both a time and region dependent manner indicating propagation of pathology along synaptically connected pathways.

[0022] Figure 6A illustrates tau aggregates (AT100 IR cells) in the medial thalamus, and CA1 hippocampus 2 months after seeding with AD lysates containing various concentrations of tau into the olfactory bulb.

[0023] Figure 6B illustrates that the concentration of tau in the injected lysate correlates with the mean number of neurons containing tau aggregates, indicating that tau concentration in the donor samples facilitates subsequent tau seeding and propagation in hTau mice.

[0024] Figure 7A illustrates the study design of an hTau propagation efficacy study with anti-tau antibody chAbA.

[0025] Figure 7B illustrates that weekly intraperitoneal (i.p.) dosing with chAbA significantly reduced numbers of neurons containing AT100 IR tau aggregates as compared to control IgG treatment in both the medial thalamus and CA1 hippocampus of female hTau mice 2 months after injection of tau lysate into the olfactory bulb.

[0026] Figure 8 illustrates that weekly intraperitoneal (i.p.) dosing chAbA significantly reduced numbers of neurons containing aggregated tau (AT100 IR) as compared to IgG treated hTau mice as well as mice dosed with N-terminal Pan-Tau antibodies HJ8.5 (mouse version of Tilavonemab), Semorinemab or IPN002. The three Pan-Tau antibodies did not affect numbers of AT100 IR neurons in any brain region analyzed.

[0027] Figure 9A illustrates that weekly intraperitoneal (i.p.) dosing with chAbA for both 2 and 8 months significantly reduced the percentage of neurons containing aggregated tau as measured by AT100 IR in the CA1 hippocampus and medial thalamus as compared to IgG treated mice.

[0028] Figure 9B illustrates that weekly intraperitoneal (i.p.) dosing with chAbA resulted in a 54% and 87% reduction in spreading of tau pathology in the hippocampus and thalamus, respectively, between 2 and 8 months post-seeding.

5. DETAILED DESCRIPTION

[0029] Described herein are embodiments of anti-tau antibodies, in particular antibodies directed to pathological species of human tau. The antibodies of the present disclosure are hypothesized to specifically target the pathological species of tau propagating transneuronally, thus inhibiting or delaying the spread of tau pathology between neurons through the synaptic cleft and slowing down tau accumulation in the brain.

[0030] In embodiments, AbA is a humanized monoclonal IgG1 antibody that binds to human tau. In embodiments, AbA is a humanized monoclonal IgG1 antibody that binds human tau peptide that is phosphorylated at threonine 231 (pT231).

[0031] In embodiments, an antibody of the present disclosure comprises variable regions and CDRs (complementarity determining regions) identified according to rules developed in the art and/or by aligning sequences against a database of known variable regions. Methods for identifying these regions are described in Kontermann and Dubel, eds., Antibody Engineering, Springer, New York, N.Y., 2001 and Dinarello et al., Current Protocols in Immunology, John Wiley and Sons Inc., Hoboken, N.J., 2000. For example, CDRs may be identified in accordance with one of the schemes provided by Kabat et al. (1991) *Sequences of Proteins of Immunological Interest* (5^{th} Ed.), U.S. Dept. of Health and Human Services, PHS, NIH, NIH Publication No. 91-3242 (referred to herein as "Kabat"); or in accordance with AbM (Oxford Molecular/MSI Pharmacopia) (referred to herein as "AbM"). AbM can be obtained from the Abysis database at www.bioinf.org.uk/abs (maintained by A.C. Martin in the Department of Biochemistry & Molecular Biology University College London).

[0032] In embodiments, anti-tau antibody AbA comprises CDRs as recited in Table 1.

Table 1. CDRs (Kabat) of anti-tau antibody AbA

CDR Identity	SEQ ID NO	Sequence Location	Sequence
Heavy Chain, CDR-H1	SEQ ID NO: 3	Residues 31-35 of SEQ ID NO: 1	DYYMN
Heavy Chain, CDR-H2	SEQ ID NO: 4	Residues 50-66 of SEQ ID NO: 1	VFYPHLGYTIYNQKFKG
Heavy Chain, CDR-H3	SEQ ID NO: 5	Residues 99-109 of SEQ ID NO: 1	PYYYGSSSLDY
Light Chain, CDR-L1	SEQ ID NO: 8	Residues 24-39 of SEQ ID NO: 6	RSSQDLVESDADTYLH
Light Chain, CDR-L2	SEQ ID NO: 9	Residues 55-61 of SEQ ID NO: 6	KVSNRFS
Light Chain, CDR-L3	SEQ ID NO: 10	Residues 94-102 of SEQ ID NO: 6	SQSTHVPFT

[0033] In embodiments, an anti-tau antibody of the present disclosure comprises a CDR-H1 having the amino acid sequence set forth as SEQ ID NO: 3; a CDR-H2 having the amino acid sequence set forth as SEQ ID NO: 4; a CDR-H3 having the amino acid sequence set forth as SEQ ID NO 5; a CDR-L1 having the amino acid sequence set forth as SEQ ID NO: 8; a CDR-L2 having the amino acid sequence set forth as SEQ ID NO 9; and a CDR-L3 having the amino acid sequence set forth as SEQ ID NO 10.

[0034] In embodiments, the anti-tau antibody of the present disclosure is an IgG isotype. In embodiments, the anti-tau antibody of the present disclosure is an IgG1 isotype or an IgG2 isotype or an IgG3 isotype or an IgG4 isotype. In embodiments, the anti-tau antibody of the present disclosure is an IgG1 isotype. In embodiments, the anti-tau antibody of the present disclosure is an IgG2 isotype.

[0035] In embodiments, the anti-tau antibody of the present disclosure is a humanized antibody.

[0036] In embodiments, an anti-tau antibody of the present disclosure comprises a heavy chain variable region having the amino acid sequence set forth as SEQ ID NO: 2 (vH CDRs underlined, and shown as SEQ ID NOs: 3, 4, and 5 respectively, in order of appearance):

EVQLVQSGAEVKKPGSSVKVSCKASGYTFT<u>DYYMN</u>WVRQAPGQGLEWIG<u>VFYPH</u>
<u>LGYTIYNQKFKG</u>RATLTVDKSTSTAYMELSSLRSEDTAVYYCAS<u>PYYYGSSSLDY</u>W
GQGTLVTVSS (SEQ ID NO: 2)

and a light chain variable region having the amino acid sequence of SEQ ID NO: 7 (vL-CDRs underlined, and shown as SEQ ID NOs: 8, 9, and 10 respectively, in order of appearance):

DVQMTQSPSSVSASVGDRVTITCRSSQDLVESDADTYLHWYQQKPGKAPKLLIYKV SNRFSGVPSRFSGSGSGTDFTLTISSLQPEDFATYFCSQSTHVPFTFGQGTKVEIK (SEQ ID NO: 7).

[0037] In embodiments, an anti-tau antibody of the present disclosure comprises a heavy chain having the amino acid sequence set forth as SEQ ID NO: 1 (variable region is **bold**; constant region is *italicized*; CDRs are underlined and shown as SEQ ID NOs: 3, 4, and 5 respectively, in order of appearance):

EVQLVQSGAEVKKPGSSVKVSCKASGYTFTDYYMNWVRQAPGQGLEWIGVFYPH LGYTIYNQKFKGRATLTVDKSTSTAYMELSSLRSEDTAVYYCASPYYYGSSSLDYW GQGTLVTVSS*ASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTF PAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAPELL GGPSVFLFPPKPKDQLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQY NSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSREEMT KNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGN VFSCSVLHEALHNHYTQKSLSLSPGK* (SEQ ID NO: 1)

[0038] In embodiments, an anti-tau antibody of the present disclosure comprises a heavy chain having the amino acid sequence set forth as SEQ ID NO: 16 (variable region is **bold**; constant region is *italicized*; CDRs are underlined and shown as SEQ ID NOs: 3, 4, and 5 respectively, in order of appearance):

EVQLVQSGAEVKKPGSSVKVSCKASGYTFTDYYMNWVRQAPGQGLEWIGVFYPH LGYTIYNQKFKGRATLTVDKSTSTAYMELSSLRSEDTAVYYCASPYYYGSSSLDYW GQGTLVTVSS*ASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTF PAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAPELL GGPSVFLFPPKPKDQLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQY NSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSREEMT KNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGN VFSCSVLHEALHNHYTQKSLSLSPG* (SEQ ID NO: 16).

[0039] In embodiments, an anti-tau antibody of the present disclosure comprises a light chain having amino acid SEQ ID NO: 6 (variable region is **bold**; constant region is *italicized*; CDRs are underlined and shown as SEQ ID NOs: 8, 9, and 10 respectively, in order of appearance):

DVQMTQSPSSVSASVGDRVTITCRSSQDLVESDADTYLHWYQQKPGKAPKLLIYKV SNRFSGVPSRFSGSGSGTDFTLTISSLQPEDFATYFCSQSTHVPFTFGQGTKVEIK*RTV AAPSVFIFPPSDEQLKSGTASVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDSTY SLSSTLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRGEC* (SEQ ID NO: 6).

[0040] In embodiments, an anti-tau antibody of the present disclosure comprises two heavy chains each having the amino acid sequence set forth as SEQ ID NO: 1 and two light chains each having the amino acid sequence set forth as SEQ ID NO: 6.

[0041] In embodiments, an anti-tau antibody of the present disclosure comprises two heavy chains each having the amino acid sequence set forth as SEQ ID NO: 16 and two light chains each having the amino acid sequence set forth as SEQ ID NO: 6.

[0042] In embodiments, an anti-tau antibody of the present disclosure comprises two heavy chains each having the amino acid sequence set forth as SEQ ID NO:1 or SEQ ID NO: 16 and two light chains each having the amino acid sequence set forth as SEQ ID NO: 6.

[0043] In embodiments, the present disclosure provides one or more nucleic acids encoding any of the polypeptides as described herein. In embodiments, the present disclosure provides a polynucleotide encoding a polypeptide of a heavy chain as described herein, such as a polynucleotide encoding a polypeptide of a heavy chain as set forth in SEQ ID NO: 1 or SEQ ID NO: 16. In embodiments, the present disclosure provides a polynucleotide encoding a polypeptide of a light chain as described herein, such as a polynucleotide encoding a polypeptide of a light chain as set forth in SEQ ID NO: 6. In embodiments, the present disclosure provides a polynucleotide encoding (1) a polypeptide of a heavy chain as set forth in SEQ ID NO: 1 or SEQ ID NO: 16, and (2) a polypeptide of a light chain as set forth in SEQ ID NO: 6.

Compositions

[0044] The antibodies of this disclosure may be provided as a composition suitable for administration to a subject. In some embodiments, the antibody composition is a

pharmaceutical composition, including an antibody of this disclosure and a pharmaceutically acceptable carrier.

Methods of Use

[0045] Provided herein are embodiments of methods for treating Alzheimer's disease (AD) comprising administering to a subject in need thereof a therapeutically effective amount of an anti-tau antibody disclosed herein.

[0046] The term "subject", as used herein, refers to a human. The terms "human," "patient," and "subject" are used interchangeably herein.

Assays for Assessing Tau Seeding and Propagation

[0047] In embodiments, disclosed herein are assays for assessing tau propagation, in particular *in vivo* assays for assessing tau propagation.

[0048] In embodiments, the activity of an antibody of the present disclosure may be evaluated in a tau seeding assay. In embodiments, the assay comprises injecting a lysate derived from brain tissue of patients having Alzheimer's disease stereotactically into the hippocampus (2.5µl, coordinates: AP: -1,9mm; ML: -1,5mm; DV: -1,6) of rTG4510 mice (Santacruz K et al, Tau suppression in a neurodegenerative mouse model improves memory function. Science. 2005 Jul 15;309(5733):476-81. doi: 10.1126/science.1113694. PMID: 16020737; PMCID: PMC1574647). In embodiments, the assay comprises evaluating tau seeding in the mouse brain, in particular by quantifying the percent area of the CA1 hippocampus covered by tau aggregates. For example, in embodiments, tau aggregates may be quantified in the CA1 hippocampus. Accordingly, in embodiments, the assay of the present disclosure may be used to evaluate tau seeding in the CA1 hippocampus.

[0049] In embodiments, the present disclosure provides assays for assessing tau propagation, in particular *in vivo* assays for assessing tau propagation.

[0050] In embodiments, the assay comprises injecting a lysate derived from brain tissue of patients having Alzheimer's disease into the olfactory bulb of a hTau mouse; in embodiments, the mouse is an hTau mouse. In embodiments, the assay comprises evaluating tau propagation over time in the mouse brain, in particular by quantifying tau aggregates in synaptically connected regions of the brain. For example, in embodiments, tau aggregates

may be quantified over time at the site of tau seeding, in the olfactory bulb. In embodiments, tau aggregates may be evaluated in both first and second synapse brain region (e.g., piriform cortex and entorhinal cortex; medial thalamus and CA1 hippocampus). Accordingly, in embodiments, the assay of the present disclosure may be used to evaluate tau progression along synaptically connected pathways over time.

[0051] In embodiments, propagation to the first synapse (e.g., piriform cortex or entorhinal cortex) may be observed after about 4 weeks post-injection of the AD lysate. In embodiments, propagation to the second synapse (e.g., thalamus or hippocampus) may be observed after about 6 weeks post-injection of the AD lysate, or after about 8 weeks post-injection, or after about 12 weeks post-injection. In embodiments, propagation to the second synapse (e.g., medial thalamus or CA1 hippocampus) is first observed after about 6 weeks post-injection of the AD lysate, and becomes more robust after about 8 - 12 weeks post-injection.

[0052] In embodiments, the assay of the present disclosure is carried out in mice which do not express murine tau. In embodiments, the assay is carried out in mice which do not express aggregated tau, even with aging. In embodiments, the assay is carried out in mice which do not express mutated human tau. In embodiments, the assay is carried out in mice which express both the 3R and 4R tau isoforms. In embodiments, the assay is carried out in mice which express all six isoforms of the human MAPT gene (including both 3R and 4R forms). For example, in embodiments, the assay is conducted in hTau mice. hTau mice express the human tau isoforms, but no endogenous mouse tau is detected. See, e.g., Andorfer et al., "Hyperphosphorylation and aggregation of tau in mice expressing normal human tau isoforms," J. Neurochemistry, 2003, 86, 582-590; Phillips et al., "Olfactory and Visuospatial Learning and Memory Performance in Two Strains of Alzheimer's Disease Model Mice—A Longitudinal Study," PLoS One, 2001; 6(5): e195657, the disclosures of each of which are incorporated by reference herein in their entireties.

[0053] In embodiments, the assay of the present disclosure can be used to evaluate tau progressing along synaptically connected pathways in hTau mice. In embodiments, the assay comprises injecting a lysate derived from brain tissue of a patient or patients having Alzheimer's disease into the olfactory bulb of an hTau mouse, and observing aggregated tau

in neurons outside the olfactory bulb after injection of the AD lysate. For example, in embodiments, propagation to the first synapse brain region (e.g., piriform cortex, entorhinal cortex) may be observed after about 4 weeks. In embodiments, propagation to second synapse brain regions (e.g., CA1 hippocampus and medial thalamus) may be observed after about 6 weeks and is more robust by about 8 weeks and about 12 weeks post-injection of the AD brain lysate.

[0054] In embodiments, the present disclosure provides a method for evaluating the effect of an anti-tau compound on tau propagation, the method comprising injecting a lysate derived from brain tissue of patients having Alzheimer's disease into the olfactory bulb of an hTau mouse. In embodiments, the method further comprises administering an anti-tau compound to the mouse after injection of the lysate. In embodiments, the anti-tau compound is an anti-tau antibody. In embodiments, aggregated tau is quantified in the olfactory bulb. In embodiments, aggregated tau is quantified in synaptically connected brain regions. For example, in embodiments, aggregated tau may be quantified in at least one region selected from the group consisting of the piriform cortex, the medial thalamus, the hippocampus, and the entorhinal cortex. In embodiments, aggregated tau may be quantified in at least one first synapse brain region selected from the group consisting of the piriform cortex and the entorhinal cortex. In embodiments, aggregated tau may be quantified at a second synapse brain region selected from the group consisting of the medial thalamus and the CA1 hippocampus. In embodiments, the method may comprise comparing the amount of aggregated tau after administration of the anti-tau compound with the amount of aggregated tau achieved with a reference compound. In embodiments, the reference compound is an IgG antibody which does not bind tau.

[0055] In embodiments, the anti-tau compound may be administered at least about 1 day post lysate injection. In embodiments, the anti-tau compound is administered at least about 2 days post lysate injection. In embodiments, the anti-tau compound may be administered at least about 3 days post lysate injection. In embodiments, the anti-tau compound may be administered at least about 4 days post lysate injection. In embodiments, the anti-tau compound may be administered at least about 5 days post lysate injection. In embodiments, the anti-tau compound may be administered at least about 6 days post lysate injection. In

embodiments, the anti-tau compound may be administered at least about 7 days post lysate injection.

[0056] In embodiments, aggregated tau is quantified in the mouse brain at least about 1 day, or at least about 2 days, or at least about 3 days, or at least about 4 days, or at least about 5 days, or at least about 6 days, or at least about 7 days, after the last dose of the anti-tau compound is administered. In embodiments, the mouse brain is collected about 7 days after the last dose of the anti-tau compound. For example, in embodiments, a mouse may be injected with a first dose of an anti-tau compound at about 7 days post-injection of the AD brain lysate, followed by weekly doses of the anti-tau compound at weeks 2-8, and the mouse brain is collected about 7 days following the dose at week 8.

[0057] In embodiments, aggregated tau is quantified in the olfactory bulb. In embodiments, aggregated tau is quantified in at least one synaptically connected region selected from the group consisting of the piriform cortex, the entorhinal cortex, the medial thalamus, and the hippocampus. For example, in embodiments, aggregated tau is quantified in at least one first synaptically connected region selected from the group consisting of the piriform cortex and the entorhinal cortex. In embodiments, aggregated tau is quantified in at least one second synaptically connected region selected from the group consisting of the hippocampus and the thalamus. In embodiments, aggregated tau is quantified in the olfactory bulb, at least one first synaptically connected region selected from the group consisting of the piriform cortex and the entorhinal cortex, and at least one second synaptically connected region selected from the group consisting of the hippocampus and the thalamus. In embodiments, aggregated tau is quantified in the piriform cortex. In embodiments, aggregated tau is quantified in the thalamus. In embodiments, aggregated tau is quantified in the entorhinal cortex. In embodiments, aggregated tau is quantified in the hippocampus. In embodiments, aggregated tau is quantified in the olfactory bulb, the piriform cortex, and the thalamus. In embodiments, aggregated tau is quantified in the olfactory bulb, the entorhinal cortex, and the hippocampus.

[0058] In embodiments, aggregated tau is quantified by AT100 immunoreactivity quantification. AT100 is an anti-tau antibody which recognizes tau protein phosphorylated at Thr212 and Ser214. In embodiments, aggregated tau is quantified by counting cell bodies that are immunoreactive to the AT100 anti-tau antibody (i.e., AT100 IR cell bodies).

[0059] In embodiments, the methods of the present disclosure involve injection of an AD brain lysate. In embodiments, the lysate of the present disclosure is a sarkosyl-insoluble AD lysate. Sarkosyl extraction of tau has been described, for example, in Guo et al., Seeding of Normal Tau by Pathological Tau Conformers Drives Pathogenesis of Alzheimer-like Tangles, Journal Biol. Chem. Vol. 286, Issue 17, 15317-15331 (2011), and Guo et al., Unique Pathological Tau Conformers from Alzheimer's brains transmit tau pathology in nontransgenic mice, J. Exp. Med. 2016 Nov 14;213(12):2635-2654, the disclosures of which are incorporated herein by reference in their entireties.

[0060] Tau propagation in the assay of the present disclosure correlates with the tau concentration in the AD lysate; that is, tau concentration in donor samples used to prepare the lysate facilitates subsequent tau aggregation in hTau mice. In embodiments, the lysate of the present disclosure has a concentration of at least about 250 nM, or at least about 350 nM, or at least about 450 nM, or at least about 550 nM, or at least about 650 nM. In embodiments, the lysate of the present disclosure has a tau concentration of at least about 750 nM, such as at least about 760 nM, or at least about 770 nM, or at least about 780 nM, or at least about 790 nM, or at least about 800 nM, or at least about 850 nM, or at least about 900 nM, or at least about 950 nM, or at least about 1000 nM, or at least about 1100 nM, or at least about 1200 nM, or at least about 1300 nM, or at least about 1400 nM, or at least about 1500 nM, or at least about 1600 nM, or at least about 1700 nM, or at least about 1800 nM, or at least about 1900 nM, or at least about 2000 nM. In embodiments, the lysate has a tau concentration of from about 750 to about 2500 nM, such as from about 780 to about 2000 nM, or about 800 to about 2000 nM. In embodiments, the lysate has a tau concentration of at least about 800 nM. In embodiments, the tau in the AD lysate is primarily pathological tau protein, i.e., nonsoluble tau.

[0061] Figure 5A provides a schematic of an *in vivo* tau propagation model for tau pathology according to the present disclosure. Lysate purified from brain tissue of AD patients is used to seed tau into the olfactory bulb of female hTau mice, which express all six isoforms of normal human tau. Evidence of tau propagation is observed by the presence of neurons containing aggregated tau in brain regions that are synaptically connected to the olfactory bulb, including the piriform cortex and entorhinal cortex, which are first synapse brain

regions. The piriform cortex sends projections into the medial thalamus (second synapse region), and the entorhinal cortex neurons project to the CA1 of the hippocampal formation (second synapse region).

6. EXAMPLES

[0062] The following Examples highlight certain features and properties of the embodiments described herein.

Example 1: Anti-Tau Antibody Generation

[0063] The murine anti-tau antibody Ab1 was identified from CD-1 mice immunized with a mixture of six phospho-peptides derived from the human tau sequence: N-terminus and C-terminus KLH conjugated pT231; N-terminus and C-terminus KLH conjugated pS235; N-terminus and C-terminus KLH conjugated pT231, pS235.

PT231/PS235: ^{224}KKVAVVR$_P$TPPK$_P$SPSSAKSR^{242}

PS235: ^{224}KKVAVVR.TPPK$_P$SPSSAKSR^{242}

PT231: ^{224}KKVAVVR$_P$TPPK.SPSSAKSR^{242}

[0064] Immune libraries were constructed from the tissues of immunized mice and screened using a phage panning strategy. The phage output was evaluated for binding by ELISA to pT231 peptide, pS235 peptides, and pT231 / pS235 peptides.

[0065] The murine antibody Ab1 was identified, which has a heavy chain sequence set forth as SEQ ID NO: 11 and a light chain sequence set forth as SEQ ID NO: 12, demonstrated specificity to the phosphorylated T231 (pT231) peptide but not to the non-phosphorylated T231 peptide.

[0066] Ab1 was subsequently humanized and subjected to CDR engineering to eliminate potential liabilities and potential deamidation sites. This yielded the humanized antibody AbA, which has a heavy chain sequence set forth as SEQ ID NO: 1 or SEQ ID NO: 16 and a light chain sequence set forth as SEQ ID NO: 6. AbA maintained specificity to the phosphorylated T231 peptide, while also demonstrating higher affinity to phosphorylated T231 peptide than the murine Ab1 parent antibody.

[0067] To facilitate *in vivo* mouse testing, a version of AbA was created on a mouse IgG backbone. This antibody, chAbA, is a mouse IgG2a/k antibody having a heavy chain

sequence set forth as SEQ ID NO: 17 and a light chain sequence set forth as SEQ ID NO: 18. chAbA maintained specificity to the phosphorylated T231 peptide.

Example 2: Anti-Tau Antibody AbA shows specificity for phosphorylated (pT231) peptide

[0068] A study was performed to examine the specificity of AbA for the tau protein derived peptide KKVAVVRTPPKSPSSAK (SEQ ID NO: 13) that was either phosphorylated at tyrosine 231 (pT231) or not phosphorylated. In the study, the peptide was biotinylated, phosphorylated (or not phosphorylated), and interacted with bound antibody in an ELISA format.

[0069] ELISA was performed using Protein G 96 well plates (Thermo Fisher Scientific, Waltham, MA, USA) washed 1X in 200 µL of phosphate-buffered saline containing 0.05% Polysorbate 20 (PBS-T) using a BioTek ELx405 plate washer (Agilent, Santa Clara, CA, USA). The plates were then incubated with AbA or a control hu IgG1-QL at 1 µg/mL concentration in 0.1% bovine serum albumin (BSA) in PBS-T for 2 hours under ambient conditions on an orbital shaker at 120 RPM. Plates were then washed 3X with PBS-T and then incubated with different concentrations of N-terminal biotinylated phospho T231 (SEQ ID NO: 13) and non-phosphorylated peptide (SEQ ID NO: 13), with concentrations ranging from 1.6 to 0.0002 ng/mL, for 1 hour under ambient conditions on a shaker at 120 RPM. Peptides were diluted threefold in 0.1% BSA in PBS-T with concentrations ranging from 1.6 to 0.002 ng/ mL. After incubation, plates were washed 3X and incubated with SA-HRP-40 (Fitzgerald Industries, Acton, MA, USA) diluted 1:6000 in 0.1% BSA in PBS-T for 1 hour at ambient conditions while on a shaker at 120 RPM. Following incubation, plates were washed 3 times in PBS-T and developed with Super Slow ELISA TMB (MilliporeSigma, Burlington, MA, USA) and absorbance was read at 650 nM after 15 minutes on BioTek Synergy H1 microplate reader (Agilent).

[0070] The quantity of antibody-bound biotinylated peptide was detected by HRP-streptavidin and binding was determined, and binding of AbA to the tau-derived peptide was evaluated (Figure 1). Each sample was performed in triplicate in each run, and values from three runs were represented as the mean +/- SD. EC_{50} was calculated from each peptide binding ELISA, and its average from three different experiments was 0.06 ng/mL. In three

independent runs, it was shown that AbA selectively and dose-dependently binds to the phospho T231 peptide with an average EC_{50} of 0.06 ng/mL. No binding to non-phosphorylated peptide was observed. Control mAb hu IgG1-QL did not bind to any of the peptides.

Example 3: Preparation of human AD lysate from donor samples

[0071] Brain homogenates were generated from frontal cortex tissues of patients diagnosed with Alzheimer's disease (n=10). The tissues were homogenized in cold phosphate buffered saline supplemented with protease (MilliporeSigma, Burlington, MA, USA) and phosphatase inhibitors (MilliporeSigma) at 5 µL/mg (v/wt) using the Precellys Evolution tissue homogenizer (Bertin Technologies, Bretonneux, France). After homogenization, the homogenates were spun at 3000 x *g* for 5 minutes at 4 °C and the clear supernatant was collected. An equal volume of clear lysates from each patient was mixed to generate a pooled lysate. Pooled AD lysates, n=13, were used for *in vitro* immunodepletion experiments (Example 4), and n=10 lysate was used for *in vivo* seeding experiments (Example 5). Lysates were aliquoted and stored at -80 °C until use.

Example 4: *In vitro* activity of the AbA antibody on pT231 peptide and human tau purified from brain tissue of AD patients

[0072] Immunodepletion of AD lysate: Pooled AD brain lysates (n=13) that were spun at 3000 x *g* were subsequently used for immunodepletion experiments. AbA or isotype control hu IgG1-QLantibodies diluted in 3-fold concentrations ranging from 100 nM to 0.01 nM were incubated with protein G magnetic beads (Thermo Fisher Scientific) for 30 minutes. Magnetic beads coated with antibodies were then incubated with 100 nM of tau concentration of pooled human AD lysate prepared as described above in Example 3 for 1.5 hours at ambient conditions on a HulaMixer™ (Thermo Fisher Scientific) at ca. 5 RPM. After incubation, samples were pulse spun in a benchtop centrifuge and placed directly on the magnetic stand for 1 minute.

[0073] Immunodepleted lysate was collected and used to evaluate the levels of tau aggregates using the Single molecule array (Simoa®) tau aggregates assay. Simoa is a bead-

based tau aggregates assay developed using the anti tau mAb HT7 (Thermo Fisher Scientific) and mouse Ab1. For the pT231 assay, mouse Ab1 was used as a capture antibody, and HT7 mAb was used as the detection antibody. For the tau aggregates assay, HT7 mAb was used as both the capture and detection antibody. Antibody labeled beads and biotinylated detection antibodies were used according to the manufacturer's protocol (Quanterix®, Billerica, MA, USA). Recombinant full-length P301L tau (SEQ ID NO: 14; Spillantini MG, et al. Tau pathology in two Dutch families with mutations in the microtubule-binding region of tau (*Am J Pathol*. 1998 Nov;153(5):1359-63.) was purified by the method of Barghorn et al. (Purification of recombinant tau protein and preparation of Alzheimer-paired helical filaments in vitro. Methods Mol Biol. 2005;299:35-51. Doi: 10.1385/1-59259-874-9:035. PMID: 15980594) and used to generate aggregates as described in Yanamandra et al. (Anti-tau antibodies that block tau aggregate seeding in vitro markedly decrease pathology and improve cognition in vivo. Neuron. 2013 Oct 16;80(2):402-414. doi: 10.1016/j.neuron.2013.07.046). A hyperphosphorylated tau was produced in SF9 cells (SEQ ID NO: 15; Tepper et al. Oligomer formation of tau protein hyperphosphorylated in cells. J Biol Chem. 2014 Dec 5;289(49):34389-407. doi: 10.1074/jbc.M114.611368). The hyperphosphorylated tau from SF9 cells was purchased as cell paste containing the recombinant protein from GenScript (GenScript ProBio, Piscataway, NJ, USA) and purified by the method of Tepper (supra). Full-length P301L tau aggregates and SF9 cell derived hyperphosphorylated tau were used as calibrators and included in each run to generate a standard curve. The Simoa HD-X instrument, buffers, helper beads, streptavidin β-galactosidase, and enzyme-substrate resorufin β-*D*-galactopyranoside were obtained from Quanterix®. Assays were performed according to the manufacturer's instructions. All samples were diluted in the Tau Calibrator Diluent (Quanterix) prior to performing Simoa.

[0074] Figure 2 illustrates the *in vitro* binding of the anti-tau antibody AbA to both phosphorylated tau pT231 and tau aggregates derived from the brain tissue of AD patients in a dose-dependent manner. As shown in Figure 2, in contrast to the IgG control, AbA-magnetic bead complexes dose dependently depleted pT231 with an IC_{50} of 2.64 nM (Figure 2A) and tau aggregates with an IC_{50} of 3.68 nM (Figure 2B) from AD lysates. IC_{50} values were calculated from three independent immunodepletion experiments. Levels of pT231 and

total tau aggregates were measured using Simoa® Ab1-HT7 and HT7-HT7 tau aggregates assay. Values represent mean +/- SD.

Example 5: Effect of an anti-tau antibody in an *in vivo* model of tau seeding

[0075] A model of tau seeding was developed in transgenic rTG4510 mice where lysate was used to directly seed tau into the hippocampus (Figure 3). Briefly, stereotactic intrahippocampal injection of protein lysate generated from brain tissue in AD patients in rTG4510 mice (Figure 3A) results in the expression of aggregated tau (AT100 IR) in the CA1 region of the hippocampus (Figure 3B).

[0076] Transgenic rTG4510 mice were used for the hippocampal seeding studies (Santacruz 2005; 10.1126/science.1113694). The original breeder mice were obtained from the Jackson Laboratory (Bar Harbor, ME, USA) under the license from Mayo Clinic (Rochester, MN, USA). Mice were bred at Charles River Laboratories (Sulzfeld, DE). Animal health and comfort were veterinary controlled. Mice were group housed in temperature- and humidity-controlled rooms with a 12:12 hour dark/light cycle with *ad libitum* access to water and food.

[0077] To evaluate the effect of antibody chAbA treatment on tau seeding, rTG4510 mice (6.5 to 7.5 weeks of age) were pre-dosed via intraperitoneal (i.p.) injection with the antibodies (chAbA or IgG control) three times within a period of 10 days, as illustrated in Figures 3 and 4, at doses X, Y, and Z (where dose X < Y < Z). chAbA represents a chimeric version of AbA on a mouse IgG backbone as described above. At the age of 8-9 weeks animals were prepared for aseptic stereotactic surgery and were unilaterally injected with protein lysate derived from human AD brain (AD lysate, prepared as described above in Example 2) into the hippocampus (2.5 µL, coordinates: AP: -1,9mm; ML: -1,5mm; DV: -1,6) using a Hamilton syringe (Hamilton, Reno, NV, USA). After recovery from surgery, mice were returned to their home cages.

[0078] Three weeks later, mice were euthanized, perfused with phosphate buffered saline (PBS), and the brains were quickly removed. For seeding studies, the trimmed forebrain was drop-fixed in 10% formalin for 24 hours before being switched to 70% ethanol:water. For propagation studies the entire brain was drop-fixed in 10% formalin for 48 hours before being switched to 70% ethanol:water. Brains were stored in 70% ethanol until all brains

from the study were collected to allow all samples to be processed for paraffin embedding at the same time (Leica Biosystems, Wetzlar, DE).

[0079] After processing, four brains were embedded together into paraffin blocks in coronal plane (Medite, Burgdorf, DE). Brains were randomized based on the test group and paraffin blocks containing 4 randomized brains were generated. Four µm paraffin sections were prepared and processed on a Leica BOND Rx automated stainer (Leica Biosystems, Wetzlar, DE). In summary, slides were placed on the BOND RX deparaffinized, rehydrated, and underwent a series of pretreatments including peroxide block, protein blocks (Abcam, Berlin, DE) and processed with a citrate-buffer based heat-induced antigen retrieval solution (Epitope Retrieval Solution 1 Bond, Leica) using AT100 antibody (ThermoFisher Scientific, Darmstadt, DE) at a concentration of 0.06µg/ mL and diaminobenzidine (DAB-) based detection (Leica) and hematoxylin counterstaining (Leica). Digital images of the stained tissue slides were collected with a P1000 scanner (3DHistec, Sysmex, Hamburg, DE).

[0080] To quantify the Tau pathology induced by the injection of the AD lysate and the therapeutic efficacy of the test antibodies, three matched sections per brain at the hippocampal level were analyzed using the Area Quantification module in HALO™ image analysis software (Indica Labs, Albuquerque, NM, USA). For each section, the CA1 region of the ipsilateral hippocampus was outlined. Using the software, the "threshold" was determined by an observer who was blind to the treatment of the animals. The threshold was set so that the positive brown DAB stain of the AT100 immunoreactivity was recognized by the software and any background/non-specific staining was excluded from the analysis. Once an appropriate threshold was set, the software measured the percentage of the area of interest (CA1 region) containing the positive immunoreactivity for AT100. The AT100 IR area values for each animal were then plotted and analyzed in GraphPad Prism (Dotmatics, Boston, MA, USA)

[0081] Figure 4 summarizes the protocol for antibody pre-dosing, seed injection, and tissue and serum collection, and presents the AT100 IR results illustrating the effect of the anti-tau antibody chAbA on hippocampal seeding in the rTG4510 seeding model. As shown in Figure 4, at doses Y and Z, chAbA significantly reduced tau pathology (measured by the % area of AT100 IR in the CA1 region of the hippocampus) as compared to IgG treatment. Pre-dosing

the animals three times with dose X chAbA did not significantly reduce AT100 IR in the CA1 region of the hippocampus.

Example 6: *In Vivo* Tau Propagation Assay

Preparation of human sarkosyl insoluble tau AD lysate from donor samples

[0082] Donor brain tissue was weighed and homogenized three times in TBS lysate buffer (50 mM TRIS, 150 mM NaCl, 20 mM NaF, 1 mM Na_3VO_4, 0.5 mM $MgSO_4$ (pH 7.4) containing protease and phosphatase inhibitors using a Precellys Evolution homogenizer (Bertin Technologies, Bretonneux, FR). Following homogenization, lysates were centrifuged at 27,000 x *g* for 20 minutes at 4 °C. The supernatant was saved and the resulting pellet was resuspended in 1X salt/sucrose buffer (0.8 M NaCl, 10% sucrose, 10 mM TRIS HCl, 1 mM EGTA) and sonicated on ice. The sonicated samples were then centrifuged at 27,000 x *g* for 20 minutes at 4 °C. The two supernatants were combined and equal volumes of 2X salt/sucrose buffer (1.6 M NaCl, 20% sucrose, 20 mM TRIS HCl, 2 mM EGTA) were added. To this combined supernatant, add 10% sarkosyl detergent (in 1X salt/sucrose) to bring the final concentration of sarkosyl to 1% (w/v). The supernatants were then shaken at ambient conditions for 1.5 hours. Following shaking, the samples were centrifuged at 250,000 x *g* for 90 minutes at 4 °C. The resulting pellet was resuspended in TBS, sonicated on ice and centrifuged at 100,000 x *g* for 60 minutes at 4 °C. The resulting pellet was resuspended in sterile PBS, sonicated and then centrifuged at 10,000 x *g* for 30 minutes at 4 °C. The resulting supernatant was the sarkosyl insoluble tau AD lysate used to seed tau.

[0083] Aggregated tau species were quantified using a homogenous time-resolved fluorescence (HTRF)-based tau aggregation assay kit (PerkinElmer - Cisbio, Waltham, MA, USA). The manufacturer's protocol was followed to ensure the appropriate dilution for the donor and receptor fluorophore-tagged antibodies. Assay reactions (20 µL) were carried out in 384 microplate (Greiner Bio-One, Kremsmünster, AT), where samples were incubated with the fluorophore-tagged antibodies for 24 hours at ambient temperature. The fluorescent signal was detected using Cytation 5 (Agilent, Santa Clara, CA, USA) by excitation at 340 nm and emission at 620 nm and 665 nm. The relative FRET rate for each sample was determined by calculating the ratio of the two fluorescence intensities (665/620). Data were

plotted in GraphPad Prism (Dotmatics) and a One-way ANOVA was used to compare statistical significance between human AD and control brain lysates.

[0084] Total tau levels were measured using the R-PLEX Human total tau kit (Meso Scale Discovery, Rockville, MD) in a sandwich immunoassay. Biotinylated capture antibody was diluted in coating diluent (MSD Diluent 100) and incubated for 1 hour at ambient conditions on a shaker. A stock of loading dye was prepared using 9 parts of 4x laemmli buffer (Bio-Rad, Hercules, CA) and 1 part β-mercaptoethanol (Bio-Rad). For sample preparation, we prepared a 1:5 dilution of the sample using the loading dye and PBS, following which samples were vortexed and spun down using a bench-top microcentrifuge. Samples were then heated at 95 °C for 5 minutes on a heating block and then placed on ice. Samples were diluted to 1:80,000 in MSD Diluent 101 and assayed in duplicates. R-plex human tau calibrator was prepared as a 7-point calibration curve starting at 5000 pg/mL with a four-fold dilution series down to 1.2pg/mL, as recommended by the manufacturer. A blank was also included for background signal deduction. After 1 hour of coating with the capture antibody, plates were washed with 1x MSD Tris wash buffer at 150 µL/well. The calibrator and unknowns were added at 25 µL/replicate, plates were sealed with an adhesive plate sealer and incubated for 1 hour at ambient conditions on a shaker. After 1 hour, plates were washed with 150 µL/well with 1X MSD Tris wash buffer. The 100x SULFO-Tag Detection antibody (Meso Scale Discovery) was diluted to 1x using MSD Diluent 101 and added to the plate at 50 µL/well. After 1 hour of incubation, plates were washed with 1X MSD Tris wash buffer. In the last step, MSD Read Buffer T (4x) was diluted to 2X working solution in deionized water and added to the plates at 150 µL/well. Plates were immediately read on the Meso Sector S600 (Meso Scale Discovery).

Animals and experimental design

[0085] Female hTau mice (B6.Cg-Mapt Tg(MAPT)8cPdav/J, Jackson Laboratory, Bar Harbor, ME, USA) that were homozygous for the targeted allele and hemizygous for the transgene were used for these studies. The mice were provided at about 3 months of age and were housed five animals/cage with 12:12 hour dark/light cycle with *ad libitum* access to food and water.

[0086] At 4.5-5 months of age, female hTau mice were prepped for aseptic stereotaxic surgery and 2 µL of sarkosyl-insoluble AD lysate was injected into the olfactory bulb using a Hamilton syringe (Hamilton). After recovery from surgery, mice were returned to their home cages. Animals were dosed weekly i.p. starting 7 days after the seed injections (see Figure 7A).All experiments were performed in full compliance with the Principles of Laboratory Animal Care (NIH publication No. 86-23). Experiments performed in the U.S. were performed in full compliance with AbbVie's Institutional Animal Care and Use Committee (IACUC). All animal studies performed in Germany were approved by the government of Rhineland Palatinate (Landesuntersuchungsamt Koblenz, DE) and conducted in accordance with directive 2010/63/EU of the European Parliament and of the Council on the protection of animals used for scientific purposes, the ordinance on the protection of animals used for experimental or scientific purposes (German implementation of EU directive 2010/63). Animal studies were conducted in an Association for Assessment and Accreditation of Laboratory Animal Care International (AAALAC) accredited facility where veterinary care and oversight was provided to ensure appropriate animal care.

Tissue processing and immunohistochemistry

[0087] At the appropriate time point, mice were euthanized, perfused with phosphate buffered saline (PBS), and the brains were quickly removed.

[0088] After processing, three brains from different treatment groups or time points were embedded together into paraffin blocks (Sakura Finetek, Torrance, CA, USA) in a horizontal plane. Every 5th, 5 µm paraffin sections was collected through the entire brain and mounted onto glass slides. Twenty-five sections (every 5th section) per block were stained for AT100 immunoreactivity using the BOND RX stainer with the Refine Detection kit (Leica). Briefly, the slides were deparaffinized, rehydrated, and placed on the BOND RX where they underwent a series of pretreatments including peroxide block, 5% donkey serum blocking (Leica) and were then incubated overnight in the AT100 mouse monoclonal antibody to tau (Thermo Fisher Scientific, Waltham, MA, USA) at a concentration of 0.006 µg/mL.

[0089] Slides were then returned to the BOND RX where they underwent a series of washes before being incubated in Biotin-SP-conjugated F(ab') 2 donkey anti mouse IgG(H+L) secondary antibody (Jackson ImmunoResearch Labs, West Grove, PA, USA) at a

concentration of 2 µg/mL for 20 minutes. After additional washing steps, the slides were incubated for 20 minutes in Streptavidin/Horseradish Peroxidase (Leica) and the immunoreactivity was visualized using diaminobenzidine (DAB; Leica) and counterstained with hematoxylin (Leica).

AT100 immunoreactivity quantification

[0090] AT100 IR cells were counted in matched sections through the entire extent of the olfactory bulb (8 – 10 slides/brain), piriform cortex (6 – 8 sections/brain), entorhinal cortex (8 – 10 sections/brain), CA1 hippocampus (10-12 sections/brain) and medial thalamus (9 – 11 sections/brain) by an observer blind to the treatment or time point. Cell counts for each region were totaled to give one number/brain region/mouse.

The effect of htau concentration in AD lysate on tau aggregation (AT100 IR) following seeding in the olfactory bulb

[0091] When purified human tau derived from brain tissue from various AD patients was injected into the olfactory bulb of female hTau mice, the level of tau aggregation along synaptically connected brain regions correlated with the concentration of tau present in the lysate (Figure 4A and 4B). In this study, lysates from individual AD donor brain tissue were prepared. In addition, lysates from multiple donors were mixed and the amount of insoluble tau present in the samples determined.

Table 2: Cortical Tissues from AD donors with various concentrations of tau

Donor ID	SP tau (ug/mL)	Molarity (nM)	Total protein (ug/ml)
37526	44.4	1076	2821.2
37524	32.6	790	2698.700
01-15	13.7	331	1419.700
18-15 (F)	83.7	2026	2760.300
18-54	11.6	281	1607.300
01-45 (F)	62.3	1509	3042.300
15-29 (F)	56.3	1363	3822.600
15-29 (T)	100.3	2430	1952.2
01-45 (T)	31.0	751	1065.9
18-15 (T)	63.6	1547	987.3
37524-1 (F)	39.3	951	1505.6
37524-2 (F)	36.9	893	1336.1
37524-3 (F)	57.9	1400	1364.9

[0092] The lysates were injected into the olfactory bulb of hTau mice. Two months after tau seeding, neurons containing aggregated tau were visualized by AT100 IR and quantified in the olfactory bulb, piriform cortex, entorhinal cortex, medial thalamus, and CA1 hippocampus (Figure 6A). Analysis showed that the concentration of tau in the AD lysate correlated with the level of tau pathology with coefficients of variation between 0.67 and 0.814 (Figure 6B). The tau aggregates from the mixture samples also correlated with the results from individual samples, confirming that the level of tau seeding and propagation in the model system was dependent on the concentration of insoluble tau present in the lysate injected into the olfactory bulb. This finding led to standardization of mixed AD lysate used for subsequent studies to be approximately 800 nM human tau protein.

Efficacy of antibody treatment on tau propagation

[0093] A study was performed to determine if the chAbA anti-tau antibody could affect tau propagation and subsequent aggregation. The study design is illustrated in Figure 7A. Four and a half-month-old female hTau mice were injected with purified AD brain lysate into the olfactory bulb; intraperitoneal (i.p.) dosing with chAbA antibody started 7 days later at doses

X, Y, and Z (where X < Y < Z). Weekly dosing continued for 8 weeks post-seeding with AD lysate.

[0094] Two months after pathological tau seeding, neurons containing tau aggregates, as measured by AT100 IR, were counted in the olfactory bulb as well as second synapse brain regions, the CA1 hippocampus and the medial thalamus. In the olfactory bulb, weekly intraparitoneal dosing with chAbA did not affect tau pathology indicating that treatment had no effect on tau seeding. However, at the second synapse, weekly intraparitoneal dosing with chAbA at all doses significantly reduced aggregated tau in both the CA1 hippocampus and the medial thalamus (Figure 7B), when dosing started seven days post-seeding. Analysis of these data suggest that anti-tau chAbA antibody treatment can alter propagation without affecting tau seeding.

[0095] Comparison of efficacy of antibody treatment with N-Terminal pan-tau antibodies on tau propagation

[0096] A study was performed to compare efficacy of chAbA in the tau propagation model with N-terminal, pan-tau targeting anti-tau antibodies that were previously tested in human clinical trials. The study design is the same as illustrated in Figure 7A. Four and a half-month-old female hTau mice were injected with purified AD brain lysate into the olfactory bulb; intraperitoneal (i.p.) dosing with chAbA antibody, HJ8.5 (mouse version of Tilavonemab), mouse version of Semorinemab and IPN002 started 7 days later. Weekly dosing continued for 8 weeks post-seeding in the olfactory bulb with AD lysate.

[0097] Two months after pathological tau seeding, neurons containing tau aggregates, as measured by AT100 IR, were counted in the olfactory bulb as well as second synapse brain regions, the CA1 hippocampus and the medial thalamus. In the olfactory bulb, treatment with chAbA or N-Terminal anti-tau antibodies did not affect tau pathology indicating that treatment had no effect on tau seeding. However, at the second synapse, weekly intraperitoneal (i.p.) dosing with chAbA significantly reduced aggregated tau in both the CA1 hippocampus and the medial thalamus (Figure 8) as compared to control IgG treatment as well treatment with N-terminal reference antibodies. Semorinemab, HJ8.5 and IPN002 did not significantly affect tau

propagation in either second synapse brain region when dosing started seven days post-seeding. Analysis of these data suggests that chAbA but not the N-Terminal anti-tau antibodies tested can alter tau propagation.

Comparison of efficacy with reference antibody treatment on tau propagation

[0098] A study was performed to determine efficacy of chAbA in the tau propagation model after 8 months of weekly intraperitoneal dosing. Four and a half-month-old female hTau mice were injected with purified AD brain lysate into the olfactory bulb; intraperitoneal (i.p.) dosing with chAbA antibody started 7 days later. Weekly dosing continued for 8 weeks (2M group) or 35 weeks (8M group) post-seeding with AD lysate.

[0099] Two and eight months after pathological tau seeding, neurons containing tau aggregates, as measured by AT100 IR, were counted in the olfactory bulb as well as second synapse brain regions, the CA1 hippocampus and the medial thalamus. In the olfactory bulb, intraperitoneal (i.p.) dosing with chAbA did not affect tau pathology at either time point indicating that treatment had no effect on tau seeding. However, at the second synapse, weekly intraperitoneal (i.p.) dosing with chAbA significantly reduced numbers of neurons containing aggregated tau in both the CA1 hippocampus and the medial thalamus (Figure 9A) as compared to control IgG treatment at both time points. Analysis of these data suggests that chAbA dosing reduced spreading of tau pathology to the hippocampus and thalamus by 54-87% , respectively, between 2 and 8 months post-injection of AD lysate into the olfactory bulb (Figure 9B).

[00100]

Sequence Table				
SEQ ID NO:	**Clone Name**	**Description**	**Residue**	**Sequence**
1	AbA	Heavy Chain Amino Acid	Residues 1-450	EVQLVQSGAEVKKPGSSVKVSCKASGY TFTDYYMNWVRQAPGQGLEWIGVFYPH LGYTIYNQKFKGRATLTVDKSTSTAYME LSSLRSEDTAVYYCASPYYYGSSSLDYW GQGTLVTVSSASTKGPSVFPLAPSSKSTS GGTAALGCLVKDYFPEPVTVSWNSGALT SGVHTFPAVLQSSGLYSLSSVVTVPSSSL GTQTYICNVNHKPSNTKVDKKVEPKSC DKTHTCPPCPAPELLGGPSVFLFPPKPKD QLMISRTPEVTCVVVDVSHEDPEVKFNW YVDGVEVHNAKTKPREEQYNSTYRVVS VLTVLHQDWLNGKEYKCKVSNKALPAP IEKTISKAKGQPREPQVYTLPPSREEMTK NQVSLTCLVKGFYPSDIAVEWESNGQPE NNYKTTPPVLDSDGSFFLYSKLTVDKSR WQQGNVFSCSVLHEALHNHYTQKSLSL SPGK
2	AbA	Variable heavy chain domain	Residues 1-120 of SEQ ID NO: 1	EVQLVQSGAEVKKPGSSVKVSCKASGY TFTDYYMNWVRQAPGQGLEWIGVFYPH LGYTIYNQKFKGRATLTVDKSTSTAYME LSSLRSEDTAVYYCASPYYYGSSSLDYW GQGTLVTVSS
3	AbA	vH CDR-1	Residues 31-35 of SEQ ID NO: 2	DYYMN
4	AbA	vH CDR-2	Residues 50-66 of SEQ ID NO: 2	VFYPHLGYTIYNQKFKG
5	AbA	vH CDR-3	Residues 99-109 of SEQ ID NO: 2	PYYYGSSSLDY
6	AbA	Light Chain Amino Acid	Residues 1-219	DVQMTQSPSSVSASVGDRVTITCRSSQD LVESDADTYLHWYQQKPGKAPKLLIYK VSNRFSGVPSRFSGSGSGTDFTLTISSLQP EDFATYFCSQSTHVPFTFGQGTKVEIKRT VAAPSVFIFPPSDEQLKSGTASVVCLLNN FYPREAKVQWKVDNALQSGNSQESVTE

				QDSKDSTYSLSSTLTLSKADYEKHKVYA CEVTHQGLSSPVTKSFNRGEC
7	AbA	Variable light chain domain	Residues 1-112 of SEQ ID NO: 6	DVQMTQSPSSVSASVGDRVTITCRSSQD LVESDADTYLHWYQQKPGKAPKLLIYK VSNRFSGVPSRFSGSGSGTDFTLTISSLQP EDFATYFCSQSTHVPFTFGQGTKVEIK
8	AbA	vL CDR-1	Residues 24-39 of SEQ ID NO: 7	RSSQDLVESDADTYLH
9	AbA	vL CDR-2	Residues 55-61 of SEQ ID NO: 7	KVSNRFS
10	AbA	vL CDR-3	Residues 94-102 of SEQ ID NO: 7	SQSTHVPFT
11	Murine Ab1	Heavy Chain Amino Acid	Residues 1-450	QIQLLQSGPVLVKPGASVKMSCKASGYT FTDYYMNWVKQSRGKSLEWIGVFYPYS GITTYNQKFKGKATLTVDKSSSTAYMEL NSLTSEDSAVYYCASPYYYGSSSLDYWG QGTTLTVSSAKTTAPSVYPLAPVCGDTT GSSVTLGCLVKGYFPEPVTLTWNSGSLSS GVHTFPAVLQSDLYTLSSSVTVTSSTWPS QSITCNVAHPASSTKVDKKIEPRGPTIKPC PPCKCPAPNLLGGPSVFIFPPKIKDVLMIS LSPIVTCVVVDVSEDDPDVQISWFVNNV EVHTAQTQTHREDYNSTLRVVSALPIQH QDWMSGKEFKCKVNNKDLPAPIERTISK PKGSVRAPQVYVLPPPEEEMTKKQVTLT CMVTDFMPEDIYVEWTNNGKTELNYKN TEPVLDSDGSYFMYSKLRVEKKNWVER NSYSCSVVHEGLHNHHTTKSFSRTPGK
12	Murine Ab1	Light Chain Amino Acid	Residues 1-219	DVVMTQTPLSLPVTLGDQASISCRSSQSL VHSNGNTYLHWYLQKPGQSPKLLIYKV SNRFSGVPDRFSGSGSGTDFTLKISRVEA EDLGVYFCSQSTHVPFTFGSGTKLEIKRA DAAPTVSIFPPSSEQLTSGGASVVCFLNN FYPKDINVKWKIDGSERQNGVLNSWTD QDSKDSTYSMSSTLTLTKDEYERHNSYT CEATHKTSTSPIVKSFNRNEC
13	Human Tau	Tau protein peptide	Residues 224-240	KKVAVVRTPPKSPSSAK

	224-240			
14	Human Tau P301L	Mutated Tau P301L	Residues 1-441	MAEPRQEFEVMEDHAGTYGLGDRKDQ GGYTMHQDQEGDTDAGLKESPLQTPTE DGSEEPGSETSDAKSTPTAEDVTAPLVDE GAPGKQAAAQPHTEIPEGTTAEEAGIGD TPSLEDEAAGHVTQARMVSKSKDGTGS DDKKAKGADGKTKIATPRGAAPPGQKG QANATRIPAKTPPAPKTPPSSGEPPKSGDR SGYSSPGSPGTPGSRSRTPSLPTPPTREPK KVAVVRTPPKSPSSAKSRLQTAPVPMPDL KNVKSKIGSTENLKHQPGGGKVQIINKK LDLSNVQSKCGSKDNIKHVLGGGSVQIV YKPVDLSKVTSKCGSLGNIHHKPGGGQ VEVKSEKLDFKDRVQSKIGSLDNITHVP GGGNKKIETHKLTFRENAKAKTDHGAEI VYKSPVVSGDTSPRHLSNVSSTGSIDMV DSPQLATLADEVSASLAKQGL
15	Human SF9	Hyperphosphorylated human tau	Residues 1-440	AEPRQEFEVMEDHAGTYGLGDRKDQGG YTMHQDQEGDTDAGLKESPLQTPTEDG SEEPGSETSDAKSTPTAEDVTAPLVDEGA PGKQAAAQPHTEIPEGTTAEEAGIGDTPS LEDEAAGHVTQARMVSKSKDGTGSDDK KAKGADGKTKIATPRGAAPPGQKGQAN ATRIPAKTPPAPKTPPSSGEPPKSGDRSGY SSPGSPGTPGSRSRTPSLPTPPTREPKKVA VVRTPPKSPSSAKSRLQTAPVPMPDLKN VKSKIGSTENLKHQPGGGKVQIINKKLD LSNVQSKCGSKDNIKHVPGGGSVQIVYK PVDLSKVTSKCGSLGNIHHKPGGGQVEV KSEKLDFKDRVQSKIGSLDNITHVPGGG NKKIETHKLTFRENAKAKTDHGAEIVYK SPVVSGDTSPRHLSNVSSTGSIDMVDSPQ LATLADEVSASLAKQGL
16	AbA	Heavy Chain Amino Acid	Residues 1-449	EVQLVQSGAEVKKPGSSVKVSCKASGY TFTDYYMNWVRQAPGQGLEWIGVFYPH LGYTIYNQKFKGRATLTVDKSTSTAYME LSSLRSEDTAVYYCASPYYYGSSSLDYW GQGTLVTVSSASTKGPSVFPLAPSSKSTS GGTAALGCLVKDYFPEPVTVSWNSGALT SGVHTFPAVLQSSGLYSLSSVVTVPSSSL GTQTYICNVNHKPSNTKVDKKVEPKSC DKTHTCPPCPAPELLGGPSVFLFPPKPKD QLMISRTPEVTCVVVDVSHEDPEVKFNW YVDGVEVHNAKTKPREEQYNSTYRVVS

				VLTVLHQDWLNGKEYKCKVSNKALPAP IEKTISKAKGQPREPQVYTLPPSREEMTK NQVSLTCLVKGFYPSDIAVEWESNGQPE NNYKTTPPVLDSDGSFFLYSKLTVDKSR WQQGNVFSCSVLHEALHNHYTQKSLSL SPG
17	chAbA	Heavy Chain Amino Acid	Residues 1-450	EVQLVQSGAEVKKPGSSVKVSCKASGY TFTDYYMNWVRQAPGQGLEWIGVFYPH LGYTIYNQKFKGRATLTVDKSTSTAYME LSSLRSEDTAVYYCASPYYYGSSSLDYW GQGTLVTVSSAKTTAPSVYPLAPVCGDT TGSSVTLGCLVKGYFPEPVTLTWNSGSLS SGVHTFPAVLQSDLYTLSSSVTVTSSTWP SQSITCNVAHPASSTKVDKKIEPRGPTIKP CPPCKCPAPNLLGGPSVFIFPPKIKDVLMI SLSPIVTCVVVDVSEDDPDVQISWFVNN VEVHTAQTQTHREDYNSTLRVVSALPIQ HQDWMSGKEFKCKVNNKDLPAPIERTIS KPKGSVRAPQVYVLPPPEEEMTKKQVTL TCMVTDFMPEDIYVEWTNNGKTELNYK NTEPVLDSDGSYFMYSKLRVEKKNWVE RNSYSCSVVHEGLHNHHTTKSFSRTPGK
18	chAbA	Light Chain Amino Acid	Residues 1-219	DVQMTQSPSSVSASVGDRVTITCRSSQD LVESDADTYLHWYQQKPGKAPKLLIYK VSNRFSGVPSRFSGSGSGTDFTLTISSLQP EDFATYFCSQSTHVPFTFGQGTKVEIKRA DAAPTVSIFPPSSEQLTSGGASVVCFLNN FYPKDINVKWKIDGSERQNGVLNSWTD QDSKDSTYSMSSTLTLTKDEYERHNSYT CEATHKTSTSPIVKSFNRNEC

WHAT IS CLAIMED:

1. A full-length humanized IgG_1 anti-tau antibody, comprising a heavy chain variable region comprising a CDR-H1, a CDR-H2, and a CDR-H3, and a light chain variable region comprising a CDR-L1, a CDR-L2, and a CDR-L3, wherein:

vH CDR-H1 has the amino acid sequence set forth as SEQ ID NO: 3;

vH CDR-H2 has the amino acid sequence set forth as SEQ ID NO: 4;

vH CDR-H3 has the amino acid sequence set forth as SEQ ID NO: 5;

vL CDR-L1 has the amino acid sequence set forth as SEQ ID NO: 8;

vL CDR-L2 has the amino acid sequence set forth as SEQ ID NO: 9; and

vL CDR-L3 has the amino acid sequence set forth as SEQ ID NO: 10.

2. The full-length anti-tau antibody of claim 1, wherein the heavy chain variable region has the amino acid sequence set forth as SEQ ID NO: 2 and the light chain variable region has the amino acid sequence set forth as SEQ ID NO: 7.

3. A full-length humanized monoclonal IgG_1 anti-tau antibody comprising two heavy chains and two light chains, wherein said heavy chains each comprise the amino acid sequence set forth as SEQ ID NO: 1, and said light chains each comprise the amino acid sequence set forth as SEQ ID NO: 6.

4. A full-length humanized monoclonal IgG_1 anti-tau antibody comprising two heavy chains and two light chains, wherein said heavy chains each comprise the amino acid sequence set forth as SEQ ID NO: 16, and said light chains each comprise the amino acid sequence set forth as SEQ ID NO: 6.

5. A pharmaceutical composition comprising the anti-tau antibody of claims 1-4 and a pharmaceutically acceptable carrier.

6. A method of treating Alzheimer's disease, the method comprising administering a therapeutically effective amount of the anti-tau antibody of claims 1-4 to a patient in need thereof.

7. A method of treating Alzheimer's disease, the method comprising administering a therapeutically effective amount of the composition of claim 5 to a patient in need thereof.