

ANTIBODIES AND ANTIGEN-BINDING FRAGMENTS THAT SPECIFICALLY BIND TO
MICROTUBULE-ASSOCIATED PROTEIN TAU

Field of the Invention

5 The invention relates to medicine. The invention in particular relates to antibodies and antigen-binding fragments that specifically bind to microtubule-associated protein tau. The invention also relates to diagnostic, prophylactic and therapeutic methods using anti-tau antibodies.

Background of the Invention

10 Dementia is a syndrome that can be caused by a number of progressive disorders that affect memory, thinking, behavior and the ability to perform everyday activities. About 36 million people worldwide are suffering from dementia today. The number of people with dementia is projected to double by 2030, and more than triple to 115.4 million people by 2050. Alzheimer's disease is the most common type of dementia. Currently, one in nine people age 65 and older (11 percent) and nearly half of those over age 85 have Alzheimer's disease. According to Alzheimer's Disease
15 International, current global costs of caring for these patients exceeds \$600 billion annually. These costs are likely to rise even faster than the prevalence of disease, especially in the developing world, as more formal social care systems emerge, and rising incomes lead to higher opportunity costs (Winblad, B and Jonsson, L, World Alzheimer Report 2010).

20 The brains of AD patients have an abundance of two abnormal structures, amyloid plaques and neurofibrillary tangles. This is especially true in certain regions of the brain that are important in memory. There is also a substantial loss of neurons and synapses in the cerebral cortex and certain subcortical regions. Both neurofibrillary tangles and neuronal loss increase in parallel with the duration and severity of illness (Gomez-Isla, t. et al, Ann Neurol 1997; 41:17-24) and neurofibrillary load has been shown to correlate with cognitive decline. (Braak, H. and Braak, E, Neurobiol Aging.
25 1997 Jul-Aug;18(4):351-7.

 Neurofibrillary tangles are intraneuronal lesions that are composed of hyperphosphorylated and insoluble accumulations of the microtubule-associated protein, tau. These accumulations are a

histopathological feature of many neurodegenerative diseases, which are collectively known as tauopathies. Tauopathies include, e.g., Alzheimer's disease (AD), Pick's disease (PiD), progressive supranuclear palsy (PSP), corticobasal degeneration (CBD), and frontotemporal lobar degeneration (FTLD). In human tauopathies, pathology progresses from one brain region to another in disease-specific patterns (Braak, H. and Braak, E, *Neurobiol Aging*. 1997 Jul-Aug;18(4):351-7, Raj et.al. *Neuron* 2012; 73:1204-1215, Seeley et.al. *Neuron* 2009; 62: 42-52. and Zhou et.al., *Neuron* 2012; 73:1216-1227), the underlying mechanism of which is not yet clear.

Tau pathology is involved in and may be a cause of many tauopathies. In its normal form, tau is a highly soluble microtubule-associated protein (Jeganathan et al., *Biochemistry* 2008; 47:10526-10539.) that binds and promotes the assembly of microtubules (Drechsel et al., *Mol Biol Cell* 1992; 3:1141-1154.). However, in tauopathies, tau becomes hyperphosphorylated, causing detachment from microtubules, and ultimately accumulation as neurofibrillary tangles that are visualized within dystrophic neurites and cell bodies (Mandelkow and Mandelkow, *Cold Spring Harbor Perspect Med* 2, 2012: a006247). The amount of tau pathology correlates with progressive neuronal dysfunction, synaptic loss, and functional decline in humans and transgenic mouse models (Arriagada et al., *Neurology*. 1992 Mar;42(3 Pt 1):631-9, Bancher et al., *Neurosci Lett* 1993; 162:179-182., Polydoro et al., *J. Neuroscience* 2009; 29:10741-10749. and Small and Duff, *Neuron*. 2008 Nov 26;60(4):534-42). While there have been no tau mutations observed in Alzheimer's disease, mutations in the tau gene appear to cause some forms of frontotemporal dementia (Cairns et al, *Am J Pathol*, 2007; 171: 227-40), presenting with tau positive inclusions and signifying that tau dysfunction is sufficient to cause neurodegeneration. Moreover, pathological tau appears to be an integral part of A β -induced neurotoxicity in cell culture and transgenic animal models (Rapoport, M, *PNAS*, 2002; 99:9, 6364-6369., Roberson ED, et al, *Science*, 2007; 316:750-754, Nicholson AM, and Ferreira A, *J Neurosci* 2009; 29:4640-4651, Oakley H, *J Neurosci* 2006;26(40):10129-10140.).

Passive and active immunizations against tau have been analyzed in mice using several different mouse models, including different phospho-tau peptides for active immunizations and anti-tau antibodies for passive immunotherapy (Asuni AA, et al, *J Neurosci*. 2007;27(34):9115-9129., Sigurdsson EM. *Curr Alzheimer Res*. 2009;6(5):446-450., Boutajangout A, et al, *J Neurosci*. 2010;30(49):16559-16566., Rosenmann H, et al. *Arch Neurol*. 2006;63(10):1459-1467., Boimel M, et al, *Exp Neurol*. 2010;224(2): 472-485.). In the first report describing immunizations with a 30-

amino acid phosphorylated tau peptide, an effect on the ratios of soluble and insoluble tau, reduction of tangle formation in the immunized mice, and functional benefits observed in behavior testing for these mice were shown (Boutajangout A. et al, J. Neuroscience, 2010; 30:16559-16566). Passive immunization with well-characterized anti-tau antibodies which react with phosphorylated Ser396 and Ser404 of the hyperphosphorylated tau protein at an early pathologic conformational epitope on tau, confirmed the results seen in active immunization studies. Mice treated with these antibodies showed marked reductions in tau pathology, which was measured by biochemical methods and histology, as well as a significant delay in loss of motor-function decline which was assessed in behavioral testings. (Boutajangout A, et al, J Neurochem. 2011;118(4):658–667., Chai X, et al. J Biol Chem. 2011;286(39):34457–34467.)

Tau-based therapies have been analyzed only in mouse models to date. But in view of the severity of tauopathies in general, and to the cost to society of Alzheimer's disease specifically, there is an ongoing need for effective means to diagnose, monitor, prevent and treat tauopathies.

Summary of the Invention

The present invention provides antibodies comprising an antigen binding variable region that binds specifically to tau. The present invention in particular provides anti-tau antibodies, and antigen-binding fragments thereof, that detect tau in normal (i.e. healthy) human brain tissue, but do not detect tau deposits in human Alzheimers Disease (AD) brain tissue. The anti-tau antibodies, and antigen-binding fragments thereof, bind to recombinant tau and PHF-tau by Western assay and do not bind to PHF-tau by ELISA. The antibodies, and antigen-binding fragments, are capable of specifically binding to a non-phosphorylated tau peptide. The antibodies and antigen binding fragments are capable of binding to dephosphorylated AD brain tissue.

In certain embodiments, the antibodies and antigen-binding fragments are capable of binding to dephosphorylated AD brain tissue and dephosphorylated PHF tau. In still other embodiments, the antibodies of the invention are non-phospho-selective and do not bind to a peptide phosphorylated at Serine 316. In still other embodiments, the antibodies of the invention are non-phospho-selective and do not bind to a peptide phosphorylated at Serine 61 and /or Threonine 63.

In certain embodiments, the present invention provides chimeric antibodies comprising an antigen binding variable region from a naturally occurring human antibody that binds specifically to tau, and a recombinant constant region of a human IgG1, wherein the constant region of the chimeric antibody is different from the naturally occurring antibody.

5 In certain embodiments, the present invention provides anti-tau antibodies, and antigen-binding fragments thereof, that detect tau in normal human brain tissue, but do not detect tau deposits in human Alzheimers Disease (AD) and Progressive Supranuclear Palsy (PSP) brain tissue.

In certain embodiments, the chimeric anti-tau antibodies and antigen-binding fragments thereof, bind to recombinant tau or PHF-tau by Western assay. In other embodiments, the antibodies
10 and antigen-binding fragments are preferentially capable of specifically binding to a non-phosphorylated tau peptide.

Preferably, the antibodies are human.

The antibodies and antigen-binding fragments of the invention are useful as diagnostic, prophylactic and/or therapeutic agents, both alone and in combination with other diagnostics,
15 prophylactic and/or therapeutic agents.

In one aspect, the invention relates to an anti-tau antibody, comprising an antigen-binding site comprising a heavy chain CDR1 region of SEQ ID NO:201, a heavy chain CDR2 region of SEQ ID NO:202, and a heavy chain CDR3 region of SEQ ID NO:203, a light chain CDR1 region of SEQ ID NO:204, a light chain CDR2 region of SEQ ID NO:205 and a light chain CDR3 region of SEQ
20 ID NO:206, and to antigen-binding fragments thereof. Another embodiment of the invention relates to an anti-tau antibody, comprising a heavy chain CDR1 region of SEQ ID NO:207, a heavy chain CDR2 region of SEQ ID NO:208, and a heavy chain CDR3 region of SEQ ID NO:209, a light chain CDR1 region of SEQ ID NO:210, a light chain CDR2 region of SEQ ID NO:211 and a light chain CDR3 region of SEQ ID NO:212, and to antigen-binding fragments thereof. Another embodiment of
25 the invention relates to an anti-tau antibody, comprising an antigen-binding site comprising a heavy chain CDR1 region of SEQ ID NO:222, a heavy chain CDR2 region of SEQ ID NO:223, and a heavy chain CDR3 region of SEQ ID NO:224, a light chain CDR1 region of SEQ ID NO:225, a light chain CDR2 region of SEQ ID NO:173 and a light chain CDR3 region of SEQ ID NO:226, and to antigen-binding fragments thereof. Another embodiment of the invention relates to an anti-tau

antibody, comprising an antigen-binding site comprising a heavy chain CDR1 region of SEQ ID NO:238, a heavy chain CDR2 region of SEQ ID NO:239, and a heavy chain CDR3 region of SEQ ID NO:240, a light chain CDR1 region of SEQ ID NO:241, a light chain CDR2 region of SEQ ID NO:173 and a light chain CDR3 region of SEQ ID NO:242, and to antigen-binding fragments thereof. Another embodiment of the invention relates to an anti-tau antibody, comprising a heavy chain CDR1 region of SEQ ID NO:243, a heavy chain CDR2 region of SEQ ID NO:244, and a heavy chain CDR3 region of SEQ ID NO:245, a light chain CDR1 region of SEQ ID NO:246, a light chain CDR2 region of SEQ ID NO:173 and a light chain CDR3 region of SEQ ID NO:212, and to antigen-binding fragments thereof. Another embodiment of the invention relates to an anti-tau antibody, comprising an antigen-binding site comprising a heavy chain CDR1 region of SEQ ID NO:243, a heavy chain CDR2 region of SEQ ID NO:247, and a heavy chain CDR3 region of SEQ ID NO:248, a light chain CDR1 region of SEQ ID NO:249 a light chain CDR2 region of SEQ ID NO:173 and a light chain CDR3 region of SEQ ID NO:212, and to antigen-binding fragments thereof. Another embodiment of the invention relates to an anti-tau antibody, comprising an antigen-binding site comprising a heavy chain CDR1 region of SEQ ID NO:250, a heavy chain CDR2 region of SEQ ID NO:251, and a heavy chain CDR3 region of SEQ ID NO:252, a light chain CDR1 region of SEQ ID NO:254, a light chain CDR2 region of SEQ ID NO:254 and a light chain CDR3 region of SEQ ID NO:255, and to antigen-binding fragments thereof.

In another aspect the invention relates to an isolated anti-tau antibody comprising an antigen-binding site of a heavy chain variable region (VH) of SEQ ID NOS: 115 or 119 or 135 or 147 or 151 or 155 or 159, and an antigen-binding site of a light chain variable region (VL) of SEQ ID NOS: 116 or 120 or 136 or 148 or 152 or 156 or 160.

In another aspect the invention relates to an isolated chimeric anti-tau antibody comprising an antigen-binding site of a heavy chain variable region (VH) of SEQ ID NOS: 115 or 119 or 135 or 147 or 151 or 155 or 159, and an antigen-binding site of a light chain variable region (VL) of SEQ ID NOS: 116 or 120 or 136 or 148 or 152 or 156 or 160.. In another aspect of the invention, the antibody is non-naturally occurring.

In a further aspect, the invention relates to an anti-tau antibody comprising a heavy chain variable region comprising the amino acid sequence of SEQ ID NO: 115 and a light chain variable region comprising a amino acid sequence of SEQ ID NO: 116 and to antigen-binding fragments thereof. In a further aspect, the invention relates to an anti-tau antibody comprising a heavy chain

variable region comprising the amino acid sequence of SEQ ID NO: 119 and a light chain variable region comprising a amino acid sequence of SEQ ID NO: 120 and to antigen-binding fragments thereof. In a further aspect, the invention relates to an anti-tau antibody comprising a heavy chain variable region comprising the amino acid sequence of SEQ ID NO: 115 and a light chain variable
5 region comprising an amino acid sequence of SEQ ID NO: 116 and to antigen-binding fragments thereof. In a further aspect, the invention relates to an anti-tau antibody comprising a heavy chain variable region comprising the amino acid sequence of SEQ ID NO: 119 and a light chain variable region comprising an amino acid sequence of SEQ ID NO: 120 and to antigen-binding fragments thereof. In a further aspect, the invention relates to an anti-tau antibody comprising a heavy chain
10 variable region comprising the amino acid sequence of SEQ ID NO: 135 and a light chain variable region comprising an amino acid sequence of SEQ ID NO: 136 and to antigen-binding fragments thereof. In a further aspect, the invention relates to an anti-tau antibody comprising a heavy chain variable region comprising the amino acid sequence of SEQ ID NO: 147 and a light chain variable region comprising an amino acid sequence of SEQ ID NO: 148 and to antigen-binding fragments
15 thereof. In a further aspect, the invention relates to an anti-tau antibody comprising a heavy chain variable region comprising the amino acid sequence of SEQ ID NO: 151 and a light chain variable region comprising an amino acid sequence of SEQ ID NO: 152 and to antigen-binding fragments thereof. In a further aspect, the invention relates to an anti-tau antibody comprising a heavy chain variable region comprising the amino acid sequence of SEQ ID NO: 155 and a light chain variable
20 region comprising an amino acid sequence of SEQ ID NO: 156 and to antigen-binding fragments thereof. In a further aspect, the invention relates to an anti-tau antibody comprising a heavy chain variable region comprising the amino acid sequence of SEQ ID NO: 159 and a light chain variable region comprising an amino acid sequence of SEQ ID NO: 160 and to antigen-binding fragments thereof.

25 In one embodiment, the IgG1 heavy chain constant region is comprised of the amino acid sequence of SEQ ID NO: 83. In another embodiment, the IgG1 light chain constant region is comprised of the amino acid sequence of SEQ ID NO: 84.

The invention also provides nucleic acid molecules encoding the antibodies or antigen-binding fragments thereof. Another aspect of the invention is a vector comprising the nucleic acid

molecules of the invention. A further feature of the invention is a host cell comprising the vector of the invention.

The invention also provides a method of producing an anti-tau antibody comprising culturing the host cell of the invention and recovering the antibody produced by the host cell.

5 The invention further provides for functional variants of the antibodies and immunoconjugates comprising the antibody and/or antigen binding fragment thereof.

The invention further provides compositions and kits which comprise one or more antibodies of the invention and/or antigen binding fragments thereof. The invention additionally provides diagnostic, prophylactic and therapeutic methods that employ the anti-tau antibodies.

10 Prophylactic and therapeutic methods include administering to human subjects the anti-tau antibodies and/or antigen-binding fragments thereof for the prevention or treatment of a tauopathy and/or tau-mediated diseases or conditions, and/or amelioration of one or more symptoms of a tauopathy or tau-mediated disease. Combinations of a plurality of different anti-tau antibodies and/or antigen-binding fragments thereof and/or with other anti-tau antibodies can be used for
15 combination therapy. Compositions comprising the anti-tau antibodies and/or antigen-binding fragments thereof in combination with other prophylactic or therapeutic agents are also provided.

The antibodies of the invention are unique in that the variable regions are recovered from anti-tau specific memory B-cells from healthy individuals and detect tau in normal human brain, but do not detect tau deposits in human Alzheimer's brain. The anti-tau antibodies are also unique in
20 that they bind to denatured PHF tau in a Western assay, but do not bind to non-denatured PHF tau in an ELISA. The anti-tau antibodies bind to a non-modified tau peptide 299 – 369 (SEQ ID NO:331) or a tau peptide 42-103 (SEQ ID NO: 325). The chimeric antibodies further bind to a non-modified tau peptide tau 52-71 (SEQ ID NO:382) or tau 299-323 (SEQ ID NO:458) or tau 82-103 (SEQ ID NO:386). The antibodies of the invention are unique in that they bind to dephosphorylated AD brain
25 and do not bind to a tau peptide phosphorylated on Serine 316 or Serine 61 or Threonine 63 of tau.

Brief Description of the Drawings

Figures 1a-t show the reactivity of CBTAU-7.1, 8.1, 16.1, 18.1, 20.1, 22.1, 24.1, 27.1, 28.1, 41.1, 41.2, 42.1, 43.1, 44.1, 45.1, 46.1, 47.1, 47.2, and 49.1 against corresponding cognate and non-cognate peptide.
30

Figures 2a-j show the reactivity of CBTAU-7.1, 8.1, 16.1, 18.1, 20.1, 22.1, 24.1, 27.1, and 28.1 against recombinant tau (rTau), enriched immunopurified paired helical filaments (ePHF), and immunopurified paired helical filaments (iPHF) by ELISA. Anti-tau mAb, AT8, was used as positive control.

Figure 3 shows the immunoreactivity of CBTAU-7.1, 18.1, 22.1, 24.1, 27.1, and 28.1 against rtau, ePHF, and iPHF by Western blot analysis.

Figures 4a-g show the epitope mapping of CBTAU-27.1, 28.1, 43.1, 46.1, 47.1, 47.2, and 49.1 using overlapping peptides that correspond to regions 42-103 and 299-369 on human tau441.

Figures 5a-d show the immunohistochemical results for the CBTAU mAbs detailed in this application. Figures 5a-b shows the immunostaining of CBTAU-7.1, 8.1, 16.1, 18.1, 20.1, 22.1, 24.1, 27.1, and 28.1 on non-AD versus AD hippocampal and cortical tissue sections, respectively. Figure c shows the immunostaining of CBTAU-7.1, 8.1, 16.1, 18.1, 20.1, 22.1, and 24.1 on non-PSP and PSP cortical tissue sections. Figure 5d shows the immunostaining of CBTAU-43.1, 46.1, 47.2, and 49.1 against non-AD and AD cortical tissue sections.

Figures 6a shows immunoreactivity of CBTAU-28.1 and control mAbs against non-AD (54 y.o. male; no clinical symptoms) and AD (93 y.o. Hispanic female) hippocampal tissue sections.

Figure 6b shows reactivity of CBTAU-28.1 and control mAbs to AD (93 y.o. Hispanic female) hippocampal tissue sections with and without calf intestinal phosphatase treatment.

Figure 7 shows reactivity of CBTAU-28.1 and phospho-tau mAb, AT8, against iPHF (immunopurified paired helical filaments) and calf intestinal phosphatase treated iPHF samples.

Figures 8a- e show reactivity of CBTAU-27.1, 28.1, 43.1, 46.1, 47.1, 47.2, and 49.1 to the tau phosphopeptides detailed on Tables 30-34.

Detailed Description of the Invention

Definitions

Definitions of terms as used in the present invention are given below.

5 The term “included” or “including” as used herein is deemed to be followed by the words “without limitation”.

The term “tau” as used herein, is used interchangeably to specifically refer to the native monomer form of tau. The term "tau" is also used to generally identify other conformers of tau, for example, oligomers or aggregates of tau. The term "tau" is also used to refer collectively to all types and forms of tau. Due to alternative splicing 6 tau isoforms are present in the human brain. These
10 isoforms differ by the absence or presence of one or two 29 amino acid inserts encoded by exon 2 and 3 in the amino-terminal part, in combination with either three (R1, R3 and R4) or four (R1–R4) repeat-regions in the carboxy-terminal part. The microtubule-binding domain is encoded by exon 10. The adult tau isoforms include the longest 441-amino acids component (SEQ ID NO:1), or 4R/2N, the 410-amino acids component (SEQ ID NO:2), or 3R/2N, the 412-amino acids component
15 (SEQ ID NO:3), or 4R/1N, the 381-amino acids component (SEQ ID NO:4), or 3R/1N and the 383-amino acids component (SEQ ID NO:5) or 4R/0N. The shortest 352-amino acids isoform (SEQ ID NO:6), or 3R/0N, is found in the fetal brain, and thus is referred to as fetal tau isoform.

The "wild type" tau amino acid sequence is represented by the 441 amino acid isoform (SEQ ID NO:1) also referred to as “tau441”, “4R/2N”, "hTau40", "TauF", "Tau-4" or "full-length tau".

20 The term “recombinant tau” herein, refers to the longest isoform of human brain tau (SEQ ID NO:1) expressed in E. coli and purified to homogeneity or near homogeneity (Barghorn S., Meth Mol Biol 2004 299:35-51). Recombinant tau is soluble and is not phosphorylated.

The term “neurofibrillary tangle” (NFT) refers to the pathological structures first described by Alzheimer in the brain of dementia patient. NFT are composed of orderly arranged subunits
25 called paired helical filaments aggregates of hyperphosphorylated tau protein that are most commonly known as a primary marker of Alzheimer's Disease.

The term "paired helical filament-tau" or "PHF-tau" as used herein refers to well-known tau aggregates which make up the pathological structures called neurofibrillary tangles (NFT), first described by Alzheimer in the brain of dementia patient. Their presence is also found in numerous other diseases known as tauopathies.

5 "Enriched PHF-tau" or "ePHF tau", is prepared according to the protocol of Greenberg and Davies as detailed in the Examples. PHF tau is enriched from 27,200 x g supernatants containing 0.8M NaCl by taking advantage of their insolubility in zwitterionic detergents (Kosik, K. S., et al. (1986) PNAS USA 83, 4044-4048, Rubenstein, R., et al (1986) Brain Res. 372, 80-88) and mercaptoethanol. PHFs isolated with zwitterionic detergents appear to maintain antigenic sites that
10 may be lost during the isolation of SDS-insoluble NeuroFibular Tangles and are similar in structure and contain many antigenic properties to PHF in NFTs. "Immunopurified PHF-tau" or "iPHF tau" is affinity purified with an anti-tau monoclonal antibody. Such protocols have provided PHF-tau preparations that retain the classical paired helical filament structure by electron microscopy and are completely soluble in low concentrations of SDS (Jicha, G., 1997, 48(2):128-32). PHF-tau is also
15 formed from recombinant tau by induction of polymerization in-vitro with heparin (Mandelkow, et al Methods in Molecular Biology 299:35 – 51(2004). Alternatively, PHF-tau is isolated by various other methods from brains of patients having AD using protocols, such as described in Rostagna and Ghiso (Rostagna, A. and Ghiso, J., Curr Protoc Cell Biol. Sep 2009; CHAPTER: Unit-3.3333.). The isolated PHF-tau is characterized for its purity and hyperphosphorylation status with antibodies
20 known to react with PHF-tau. In a typical PHF-tau preparation, the hyperphosphorylated bands migrating at about 60, 64, 68 and 72kDa in western blot (Spillantini and Goedert Trends Neurosci 21:428-33, 1998) are detected by an AT8 antibody that specifically binds hyperphosphorylated PHF-tau but not dephosphorylated PHF-tau.

 The term "antibodies" as used herein is meant in a broad sense and includes immunoglobulin
25 or antibody molecules including polyclonal antibodies, monoclonal antibodies including murine, human, human-adapted, humanized and chimeric monoclonal antibodies, bispecific or multispecific antibodies and antibody fragments. In general, antibodies are proteins or peptide chains that exhibit binding specificity to a defined antigen. Antibody structures are well known. Immunoglobulins can be assigned to five major classes, namely IgA, IgD, IgE, IgG and IgM, depending on the heavy
30 chain constant domain amino acid sequence. IgA and IgG are further sub-classified as the isotypes I

gA1, IgA2, IgG 1, IgG2, IgG3 and IgG4. Antibody light chains of any vertebrate species can be assigned to one of two clearly distinct types, namely kappa (K) and lambda (λ), based on the amino acid sequences of their constant domains.

The term "antigen-binding fragments" means a portion of an intact antibody. Examples of antibody fragments include Fab, Fab', F(ab')₂ and Fv fragments, CDR, antigen-binding site, heavy or light chain variable region, diabodies, triabodies single chain antibody molecules(scFv) and multispecific antibodies formed from at least two intact antibodies or fragments thereof or (poly) peptides that contain at least a fragment of an immunoglobulin that is sufficient to confer antigen binding to the (poly) peptide, etc.. An antigen-binding fragment may comprise a peptide or polypeptide comprising an amino acid sequence of at least 2, 5, 10, 15, 20, 25, 30, 35, 40, 50, 60, 70, 80, 90, 100, 125, 150, 175, 200, or 250 contiguous amino acid residues of the amino acid sequence of the antibody. The antigen-binding fragments may be produced synthetically or by enzymatic or chemical cleavage of intact immunoglobulins or they may be genetically engineered by recombinant DNA techniques. The methods of production are well known in the art and are described, for example, in Antibodies: A Laboratory Manual, Edited by: E. Harlow and D, Lane (1988), Cold Spring Harbor Laboratory, Cold Spring Harbor, New York, which is incorporated herein by reference. An antibody or antigen-binding fragment thereof may have one or more binding sites. If there is more than one binding site, the binding sites may be identical to one another or they may be different.

An immunoglobulin light or heavy chain variable region consists of a "framework" region interrupted by "antigen-binding sites". The antigen-binding sites are defined using various terms as follows: (i) Complementarity Determining Regions (CDRs) are based on sequence variability (Wu and Kabat J Exp Med 132:211-50, 1970). Generally, the antigen binding site has three CDRs in each variable region (HCDR1, HCDR2 and HCDR3 in heavy chain variable region (VH) and LCDR1, LCDR2 and LCDR3 in light chain variable region (VL)) (Kabat et al., Sequences of Proteins of Immunological Interest, 5th Ed. Public Health Service, National Institutes of Health, Bethesda, Md., 1991). (ii) The term "hypervariable region", "HVR", or "HV" refers to the regions of an antibody variable domain which are hypervariable in structure as defined by Chothia and Lesk (Chothia and Lesk J Mol Biol 96:901-17, 1987). Generally, the antigen-binding site has three hypervariable regions in each VH (H1, H2, H3) and VL (L1, L2, L3). Chothia and Lesk refer to structurally

conserved HVs as "canonical structures". Numbering systems as well as annotation of CDRs and HVs have recently been revised by Abhinandan and Martin (Abhinandan and Martin Mol Immunol45:3832-9, 2008). (iii) Another definition of the regions that form the antigen-binding site has been proposed by Lefranc (Lefranc, et al. Dev Camp Immunol27:55-77, 2003) based on the
5 comparison of V domains from immunoglobulins and T-cell receptors. The International ImMunoGeneTics (IMGT) database (<http://www.imgt.org>) provides a standardized numbering and definition of these regions. The correspondence between CDRs, HVs and IMGT delineations is described in Lefranc et al. The antigen-binding site can also be delineated based on Specificity Determining Residue Usage (SDRU) (Almagro J Mol Recognit 17:132-43, 2004), where Specificity
10 Determining Residues (SDR), refers to amino acid residues of an immunoglobulin that are directly involved in antigen contact.

Kabat et al. also defined a numbering system for variable domain sequences that is applicable to any antibody. One of ordinary skill in the art can unambiguously assign this system of "Kabat numbering" to any variable domain sequence, without reliance on any experimental data
15 beyond the sequence itself. As used herein, "Kabat numbering" refers to the numbering system set forth by Kabat et al., U.S. Dept. of Health and Human Services, "Sequence of Proteins of Immunological Interest" (1983). Unless otherwise specified, references to the numbering of specific amino acid residue positions in an antibody or antigen-binding fragment, variant, or derivative thereof of the present invention are according to the Kabat numbering system, which however is
20 theoretical and may not equally apply every antibody of the present invention. For example, depending on the position of the first CDR the following CDRs might be shifted in either direction.

"Framework" or "framework sequence" are the remaining sequences within the variable region of an antibody other than those defined to be antigen-binding site sequences. Because the exact definition of an antigen-binding site can be determined by various delineations as described
25 above, the exact framework sequence depends on the definition of the antigen-binding site.

The term "monoclonal antibody" (mAb) as used herein means an antibody (or antibody fragment) obtained from a population of substantially homogeneous antibodies. Monoclonal antibodies are highly specific, typically being directed against a single antigenic determinant.

In one aspect, the antibody of the present invention is a chimeric human antibody. Thus, in accordance with the present invention the terms “human chimeric antibody”, or “human recombinant antibody” and the like are used to denote a binding molecule which antigen-binding features originated from a human cell, i.e. which antigen binding site is derived from nucleic acids produced from a human cell such as a B cell, or the partial cDNA of which has been cloned from mRNA of a human cell, for example a human memory B cell. A chimeric antibody is still “human” even if amino acid substitutions are made in the antibody, e.g., to improve biophysical or pharmacokinetic characteristics. Compared to artificially generated human-like antibodies such as single chain antibody fragments (scFvs) from a phage displayed antibody library or xenogeneic mice, the chimeric human antibody of the present invention is characterized by (i) the antigen-binding region being obtained using the human immune response rather than that of animal surrogates, i.e. the antigen binding region has been generated in response to natural tau in its relevant conformation in the human body, and/or (ii) having protected the individual or is at least significant for the presence of tau.

Antibodies originating from human immunoglobulin libraries or from animals transgenic for one or more human immunoglobulins and that do not express endogenous immunoglobulins, as described infra and, for example in, U.S. Pat. No. 5,939,598 by Kucherlapati et al., are denoted human-like antibodies in order distinguish them from human-derived antibodies of the present invention.

For example, the pairing of heavy and light chains of human-like antibodies such as synthetic and semi-synthetic antibodies typically isolated from phage display do not necessarily reflect the original pairing as it occurred in the original human B cell. Accordingly Fab and scFv fragments obtained from recombinant expression libraries as commonly used in the prior art can be considered as being artificial with all possible associated effects on immunogenicity and stability. In contrast, the present invention provides antigen-binding regions of affinity-matured anti-tau antibodies from selected human subjects, which in certain embodiments are recombinantly expressed as chimeras with a common IgG1 constant region.

The term “functional variant”, as used herein, refers to an antibody that comprises a nucleotide and/or amino acid sequence that is altered by one or more nucleotides and/or amino acids compared to the nucleotide and/or amino acid sequences of a reference antibody and that is capable

of competing for specific binding to the binding partner, i.e. tau, with the reference antibody. In other words, the modifications in the amino acid and/or nucleotide sequence of the reference antibody do not significantly affect or alter the binding characteristics of the antibody encoded by the nucleotide sequence or containing the amino acid sequence, i.e. the antibody is still able to specifically recognize and bind its target. The functional variant may have conservative sequence modifications including nucleotide and amino acid substitutions, additions and deletions. Examples of functional variants include de-risking a free Cysteine or amino acid with potential post-translational modification in the hypervariable region, as well as Fc engineering to increase/decrease the binding affinity of IgG antibodies to FcRn, increase/decrease serum half-life. A functional variant can also be generation of the antibody as a human chimeric IgG2, IgG3 or IgG4 isotype, or as a chimeric isotype of a different species. A functional variant can also be a mutation or mutations of the constant regions for enhancement of bispecific antibody formation. These modifications can be introduced by standard techniques known in the art, such as PCR, site-directed mutagenesis and random PCR-mediated mutagenesis, and may comprise natural as well as non-natural nucleotides and amino acids.

The term “specifically binding”, or “specifically recognize”, as used herein, in reference to the interaction of an antibody and its binding partner, e.g. an antigen, means that the interaction is dependent upon the presence of a particular amino acid sequence or structure, e.g. an antigenic determinant or epitope, on the binding partner. In other words, the antibody preferentially binds or recognizes the binding partner even when the binding partner is present in a mixture of other molecules or organisms. The binding may be mediated by covalent or noncovalent interactions or a combination of both. In yet other words, the term “specifically binding” or “specifically recognizes” means that the antibody is specifically immunoreactive with an antigenic determinant or epitope and is not immunoreactive with other antigenic determinants or epitopes. An antibody that (immuno)specifically binds to an antigen may bind to other peptides or polypeptides with lower affinity as determined by, e.g., radioimmunoassays (RIA), enzyme-linked immunosorbent assays (ELISA), BIACORE, or other assays known in the art. Antibodies or fragments thereof that specifically bind to an antigen may be cross-reactive with related antigens, carrying the same epitope. Preferably, antibodies or fragments thereof that specifically bind to an antigen do not cross-react with other antigens.

The term "epitope" as used herein means that part of the antigen that is contacted by the CDR loops of antibody. A "structural epitope" comprises about 15 – 22 contact residues on the the antigen surface and involves many amino acid residues that make contact with a large group of residues on CDRs collectively referred to as the paratope of antibody. Direct contact between epitope and paratope residues is established through electrostatic forces such as hydrogen bonds, salt bridges, van der Waals forces of hydrophobic surfaces and shape complementarity. The interface has also bound water molecules or other co-factors that contribute to the specificity and affinity of antigen-antibody interactions. The binding energy of an antigen-antibody complex is primarily mediated by a small subset of contact residues in the epitope-paratope interface. These "energetic residues" are often located in the center of the epitope-paratope interface and make up the functional epitope. Contact residues in the periphery of the interface make generally minor contributions to the binding energy; their replacements have frequently little effect on the binding with antigen. Thus, the binding or functional activity of an epitope involves a small subset of energetic residues centrally located in the structural epitope and contacted by the specificity-determining CDRs. The assignment of a functional epitope on an antigenic protein can be made using several methods including Alanine scan mutagenesis or by solving the crystal structure of the antigen with the antibody. An epitope can be linear in nature or can be a discontinuous epitope, e.g., a conformational epitope, which is formed by a spatial relationship between non-contiguous amino acids of an antigen rather than a linear series of amino acids. A conformational epitope includes epitopes resulting from folding of an antigen, where amino acids from differing portions of the linear sequence of the antigen come in close proximity in 3-dimensional space. For discontinuous epitopes, it may be possible to obtain binding of one or more linear peptides with decreased affinity to a so-called partial epitope, e. g. dispersed at different regions of the protein sequence (Cragg, M. S. (2011) *Blood* 118 (2): 219–20.).

As used herein, the term "affinity" refers to a measure of the strength of the binding of an individual epitope or partial epitope with the CDRs of a binding molecule, e.g., an immunoglobulin molecule; see, e.g., Harlow et al., *Antibodies: A Laboratory Manual*, Cold Spring Harbor Laboratory Press, 2nd ed. (1988) at pages 27-28. As used herein, the term "avidity" refers to the overall stability of the complex between a population of immunoglobulins and an antigen, that is, the functional combining strength of an immunoglobulin mixture with the antigen; see, e.g., Harlow at pages 29-34. Avidity is related to both the affinity of individual immunoglobulin molecules in the

population with specific epitopes, and also the valences of the immunoglobulins and the antigen. For example, the interaction between a bivalent monoclonal antibody and an antigen with a highly repeating epitope structure, such as a polymer, would be one of high avidity. The affinity or avidity of an antibody for an antigen can be determined experimentally using any suitable method; see, for example, Berzofsky et al., "Antibody-Antigen Interactions" In Fundamental Immunology, Paul, W. E., Ed., Raven Press New York, N.Y. (1984), Kubly, Janis Immunology, W.H. Freeman and Company New York, N Y (1992), and methods described herein. General techniques for measuring the affinity of an antibody for an antigen include ELISA, RIA, and surface plasmon resonance. The measured affinity of a particular antibody-antigen interaction can vary if measured under different conditions, e.g., salt concentration, pH. Thus, measurements of affinity and other antigen-binding parameters, e.g., KD, IC50, are preferably made with standardized solutions of antibody and antigen, and a standardized buffer.

Antibodies or antigen-binding fragments or variants thereof of the invention may also be described or specified in terms of their ability to specifically detect the presence of antigen. The term "detect" or "detecting" is used in the broadest sense to include quantitative, semi-quantitative or qualitative measurements of a target molecule. In one aspect, antibodies described herein may only determine the presence or absence of tau polypeptide in a biological sample, e.g. by immunohistochemistry, and, thus, the tau polypeptide is detectable or, alternatively, undetectable in the sample as determined by the method.

The term "phospho-specific antibody or "phospho-dependent antibody" herein used means a specific antibody in which at least part or the entire epitope relies on a phosphorylated amino acid residue. A phospho-specific or phospho-dependent antibody does not detect un-phosphorylated antigen. The term "phospho-selective antibody" means a specific antibody that preferentially binds to the phosphorylated residue and has higher affinity to the phosphorylated versus the non-phosphorylated antigen. The term "non-phospho-selective antibody" means a specific antibody that preferentially binds to the non-phosphorylated residue and has higher affinity to the non-phosphorylated versus the phosphorylated antigen. In certain embodiments, the anti-tau antibodies of the invention, or antigen-binding fragments thereof, are non-phospho-specific.

The term "polynucleotide" is intended to encompass a singular nucleic acid as well as plural nucleic acids, and refers to an isolated nucleic acid molecule or construct, e.g., messenger RNA

(mRNA) or plasmid DNA (pDNA). A polynucleotide may comprise a conventional phosphodiester bond or a non-conventional bond (e.g., an amide bond, such as found in peptide nucleic acids (PNA)). The term “nucleic acid molecule” refers to any one or more nucleic acid segments, e.g., DNA or RNA fragments, present in a polynucleotide. By “isolated” nucleic acid or polynucleotide is intended a nucleic acid molecule, DNA or RNA, which has been removed from its native environment. For example, a recombinant polynucleotide encoding an antibody contained in a vector is considered isolated for the purposes of the present invention.

In certain embodiments, the polynucleotide or nucleic acid is DNA. In the case of DNA, a polynucleotide comprising a nucleic acid which encodes a polypeptide normally may include a promoter and/or other transcription or translation control elements operably associated with one or more coding regions. An operable association is when a coding region for a gene product, e.g., a polypeptide, is associated with one or more regulatory sequences in such a way as to place expression of the gene product under the influence or control of the regulatory sequence(s). Thus, a promoter region would be operably associated with a nucleic acid encoding a polypeptide if the promoter was capable of effecting transcription of that nucleic acid. The promoter may be a cell-specific promoter that directs substantial transcription of the DNA only in predetermined cells. Other transcription control elements, besides a promoter, for example enhancers, operators, repressors, and transcription termination signals, can be operably associated with the polynucleotide to direct cell-specific transcription. Suitable promoters and other transcription control regions are disclosed herein.

As used herein, the terms “treat” or “treatment” refer to both therapeutic treatment and prophylactic or preventative measures, wherein the object is to prevent or slow down (lessen) an undesired physiological change or disorder, such as the development of Parkinsonism or Alzheimer’s Disease. Beneficial or desired clinical results include, but are not limited to, alleviation of symptoms, diminishment of extent of disease, stabilized (i.e., not worsening) state of disease, delay or slowing of disease progression, amelioration or palliation of the disease state, and remission (whether partial or total), whether detectable or undetectable. “Treatment” can also mean prolonging survival as compared to expected survival if not receiving treatment. Those in need of treatment include those already with the condition or disorder as well as those prone to have the condition or disorder or those in which the manifestation of the condition or disorder is to be prevented. A

“medicament” as used herein, is an agent used in the treatment of an undesirable physiological change or disorder.

By “subject” or “individual” or “animal” or “patient” or “mammal,” is meant any subject, particularly a mammalian subject, e.g., a human patient, for whom diagnosis, prognosis, prevention,
5 or therapy is desired.

Description

Tau is an abundant central and peripheral nervous system protein having multiple well known isoforms. In the human CNS, six major tau isoforms ranging in size from 352 to 441 exist due to alternative splicing (Hanger, et al. Trends Mol Med 15:112-9, 2009). These isoforms differ
10 from each other by the regulated inclusion of 0-2 N-terminal inserts, and 3 or 4 tandemly arranged microtubule-binding repeats, and are referred to as ON3R (SEQ ID NO: 6), 1N3R (SEQ ID NO:4), 2N3R (SEQ ID NO:2), ON4R (SEQ ID NO:5), 1N4R (SEQ ID NO:3) and 2N4R (SEQ ID NO:1). The term "recombinant tau" as used herein refers to the tau isoform of SEQ ID NO:1 that is devoid of phosphorylation and other posttranslational modifications. The tau protein can be recombinantly
15 expressed in high quantities, for example, in E. coli, baculovirus, mammalian or cell-free systems. ‘Recombinant tau’ may be recombinantly expressed and purified using standard methods. (Barghorn, et al 2004, Meth Mol Biol 35-51)

Tau binds microtubules and regulates transport of cargo through cells, a process that can be modulated by tau phosphorylation which occurs at many of the 79 potential serine (Ser) and
20 threonine (Thr) phosphorylation sites. Tau is highly phosphorylated during brain development. The degree of phosphorylation declines in adulthood. Some of the phosphorylation sites are located within the microtubule binding domains of tau, and it has been shown that an increase of tau phosphorylation negatively regulates the binding of microtubules. For example, Ser262 and Ser396, which lie within or adjacent to microtubule binding motifs, are hyperphosphorylated in the tau
25 proteins of the abnormal paired helical filaments (PHFs), a major component of the neurofibrillary tangles (NFTs) in the brain of AD patients. PHFs are filamentous aggregates of tau proteins which are abnormally hyperphosphorylated and can be stained with specific anti-tau antibodies and detected by light microscopy. The same holds true for so called straight tau filaments. PHFs form twisted ribbons consisting of two filaments twisted around one another with a periodicity of about

80 nm. These pathological features are commonly referred to as “tau-pathology”, “tauopathology” or “tau-related pathology”. For a more detailed description of neuropathological features of tauopathies refer to Lee et al., *Annu. Rev. Neurosci.* 24 (2001), 1121-1159 and Götz, *Brain. Res. Rev.* 35 (2001), 266-286, the disclosure content of which is incorporated herein by reference.

- 5 Physiological tau protein stabilizes microtubules in neurons. Pathological phosphorylation leads to abnormal tau localization and aggregation, which causes destabilization of microtubules and impaired cellular transport. Aggregated tau is neurotoxic in vitro (Khlistunova et al., *J. Biol. Chem.* 281 (2006), 1205-1214). The exact neurotoxic species remains unclear, however, as do the mechanism(s) by which they lead to neuronal death. Aggregates of tau can be observed as the main
- 10 component of neurofibrillary tangles (NFT) in many tauopathies, such as Alzheimer's disease (AD), Frontotemporal dementias, supranuclear palsy, Pick's disease, Argyrophilic grain disease (AGD), corticobasal degeneration, FTDP-17, Parkinson's disease, Dementia pugilistica (Reviewed in Gendron and Petrucelli, *Mol. Neurodegener.* 4:13 (2009)). Besides these observations, evidence emerges that tau-mediated neuronal death can occur even in the absence of tangle formation.
- 15 Soluble phospho-tau species are present in CSF (Aluise et al., *Biochim. Biophys. Acta.* 1782 (2008), 549-558). Tau aggregates can transmit a misfolded state from the outside to the inside of a cell and transfer between co-cultured cells (Frost et al., *J. Biol. Chem.* 284 (2009), 12845-12852).

In addition to the involvement in neurodegenerative tauopathies, observed alterations in tau phosphorylation during and after ischemia/reperfusion and after concussive head injury suggest tau

20 plays a crucial role in neuronal damage and clinical pathophysiology of neurovascular disorders such as ischemic stroke (Zheng et al., *J. Cell. Biochem.* 109 (2010), 26-29), as well as changes in tau found in chronic traumatic encephalopathy, a tauopathy in concussed athletes and military veterans with Traumatic Brain Injury (TBI)

The anti-tau antibodies disclosed herein specifically bind tau and epitopes thereof and to

25 various conformations of tau and epitopes thereof. For example, disclosed herein are antibodies that specifically bind tau found in normal adult human brain. In one example, a tau antibody disclosed herein binds to tau or an epitope thereof and shows no binding above about 3 times background for other proteins. An antibody that “specifically binds” or “selectively binds” a tau conformer refers to an antibody that does not bind all conformations of tau, i.e., does not bind at least one other tau

30 conformer such as recombinant tau.

The variable domains of the chimeric anti-tau monoclonal antibodies of the present invention have origin from a pool of healthy human subjects exhibiting a tau-specific immune response. The tau antibodies of the present invention may also be called “human-derived antibodies” in order to emphasize that those antibody antigen binding regions were indeed expressed by the subjects and have not been isolated from, for example a human immunoglobulin expressing phage library, which hitherto represented one common method for trying to provide human-like antibodies. For example, the antibodies of the present invention differ from mAb AT8, MC1, and AT100, in that they are human-derived antibodies.

The present invention provides monoclonal antibodies, wherein the antibodies a) bind tau in normal human brain tissue and b) do not binds tau in human AD brain tissue. In certain embodiments, the antibodies : a) formsan immunological complex with tau in normal (i.e. healthy) human brain tissue, and b) do not form an immunological complex with tau in human AD brain tissue.

Anti-tau antibodies of the current invention can for example be characterized by their binding properties to recombinant tau in an ELISA. Recombinant tau purified from E. coli is highly soluble owing to its hydrophilic character. It lacks phosphorylation of Ser, Thr and Tyr residues characteristic of tau found in tauopathies. In one example, the human anti-tau antibodies disclosed herein specifically bind recombinant tau in an ELISA.

In an embodiment, the anti-tau antibody of the invention has been shown to specifically bind to a non-phosphorylated tau peptide of SEQ ID NO:325 or SEQ ID NO: 331 or SEQ ID NO: 382 or SEQ ID NO: 458 or SEQ ID NO: 386. In a further embodiment, the anti-tau antibody of the invention has been shown to specifically bind to phosphorylated peptides when the serines at positions 316, 61 or the threonine at position 63 are not phosphorylated.

Thus, in certain embodiments, the anti-tau antibodies disclosed herein specifically bind tau peptide in a peptide ELISA. In one embodiment, an anti-tau antibody binds to a tau peptide, e. g. HVPGGGSVQIVYKPVDSLKVT SKCGSLGNIHHKPGGGQVEVKSEKLDFKDRVQSKIGSLDN ITHVPGGGNK (SEQ ID NO: 331), corresponding to amino acids 299-369 of tau441. In another embodiment, an anti-tau antibody binds to a tau peptide, e. g. GLKESPLQTPTEGSEEPGSETSDAKSTPTAEDVTAPLVDEGAPGKQAAQPHTEIPEGTTA

(SEQ ID NO: 325), corresponding to amino acids 42-103 of tau441. In another example, an anti-tau antibody binds to a tau peptide, e. g. TEDGSEEPGSETSDAKSTPT (SEQ ID NO: 382), corresponding to amino acids 52-71 of tau441. In another embodiment, an anti-tau antibody binds to a tau peptide, e. g. HVPGGGSVQIVYKPVDLSKVTSCG (SEQ ID NO: 458), corresponding to amino acids 299-323 of tau441. In another example, an anti-tau antibody binds to a tau peptide, e. g. EGAPGKQAAAQPHTEIPEGTTA (SEQ ID NO: 386), corresponding to amino acids 82-103 of tau441. The anti-tau antibody of the invention is unlike previously disclosed human anti-tau monoclonal antibodies (US20130295021) which have been reported to bind to different tau peptides. Monoclonal antibodies NI-105.4E4, NI-105.4A3 and NI-105.4E4 bind to peptides 329-351+ 387-397, 337 – 343, and 35 – 49 of tau441, respectively.

In certain embodiments, the antibodies of the invention are chimeric.

In certain embodiments, the antibody is a chimeric antibody comprising an antigen binding variable region from a human antibody which binds specifically to tau, and a recombinant constant region of a human IgG1, wherein the chimeric antibody is different from the human antibody.

In certain embodiments, the antibody is a chimeric antibody comprising an antigen binding variable region from a human antibody which binds specifically to tau, and a recombinant constant region of a human IgG1, wherein the constant region of the chimeric antibody differs from the constant region of the human antibody.

In certain embodiments, the antibody is a chimeric antibody comprising a naturally occurring human antigen binding variable region which binds specifically to tau, and a recombinant constant region of a human IgG1 antibody.

In certain embodiments, the antibody is a chimeric antibody, wherein the antibody comprises naturally occurring human light and heavy chain variable regions from a human antibody, and recombinant human IgG 1 heavy and light chain constant regions.

In certain embodiments, the antibody is a chimeric antibody, wherein the chimeric antibody comprises heavy and light chain variable regions from a naturally occurring human antibody, and recombinant human IgG1 heavy and light chain constant regions.

In certain embodiments, the antibody is a chimeric antibody comprising heavy and light chain variable regions from a human antibody, and recombinant human IgG 1 heavy and light chain constant regions.

In certain embodiments, the antibody is a non-naturally occurring variant of a human monoclonal antibody.

In certain embodiments, the antibodies bind to phosphatase-treated tau deposits in human AD brain. Thus, in certain embodiments, the antibodies recognize tau in AD brain following phosphatase treatment. In certain embodiments, the antibodies form an immunological complex with tau deposits in phosphatase-treated human AD tissue.

Anti-tau antibodies of the invention can for example be characterized by their binding properties to PHF-tau in an ELISA. Anti-PHF-tau antibody clone AT8 binds to PHF-tau and has been used extensively to detect PHF -tau in neurofibrillary tangles in samples from Alzheimer's patients. AT8 is a phospho-specific monoclonal antibody and binds to phosphorylated Ser202 and Thr 205 of PHF-tau and is well-published for use in ELISA, immunohistochemistry, immunoblot, Western blot, and related applications. Clone AT8 recognizes Alzheimer Disease tau, as well as PHF-tau by ELISA and does not bind nonphosphorylated tau from healthy individuals or recombinant tau. In one embodiment, the anti-tau monoclonal antibody of the invention does not bind to PHF tau by ELISA. In another embodiment, the anti-tau monoclonal antibody of the invention binds to dephosphorylated PHF tau by ELISA.

Anti-tau antibodies of the invention can be characterized by their binding properties to PHF-tau and recombinant tau by Western blot. In neurodegenerative disorders, several mechanisms (phosphorylation, ubiquitination, acetylation, oxidation, glycation) are involved in the aggregation of tau proteins into PHF (Martin, L, et al, Neurochem Int, 58(4):458-71, 2011). These pathological tau proteins are visualized by western blotting as three major bands between 55 and 69 kDa, and a minor band at 74 kDa. Tau 55 results from the phosphorylation of the shortest isoform (SEQ ID NO:6), tau 64 from the phosphorylation of tau variants with one cassette exon (SEQ ID NO:4 and/or SEQ ID NO:5), tau 69 from the phosphorylation of tau variants with two cassette exons (SEQ ID NO:2 and/or SEQ ID NO:3). Phosphorylation of the longest tau isoform (SEQ ID NO:1) induces the formation of the additional hyperphosphorylated tau74 variant. In certain embodiments, the anti-tau antibodies of the invention bind to PHF tau and recombinant tau by Western analysis, and do not bind to PHF tau by ELISA.

In certain embodiments, the antibody a) binds denatured PHF tau and b) does not bind non-denatured PHF tau.

In certain embodiments, the antibody binds phosphatase-treated, non-denatured PHF tau. In certain embodiments, the antibodies bind with higher affinity to phosphatase-treated PHF tau than PHF tau isolated from human donor tissue.

5 In certain embodiments, the antibody a) binds PHF tau by Western blot and d) does not bind PHF-tau by ELISA. In certain embodiments, the antibodies bind PHF tau isolated from human AD tissue by Western Blot and b) do not bind PHF tau by ELISA.

In certain embodiments, the antibody binds phosphatase-treated PHF tau by ELISA. IN certain embodiments, the antibodies bind with higher affinity to phosphatase-treated PHF tau isolated from human AD tissue by ELISA.

10 Anti-tau antibodies of the invention can be utilized in and characterized by immunohistochemistry (IHC) of tissue sections from normal or AD brain. Phospho-tau antibodies in particular, highlight neurofibrillary pathology with a high degree of sensitivity and specificity, whereas, no detection of tau in normal healthy brain is observed. Clinicopathological studies have demonstrated that phospho-tau deposits or accumulations correspond more closely to clinical signs
15 compared to amyloid- β accumulations, and progress in a stepwise fashion from transentorhinal, to limbic, to isocortical areas, forming the basis for AD staging [R.J. Castellani, et al, *Acta Neuropathol* (Berl) 111, 503(2006); H. Braak and E. Braak, *Acta Neuropathol* (Berl) 82, 239 (1991) Tau monoclonal antibodies that are commonly used in immunohistochemistry include AT8 (p202/p205 tau), AT180 (p231 tau), AT270 (p181 tau), AT100(pT212 and S214), and MC-1, (Mercken M, et al, 1992 *Acta Neuropatho* 84:265-272, Zheng-Fischhofer 1998 *Eur J Biochem* 252:542-552, Goedert M, et. Al. 1994 *Biochem J* 301:871-877). In one embodiment, the anti-tau monoclonal antibodies of the invention detect tau in normal human brain tissue and do not detect tau deposits in human AD brain tissue. In another example, the anti-tau monoclonal antibodies of the invention detect tau deposits in dephosphorylated or phosphatase-treated human AD brain tissue.

25 Anti-tau antibodies can also be utilized in and characterized by immunohistochemistry of additional tauopathies, including Progressive Supranuclear Palsy, Pick's Disease and others. The pathological filamentous tau inclusions in PSP are composed of aberrantly phosphorylated tau proteins, but there is a preferential accumulation of abnormal 4R tau isoforms. . A panel of anti-tau monoclonal antibodies, including Alz50, Tau-2, T46, PHF-1, PHF-6, 12E8, PHF-1, RD4 and AT8,
30 has been used to characterize PSP deposits(*J Neuropathol Exp Neurol*. 1998 (6):588-601.). All of the monoclonal antibodies stained intraneuronal and glial inclusions, however, 12E8 and PHF-6

stained with less intensity. These antibodies detect different epitopes of tau, e.g., phospho-specific, isoform specific, and also detect tau deposits in AD brain. RD3, an anti-tau monoclonal antibody that specifically detects the 3-repeat tau isoform, shows limited IHC detection of PSP, yet intensely stains tau deposits in human AD brain tissue. The limited detection of PSP by this antibody is due to reduced levels of the 3-repeat tau isoform in PSP (De Silva, R. et al, Neuropath and Appl Neurobio (2003) 29(3)288-302).

In certain embodiments, the antibody comprises a heavy chain comprising: a) heavy chain CDR1 region of SEQ ID NO:201, a heavy chain CDR2 region of SEQ ID NO:202, and a heavy chain CDR3 region of SEQ ID NO:203, or b) a heavy chain CDR1 region of SEQ ID NO:207, a heavy chain CDR2 region of SEQ ID NO:208, and a heavy chain CDR3 region of SEQ ID NO:209, c) a heavy chain CDR1 region of SEQ ID NO:222, a heavy chain CDR2 region of SEQ ID NO:223, and a heavy chain CDR3 region of SEQ ID NO:224, d) a heavy chain CDR1 region of SEQ ID NO:238, a heavy chain CDR2 region of SEQ ID NO:239, and a heavy chain CDR3 region of SEQ ID NO:240 e) a heavy chain CDR1 region of SEQ ID NO:243, a heavy chain CDR2 region of SEQ ID NO:244, and a heavy chain CDR3 region of SEQ ID NO:245, f) a heavy chain CDR1 region of SEQ ID NO:243, a heavy chain CDR2 region of SEQ ID NO:247, and a heavy chain CDR3 region of SEQ ID NO:248, and g) a heavy chain CDR1 region of SEQ ID NO:250, a heavy chain CDR2 region of SEQ ID NO:251, and a heavy chain CDR3 region of SEQ ID NO:252, a light chain CDR1 region of SEQ ID NO:254, a light chain CDR2 region of SEQ ID NO:254 and a light chain CDR3 region of SEQ ID NO:255.

In certain embodiments, the antibody comprises a light chain comprising: a) a light chain CDR1 region of SEQ ID NO:204, a light chain CDR2 region of SEQ ID NO:205 and a light chain CDR3 region of SEQ ID NO:206, b), a light chain CDR1 region of SEQ ID NO:210, a light chain CDR2 region of SEQ ID NO:211 and a light chain CDR3 region of SEQ ID NO:212, c) a light chain CDR1 region of SEQ ID NO:225, a light chain CDR2 region of SEQ ID NO:173 and a light chain CDR3 region of SEQ ID NO:226, d) a light chain CDR1 region of SEQ ID NO:241, a light chain CDR2 region of SEQ ID NO:173 and a light chain CDR3 region of SEQ ID NO:242, e) a light chain CDR1 region of SEQ ID NO:246, a light chain CDR2 region of SEQ ID NO:173 and a light chain CDR3 region of SEQ ID NO:212, f) a light chain CDR1 region of SEQ ID NO:249 a light chain CDR2 region of SEQ ID NO:173 and a light chain CDR3 region of SEQ ID NO:212, and g) a light

chain CDR1 region of SEQ ID NO:254, a light chain CDR2 region of SEQ ID NO:254 and a light chain CDR3 region of SEQ ID NO:255.

In certain embodiments, the antibody is selected from the group consisting of: a) an antibody comprising a heavy chain CDR1 region of SEQ ID NO:201, a heavy chain CDR2 region of SEQ ID NO:202, and a heavy chain CDR3 region of SEQ ID NO:203, a light chain CDR1 region of SEQ ID NO:204, a light chain CDR2 region of SEQ ID NO:205 and a light chain CDR3 region of SEQ ID NO:206, b) an antibody comprising a heavy chain CDR1 region of SEQ ID NO:207, a heavy chain CDR2 region of SEQ ID NO:208, and a heavy chain CDR3 region of SEQ ID NO:209, a light chain CDR1 region of SEQ ID NO:210, a light chain CDR2 region of SEQ ID NO:211 and a light chain CDR3 region of SEQ ID NO:212, c) an antibody comprising a heavy chain CDR1 region of SEQ ID NO:222, a heavy chain CDR2 region of SEQ ID NO:223, and a heavy chain CDR3 region of SEQ ID NO:224, a light chain CDR1 region of SEQ ID NO:225, a light chain CDR2 region of SEQ ID NO:173 and a light chain CDR3 region of SEQ ID NO:226, d) an antibody comprising a heavy chain CDR1 region of SEQ ID NO:238, a heavy chain CDR2 region of SEQ ID NO:239, and a heavy chain CDR3 region of SEQ ID NO:240, a light chain CDR1 region of SEQ ID NO:241, a light chain CDR2 region of SEQ ID NO:173 and a light chain CDR3 region of SEQ ID NO:242, e) an antibody comprising a heavy chain CDR1 region of SEQ ID NO:243, a heavy chain CDR2 region of SEQ ID NO:244, and a heavy chain CDR3 region of SEQ ID NO:245, a light chain CDR1 region of SEQ ID NO:246, a light chain CDR2 region of SEQ ID NO:173 and a light chain CDR3 region of SEQ ID NO:212, f) an antibody comprising a heavy chain CDR1 region of SEQ ID NO:243, a heavy chain CDR2 region of SEQ ID NO:247, and a heavy chain CDR3 region of SEQ ID NO:248, a light chain CDR1 region of SEQ ID NO:249 a light chain CDR2 region of SEQ ID NO:173 and a light chain CDR3 region of SEQ ID NO:212, and g) an antibody comprising a heavy chain CDR1 region of SEQ ID NO:250, a heavy chain CDR2 region of SEQ ID NO:251, and a heavy chain CDR3 region of SEQ ID NO:252, a light chain CDR1 region of SEQ ID NO:254, a light chain CDR2 region of SEQ ID NO:254 and a light chain CDR3 region of SEQ ID NO:255.

In certain embodiments, the antibody comprises a heavy chain variable region comprising the amino acid sequence of SEQ ID NO: 115, a heavy chain variable region comprising the amino acid sequence of SEQ ID NO: 119, a heavy chain variable region comprising the amino acid sequence of SEQ ID NO: 135, a heavy chain variable region comprising the amino acid sequence of SEQ ID NO:

147, a heavy chain variable region comprising the amino acid sequence of SEQ ID NO: 151, a heavy chain variable region comprising the amino acid sequence of SEQ ID NO: 155, a heavy chain variable region comprising the amino acid sequence of SEQ ID NO: 159. In certain embodiments, the antibody comprises a light chain variable region comprising the amino acid sequence of SEQ ID NO: 116, a
5 light chain variable region comprising the amino acid sequence of SEQ ID NO: 120, comprises a light chain variable region comprising the amino acid sequence of SEQ ID NO: 136, a light chain variable region comprising the amino acid sequence of SEQ ID NO: 148, comprises a light chain variable region comprising the amino acid sequence of SEQ ID NO: 152, a light chain variable region comprising the amino acid sequence of SEQ ID NO: 156, comprises a light chain variable region
10 comprising the amino acid sequence of SEQ ID NO: 169.

In certain embodiments, the antibodies bind to a peptide selected from the group consisting of SEQ ID NO: 325 and SEQ ID NO: 331.

In certain embodiments, the antibodies bind to a peptide selected from the group consisting of 382, 458 and 386.

15 In certain embodiments, the anti-tau antibody of the invention has been shown to specifically bind to an epitope comprising D314, L315, and K317 or L315, K317 and P312 or P59, S61, E62, T63, D65, and K67 of tau 441.

In certain embodiments, antigen-binding fragments of the above described antibodies are provided. The antigen-binding fragments preferably bind to the same epitope. The anti-tau
20 monoclonal antibodies and antigen-binding fragments of the present invention bind to different epitopes as compared to the epitopes of known human anti-tau antibodies, such as e.g. NI-105.4E4, and NI-105.4A3. With binding to a different epitope it is meant that the antibody binds to different critical amino acid residues as compared to known antibodies. It has furthermore been shown that the antibodies of the invention are non-phospho-selective.

25 In certain embodiments, the antibodies act synergistically when used in combination with other antibodies binding to tau protein. As used herein, the term "synergistic" means that the combined effect of the antibodies or antigen-binding fragments when used in combination is greater than their additive effects when used individually. A way of calculating synergy is by means of the

combination index. The concept of the combination index (CI) has been described by Chou and Talalay (Adv Enzyme Regul., 22:27-55, 1984).

In certain embodiments, the antibodies and antigen-binding fragments are for use as a medicament, and preferably for use in the diagnostic, therapeutic and/or prophylactic treatment of neurodegenerative diseases. Human anti -tau antibodies of the invention or fragments thereof, including Fab, (Fab')₂, scFv fragments, or antibodies comprising antigen-binding sites of the antibodies of the invention can be used to treat, reduce or prevent symptoms in patients having a neurodegenerative disease that involves accumulation of tau or pathological tau or tau aggregation within the brain, such as patients suffering from AD as well as any other tauopathy or other tau-related pathologies in which tau may be overexpressed. While not wishing to be bound by any particular theory, the antibodies of the invention may exert their beneficial effect by reducing or eliminating pathological tau or tau aggregation and hence the amount of PHF-tau in the brain. The antibodies of the invention may be used to treat an animal patient belonging to any classification. Examples of such animals include mammals such as humans, rodents, dogs, cats and farm animals. For example, the antibodies of the invention are useful in the preparation of a medicament for treatment of AD wherein the medicament is prepared for administration in dosages defined herein.

Another embodiment of the invention is a method of treating or reducing symptoms of a neurodegenerative disease that involves aggregation of tau in a patient comprising administering to the patient a therapeutically effective amount of the isolated antibody of the invention for a time sufficient to treat or reduce symptoms of the neurodegenerative disease. Another embodiment of the invention is a method of reducing tau in patients in need thereof comprising administering to the patient a therapeutically effective amount of the isolated antibody of the invention for a time sufficient to reduce tau.

In any of the embodiments above, the neurodegenerative disease that involves aggregation of tau is a tauopathy. As used herein a "tauopathy" encompasses any neurodegenerative disease that involves the pathological aggregation of tau within the brain. In addition to familial and sporadic AD, other exemplary tauopathies are frontotemporal dementia with parkinsonism linked to chromosome 17 (FTDP-17), progressive supranuclear palsy, corticobasal degeneration, Pick's disease, progressive subcortical gliosis, tangle only dementia, diffuse neurofibrillary tangles with calcification, argyrophilic grain dementia, amyotrophic lateral sclerosis parkinsonism-dementia complex, Down syndrome, Gerstmann-StrausslerScheinker disease, Hallervorden-Spatz disease,

inclusion body myositis, Creutzfeld-Jakob disease, multiple system atrophy, Niemann-Pick disease type C, prion protein cerebral amyloid angiopathy, subacute sclerosing panencephalitis, myotonic dystrophy, nonguanamian motor neuron disease with neurofibrillary tangles, postencephalitic parkinsonism, and chronic traumatic encephalopathy, such as dementia pugilistica (boxing disease).
5 (Morris, et al. Neuron 70:410-26, 2011).

A tauopathy-related behavioral phenotype includes cognitive impairments, early personality change and disinhibition, apathy, abulia, mutism, apraxia, perseveration, stereotyped movements/behaviors, hyperorality, disorganization, inability to plan or organize sequential tasks, selfishness/callousness, antisocial traits, a lack of empathy, halting, agrammatic speech with
10 frequent paraphasic errors but relatively preserved comprehension, impaired comprehension and word-finding deficits, slowly progressive gait instability, retropulsions, freezing, frequent falls, non-levodopa responsive axial rigidity, supranuclear gaze palsy, square wave jerks, slow vertical saccades, pseudobulbar palsy, limb apraxia, dystonia, cortical sensory loss, and tremor.

Patients amenable to treatment include asymptomatic individuals at risk of AD or other
15 tauopathy, as well as patients presently showing symptoms. Patients amenable to treatment include individuals who have a known genetic risk of AD, such as a family history of AD or presence of genetic risk factors in the genome. Exemplary risk factors are mutations in the amyloid precursor protein (APP), especially at position 717 and positions 670 and 671 (Hardy and Swedish mutations, respectively). Other risk factors are mutations in the presenilin genes, PS 1 and PS2, and ApoE4,
20 family history of hypercholesterolemia or atherosclerosis. Individuals presently suffering from AD can be recognized from characteristic dementia by the presence of risk factors described above. In addition, a number of diagnostic tests are available to identify individuals who have AD. These include measurement of cerebrospinal fluid tau and A β 42 levels. Elevated tau and decreased A β 42 levels signify the presence of AD. Individuals suffering from AD can also be diagnosed by AD and
25 Related Disorders Association criteria.

Another embodiment of the invention is a method of reducing tau in patients in need thereof comprising administering to the patient a therapeutically effective amount of the isolated anti-tau antibody of the invention for a time sufficient to reduce tau. Patients amenable to treatment may suffer from an ailment associated with overexpression of tau. Some mutations, including mutations
30 in intron 10, induce increased levels of the functionally normal four-repeat tau protein isoform, leading to neurodegeneration. Overexpression of the four-repeat human tau protein isoform

specifically in neurons in a transgenic mouse led to development of axonal degeneration in brain and spinal cord. In the model, axonal dilations with accumulation of neurofilaments, mitochondria, and vesicles were documented. The axonopathy and the accompanying dysfunctional sensorimotor capacities were transgene-dosage related. These findings proved that merely increasing the concentration of the four-repeat tau protein isoform is sufficient to injure neurons in the central nervous system, without formation of intraneuronal neurofibrillary tangles (Spittaels, et al, Am J Pathology 155(6) 2153-2165,1999).

Administration/Pharmaceutical Compositions

Anti-tau antibodies of the invention are suitable both as therapeutic and prophylactic agents for treating or preventing neurodegenerative diseases that involves accumulation of tau, and/or pathological aggregation of tau, such as AD or other tauopathies or tau-associated ailments. In asymptomatic patients, treatment can begin at any age (e.g., at about 10, 15, 20, 25, 30 years). Usually, however, it is not necessary to begin treatment until a patient reaches about 40, 50, 60, or 70 years. Treatment typically entails multiple dosages over a period of time. Treatment can be monitored by assaying antibody, or activated T-cell or B-cell responses to the therapeutic agent over time. If the response falls, a booster dosage is indicated.

In prophylactic applications, pharmaceutical compositions or medicaments are administered to a patient susceptible to, or otherwise at risk of, AD or other ailment involving tau, in an amount sufficient to eliminate or reduce the risk, lessen the severity, or delay the outset of the disease, including biochemical, histologic and/or behavioral symptoms of a disease, its complications and intermediate pathological phenotypes presented during development of the disease. In therapeutic applications, compositions or medicaments are administered to a patient suspected of, or already suffering from, such a disease in an amount sufficient to reduce, arrest, or delay any of the symptoms of the disease (biochemical, histologic and/or behavioral). Administration of a therapeutic may reduce or eliminate mild cognitive impairment in patients that have not yet developed characteristic Alzheimer's pathology. An amount adequate to accomplish therapeutic or prophylactic treatment is defined as a therapeutically- or prophylactically-effective dose. In both prophylactic and therapeutic regimes, compositions or medicaments are usually administered in several dosages until a sufficient immune response has been achieved.

Anti- tau antibodies or fragments thereof of the invention may be administered in combination with other agents that are effective for treatment of related neurodegenerative diseases.

In the case of AD, antibodies of the invention may be administered in combination with agents that reduce or prevent the deposition of amyloid beta (A β). It is possible that PHF-tau and A β pathologies are synergistic. Therefore, combination therapy targeting the clearance of both PHF-tau and A β - related pathologies at the same time may be more effective than targeting each individually.

5 In the case of Parkinson's Disease and related neurodegenerative diseases, immune modulation to clear aggregated forms of the α -synuclein protein is also an emerging therapy. A combination therapy which targets the clearance of both tau and α -synuclein proteins simultaneously may be more effective than targeting either protein individually. In the methods of the invention, the "therapeutically effective amount" of the antibody in the treatment or ameliorating
10 symptoms of a tauopathy can be determined by standard research techniques. For example, the dosage of the antibody can be determined by administering the agent to relevant animal models well known in the art.

In addition, *in vitro* assays can optionally be employed to help identify optimal dosage ranges. Selection of a particular effective dose can be determined (e.g., via clinical trials) by those
15 skilled in the art based upon the consideration of several factors. Such factors include the disease to be treated or prevented, the symptoms involved, the patient's body mass, the patient's immune status and other factors known by the skilled artisan. The precise dose to be employed in the formulation will also depend on the route of administration, and the severity of disease, and should be decided according to the judgment of the practitioner and each patient's circumstances. Effective doses can
20 be extrapolated from dose-response curves derived from *in vitro* or animal model test systems. The mode of administration for therapeutic use of the antibodies of the invention may be any suitable route that delivers the agent to the host. Pharmaceutical compositions of these antibodies are useful for parenteral administration, e.g., intradermal, intramuscular, intraperitoneal, intravenous, subcutaneous, intranasal or intracranial or they can be administered into the cerebrospinal fluid of
25 the brain or spine.

The antibodies of the invention may be prepared as pharmaceutical compositions containing an effective amount of the antibody as an active ingredient in a pharmaceutically acceptable carrier. The term "carrier" refers to a diluent, adjuvant, excipient, or vehicle with which the antibody is administered. Such pharmaceutical vehicles can be liquids, such as water and oils, including those of
30 petroleum, animal, vegetable or synthetic origin, such as peanut oil, soybean oil, mineral oil, sesame oil and the like. For example, 0.4% saline and 0.3% glycine can be used. These solutions are sterile

and generally free of particulate matter. They may be sterilized by conventional, well-known sterilization techniques (e.g., filtration). The compositions may contain pharmaceutically acceptable auxiliary substances as required to approximate physiological conditions such as pH adjusting and buffering agents, stabilizing, thickening, lubricating and coloring agents, etc. The concentration of the antibodies of the invention in such pharmaceutical formulation can vary widely, *i.e.*, from less than about 0.5%, usually at or at least about 1% to as much as 15 or 20% by weight and will be selected primarily based on required dose, fluid volumes, viscosities, etc., according to the particular mode of administration selected.

The treatment may be given in a single dose schedule, or as a multiple dose schedule in which a primary course of treatment may be with 1-10 separate doses, followed by other doses given at subsequent time intervals required to maintain and or reinforce the response, for example, at 1-4 months for a second dose, and if needed, a subsequent dose(s) after several months. Examples of suitable treatment schedules include: (i) 0, 1 month and 6 months, (ii) 0, 7 days and 1 month, (iii) 0 and 1 month, (iv) 0 and 6 months, or other schedules sufficient to elicit the desired responses expected to reduce disease symptoms, or reduce severity of disease. Thus, a pharmaceutical composition of the invention for intramuscular injection could be prepared to contain 1 ml sterile buffered water, and between about 1 ng to about 100 mg, about 50 ng to about 30 mg or about 5 mg to about 25 mg of an antibody of the invention. Similarly, a pharmaceutical composition of the invention for intravenous infusion could be made up to contain about 250 ml of sterile Ringer's solution, and about 1 mg to about 30 mg or about 5 mg to about 25 mg of an antibody of the invention. Actual methods for preparing parenterally administrable compositions are well known and are described in more detail in, for example, "Remington's Pharmaceutical Science", 15th ed., Mack Publishing Company, Easton, PA.

The antibodies of the invention can be lyophilized for storage and reconstituted in a suitable carrier prior to use. This technique has been shown to be effective with antibody and other protein preparations and art-known lyophilization and reconstitution techniques can be employed.

Diagnostic methods and kits

Antibodies of the invention may be used in methods of diagnosing AD or other tauopathy in a subject. This method involves detecting, in the subject, the presence of tau using a diagnostic reagent such as an antibody or a fragment thereof of the present invention. Tau may be detected in a biological sample from a subject (e.g., blood, urine, cerebral spinal fluid) by contacting the

biological sample with the diagnostic antibody reagent, and detecting binding of the diagnostic antibody reagent to PHF-tau in the sample from the subject. Assays for carrying out the detection include well known methods such as ELISA, immunohistochemistry, western blot, or *in vivo* imaging. Exemplary diagnostic antibodies are antibodies CBTAU-27.1, CBTAU-28.1, CBTAU-43.1, CBTAU-46.1, CBTAU-47.1, CBTAU-47.2 and CBTAU-49.1 of the invention, and are of IgG 5 1,K type.

Diagnostic antibodies or similar reagents can be administered by intravenous injection into the body of the patient, or directly into the brain by any suitable route that delivers the agent to the host as exemplified above. The dosage of antibody should be within the same ranges as for 10 treatment methods. Typically, the antibody is labeled, although in some methods, the primary antibody with affinity for tau is unlabelled and a secondary labeling agent is used to bind to the primary antibody. The choice of label depends on the means of detection. For example, a fluorescent label is suitable for optical detection. Use of paramagnetic labels is suitable for tomographic detection without surgical intervention. Radioactive labels can also be detected using PET or 15 SPECT.

Diagnosis is performed by comparing the number, size, and/or intensity of labeled tau, tau accumulation, tau aggregates, and/or neurofibrillary tangles in a sample from the subject or in the subject, to corresponding baseline values. The baseline values can represent the mean levels in a population of undiseased individuals. Baseline values can also represent previous levels determined 20 in the same subject.

The diagnostic methods described above can also be used to monitor a subject's response to therapy by detecting the presence of tau in a subject before, during or after the treatment. A change in values relative to baseline signals a response to treatment. Values can also change temporarily in biological fluids as pathological tau is being cleared from the brain.

25 The present invention is further directed to a kit for performing the above described diagnostic and monitoring methods. Typically, such kits contain a diagnostic reagent such as the antibodies of the invention, and optionally a detectable label. The diagnostic antibody itself may contain the detectable label (e.g., fluorescent molecule, biotin, etc.) which is directly detectable or detectable via a secondary reaction (e.g., reaction with streptavidin). Alternatively, a second reagent 30 containing the detectable label may be utilized, where the second reagent has binding specificity for the primary antibody. In a diagnostic kit suitable for measuring tau in a biological sample, the

antibodies of the kit may be supplied pre bound to a solid phase, such as to the wells of a microtiter dish.

The contents of all cited references (including literature references, issued patents, published patent applications, and co-pending patent applications) cited throughout this application are hereby
5 expressly incorporated by reference.

Examples

EXAMPLE 1

10 **Tau peptide design and labeling**

Hyperphosphorylation of tau protein resulting in release from microtubules and leading to depolymerization is a pathological hallmark occurring in Alzheimer's disease (AD) and other related tauopathies. As the equilibrium of free tau to microtubule-bound tau shifts in favor of the former, unassociated tau protein is thought to accumulate in a misfolded, aggregated state. During
15 the disease process, tau is thought to adopt a variety of conformations, progressing from soluble dimeric and oligomeric forms to higher-order insoluble aggregates such as paired helical filaments (PHFs) and neurofibrillary tangles (NFTs). However, the exact forms of tau that contribute to pathology, and hence optimal for therapeutic targeting, remain unknown. Consequently, attempts to target disease-promoting tau are often limited by the choice of target. In an effort to prepare novel
20 anti-tau binding molecules, antibody variable regions to tau were recovered from human memory B-cells using phosphorylated and non-phosphorylated tau peptides as bait antigens using a single-cell based approach.

Human memory B-cells to tau are likely rare in the human repertoire; therefore, we decided to label the tau baits with the brightest fluorophores. All tau peptides were synthesized with an
25 amino-terminal biotin group to aid labeling with two bright fluorophores, streptavidin-APC or streptavidin-PE (aka, tau peptide tetramers). Each tau peptide was labeled with both fluorophores to increase the signal-to-noise during the screening of human memory B-cells (detailed in Example 2) from donor samples. Labeled tau peptide tetramers were prepared by mixing biotinylated peptide at a 35:1 molar ratio of peptide to streptavidin label overnight at 4°C with gentle mixing. Free peptide

was removed by separation over a BioSpin 30 column (Biorad). All tau peptide tetramers were stored at 4°C for up to 2 months.

EXAMPLE 2

5 Recovery of anti-tau specific memory B-cells by FACs sorting with labelled peptide tetramers

Monoclonal antibodies against tau were recovered from memory B-cells (CD22+CD19+CD27+IgG+) isolated from peripheral blood mononuclear cells (PBMCs) obtained from presumably asymptomatic (non-AD) human blood donors obtained from San Diego Blood Bank and TSRI Normal Blood Donor Services. In addition, AD patient blood samples were obtained
10 through the CRO, Quintiles, from which three antibodies detailed here were recovered. PBMCs were isolated on Ficoll-Paque Plus (GE Healthcare) and cryopreserved at 50 million cells per ml in 90% FBS and 10% DMSO. An aliquot of plasma was heat inactivated at 56°C and stored at -20°C for downstream assessment of plasma reactivity.

For each sorting experiment, PBMCs from 3-4 donors were thawed and transferred to tubes
15 containing pre-warmed RPMI complete (RPMI, 10% heat inactivated FBS and 1% penicillin /streptomycin), washed and incubated separately at 37°C for 16 hrs. Pooled PBMCs were enriched for mature B-cells by positive selection using CD22+ magnetic beads (Miltenyi Biotec). Cells were resuspended in Tris-buffered saline pH 7.4, containing 2 mM EDTA and 0.25% bovine serum albumin Fraction V (TBS Buffer). The cells were stained with the extracellular markers IgG-FITC,
20 CD19-PerCPCy5.5 and CD27-PECy7 (all from BD Biosciences) to label B-cells. Ten million cells were removed and as a negative control, biotin streptavidin labeled conjugates were used. The remaining cells were incubated with a pool of ten dual labeled tau peptide tetramers (SA-APC and SA-PE) at 16.8 nM each. Cells were incubated for 60 min at 4°C with gentle mixing, washed twice and re-suspended at 20 million cells per ml in TBS buffer. Prior to sorting, DAPI (Thermo Fisher)
25 was added as a live cell marker and cells were sorted on a Beckman Coulter MoFlo XDP. Negative control samples were used to determine nonspecific binding and signal-to-noise ratio. CD19+, IgG+, CD27hi, and antigen double-positive cells were collected and deposited into individual wells of a 96-well PCR plate and stored at -80°C.

EXAMPLE 3

Recovery of heavy and light chain genes from tau-specific single B-cells

As detailed in Example 2, memory B-cells with reactivity to tau peptide tetramers were identified, isolated and sorted into individual microtiter wells. Heavy and light chain cDNAs were then recovered by a two-step PCR approach from individual B-cells, and variable domain sequences were cloned and expressed *in vitro* as full-length recombinant IgG1 antibodies and are thus human chimeric antibodies.

First strand cDNA synthesis

First-strand complementary DNA (cDNA) was generated from single sorted cells according to manufacturer's protocol (Superscript III, Invitrogen Corp.) with the following modifications: to each well containing a single B-cell, 0.5 µl of 10% NP-40, 1.0 µl of oligo dT, 1.0 µl of dNTP was added and samples were incubated at 65°C for 5 min. After incubation, samples were placed on ice for 1 min. The following was then added to each well: 2.0 µl of DTT, 4.0 µl of MgCl₂, 1.0 µl of SuperScript RT, and 0.5 µl of RNaseOut. Samples were incubated at 50°C for 50 min, followed by incubation at 85°C for 5 min.

Step I Amplification

For the initial PCR (Step I), 2.5 µl of cDNA preparation was used as a template to amplify heavy and kappa or lambda light chains. Primer pools specific to the leader regions of antibody heavy (CB-5'LVH primers, **Table 1**), kappa light chain (CB-5'LVk primers, **Table 2**), and lambda light chain (CB-5' LVlam primers, **Table 3**) were used. A single reverse primer specific to the CH1 region, CK, and CL regions of the heavy, kappa light and lambda light chain, respectively, were used in the Step I PCR reaction.

Table 1 **VH STEP I FORWARD PRIMERS**

Primer ID	DNA SEQUENCE (5'-3')	SEQ ID NO:
CB-5'LVH1a	ATGGACTGGACCTGGAGGTTCTC	7
CB-5'LVH1b	ATGGACTGGACCTGGAGGATCTC	8

CB-5'LVH1c	ATGGACTGGACCTGGAGGGTCTTC	9
CB-5'LVH1d	ATGGACTGGACCTGGAGCATCC	10
CB-5'LVH2	GGACATACTTTGTTCCACGCTCCTGC	11
CB-5'LVH3a	AGGTGTCCAGTGTGAGGTGCAGC	12
CB-5'LVH3b	AGGTGTCCAGTGTGAGGTGCAGC	13
CB-5'LVH3c	AGGTGTCCAGTGTGAGGTACAGC	14
CB-5'LVH4	GCAGCTCCCAGATGGGTCCTG	15
CB-5'LVH5	TCAACCGCCATCCTCGCCCTC	16
CB-5'LVH6	GTCTGTCTCCTTCCTCATCTTCCTGC	17
3'CgCH1	GGAAGGTGTGCACGCCGCTGGTC	18

Table 2 VK STEP I FORWARD PRIMERS

Primer ID	DNA SEQUENCE (5'-3')	SEQ ID NO:
CB-5'LVk1a	ATGAGGGTCCCCGCTCAGCTC	19
CB-5'LVk1b	ATGAGGGTCCCTGCTCAGCTC	20
CB-5'LVk1c	ATGAGAGTCCTCGCTCAGCTC	21
CB-5'LVk2	TGGGGCTGCTAATGCTCTGG	22
CB-5'LVk3	CCTCCTGCTACTCTGGCTCCAG	23
CB-5'LVk4	TCTCTGTTGCTCTGGATCTCTGGTGC	24
CB-5'LVk5	CTCCTCAGCTTCCTCCTCTTTGG	25
CB-5'LVk6	AACTCATTGGGTTTCTGCTGCTCTGG	26
3'Ck-Rev543	GTTTCTCGTAGTCTGCTTTGCTCAGC	27
3'Ck-Rev494	GTGCTGTCCCTTGCTGTCTGCTC	28
3'Ck-Rev	GCACTCTCCCCTGTTGAAGCTCTTTG	29

Table 3 VL STEP I FORWARD PRIMERS (5'-3')

Primer ID	DNA SEQUENCE (5'-3')	SEQ ID NO:
CB-5' L Vlam1	CTCCTCGCTCACTGCACAGG	30
CB-5' L Vlam2	CTCCTCTCTCACTGCACAGG	31
CB-5' L Vlam3	CTCCTCACTCGGGACACAGG	32
CB-5' L Vlam4	ATGGCCTGGACCCCTCTCTG	33

CB-5' L Vlam5	ATGGCATGGATCCCTCTCTTCCTC	34
3'Cl -Rev	CACTAGTGTGGCCTTGTGGCTTG	35

Step II Amplification

For Step II, 2.5 µl of Step I PCR product was used as a template to amplify heavy, and kappa or lambda light chain variable regions. A pool of forward and reverse primers specifically designed to the framework 1 region of antibody heavy chain (pCB-IgG-VH and 3'SalIJH primers, **Table 4**), kappa light chain (pCB-IgG-VK and 3'Jk primers, **Table 5**), and lambda light chain (CB-VL and 3'Clam-Step II primers, **Table 6**) were used to prepare DNA from the variable regions. Furthermore, Step II primers were designed to introduce XbaI (VK and VL forward primers) and XhoI (3'SalIJH primers) restriction sites for downstream cloning. Following the Step II amplification reactions, heavy and light chain variable domain PCR products were run on a 1% agarose gel. Heavy and light chain variable region fragments were purified according to the manufacturer's protocol (Qiagen) and used in the Step III PCR reaction.

Table 4 VH Step II Forward and Reverse Primers		
Primer ID	DNA SEQUENCE (5' - 3')	SEQ ID NO:
pCB-IgG-VH1a	CCTGTCTGGAATTCAGCATGGCCCAGGTGCAGCTGGTGCAGTC	36
pCB-IgG-VH1b	CCTGTCTGGAATTCAGCATGGCCCAGGTCCAGCTGGTGCAGTC	37
pCB-IgG-VH1c	CCTGTCTGGAATTCAGCATGGCCCAGGTTCAGCTGGTGCAGTC	38
pCB-IgG-VH1d	CCTGTCTGGAATTCAGCATGGCCCAGGTCCAGCTTGTGCAGTC	39
pCB-IgG-VH2a	CCTGTCTGGAATTCAGCATGGCCCAGGTCACCTTGAGGGAGTCTGG	40
pCB-IgG-VH2b	CCTGTCTGGAATTCAGCATGGCCCAGGTCACCTTGAAGGAGTCTGG	41
pCB-IgG-VH3a	CCTGTCTGGAATTCAGCATGGCCCAGGTGCAGCTGGTGGAGTC	42
pCB-IgG-VH3b	CCTGTCTGGAATTCAGCATGGCCGAGGTGCAGCTGTTGGAGTC	43
pCB-IgG-VH3c	CCTGTCTGGAATTCAGCATGGCCGAGGTGCAGCTGGTGGAGTC	44
pCB-IgG-VH3d	CCTGTCTGGAATTCAGCATGGCCCAGGTACAGCTGGTGGAGTCTG	45
pCB-IgG-VH4a	CCTGTCTGGAATTCAGCATGGCCCAGSTGCAGCTGCAGGAG	46
pCB-IgG-VH4b	CCTGTCTGGAATTCAGCATGGCCCAGGTGCAGCTACAGCAGTGG	47
pCB-IgG-VH5	CCTGTCTGGAATTCAGCATGGCCGAGGTGCAGCTGGTGCAGTC	48

pCB-IgG-VH6	CCTGTCTGGAATTCAGCATGGCCCAGGTACAGCTGCAGCAGTCAG	49
pCB-IgG-VH7	CCTGTCTGGAATTCAGCATGGCCCAGGTGCAGCTGGTGCAATCTG	50
3'SalIJH 1/2/4/5	TCGGGCCTCGAGACTCACCTGAGGAGACGGTGACCAG	51
3'SalIJH3	TCGGGCCTCGAGACTCACCTGAAGAGACGGTGACCATTG	52
3'SalIJH6	TCGGGCCTCGAGACTCACCTGAGGAGACGGTGACCGTG	53

Table 5 VK STEP II FORWARD and REVERSE PRIMERS

Primer ID	DNA SEQUENCE (5'-3')	SEQ ID NO:
pCB-IgG-VK1a	CCGGTCTAGAGTTTTCCATGGCGGACATCCAGATGACCCAGTCTCC	54
pCB-IgG-VK1b	CCGGTCTAGAGTTTTCCATGGCGGACATCCAGTTGACCCAGTCTCC	55
pCB-IgG-VK1c	CCGGTCTAGAGTTTTCCATGGCGGCCATCCAGTTGACCCAGTCTCC	56
pCB-IgG-VK2a	CCGGTCTAGAGTTTTCCATGGCGGATRTTGTGATGACTCAGTCTCCACTC	57
pCB-IgG-VK3a	CCGGTCTAGAGTTTTCCATGGCGGAAATTGTGTTGACGCAGTCTCCAG	58
pCB-IgG-VK3b	CCGGTCTAGAGTTTTCCATGGCGGAAATTGTGTTGACACAGTCTCCAG	59
pCB-IgG-VK3c	CCGGTCTAGAGTTTTCCATGGCGGAAATAGTGATGACGCAGTCTCCAG	60
pCB-IgG-VK4	CCGGTCTAGAGTTTTCCATGGCGGACATCGTGATGACCCAGTCTCC	61
pCB-IgG-VK5	CCGGTCTAGAGTTTTCCATGGCGGAAACGACACTCACGCAGTCTCC	62
pCB-IgG-VK6	CCGGTCTAGAGTTTTCCATGGCGGAAATTGTGCTGACTCAGTCTCCAG	63
3'Jk1 Rev IIa	CGCAAAGTGCACTTACGTTTGATTTCCACCTTGGTCCCTTGGC	64
3'Jk2 Rev Iib	CGCAAAGTGCACTTACGTTTGATCTCCAGCTTGGTCCCTTGGC	65
3'Jk4 Rev IIc	CGCAAAGTGCACTTACGTTTGATATCCACTTTGGTCCCAAGGC	66
3'Jk3 Rev IIc	CGCAAAGTGCACTTACGTTTGATCTCCACCTTGGTCCCTCCGC	67
3'Jk5 Rev IId	CGCAAAGTGCACTTACGTTTAATCTCCAGTCGTGTCCCTTGGC	68

TABLE 6 VL STEP II FORWARD and REVERSE PRIMERS

Primer ID	DNA SEQUENCE (5'-3')	SEQ ID NO:
CB-VL1	CCGGTCTAGAGTTTTCCATGGCGAATTTTATGCTGACTCAGCCCCACTC	69
CB-VL2	CCGGTCTAGAGTTTTCCATGGCGTCCTATGTGCTGACTCAGCC	70
CB-VL3	CCGGTCTAGAGTTTTCCATGGCGCAGTCTGTGCTGACGCAGCC	71

CB-VL4	CCGGTCTAGAGTTTTCCATGGCGCAGTCTGTCGTGACGCAGCC	72
CB-VL5	CCGGTCTAGAGTTTTCCATGGCGCAGTCTGCCCTGACTCAGCC	73
CB-VL6	CCGGTCTAGAGTTTTCCATGGCGTCTTCTGAGCTGACTCAGGACC	74
CB-VL7	CCGGTCTAGAGTTTTCCATGGCGTCCTATGAGCTGACTCAGCCACC	75
3'Clam-Step II	CTCAGAGGAGGGYGGGAACAGAGTGAC	76

Step III Amplification: Overlap Extension PCR

For Step III, the heavy and light chain variable region DNA fragments produced in Step II were linked into a single cassette via overlap extension PCR using: 1) a kappa linker or lamda linker (see linker preparation method below), which anneals to the 3' end of the light chain Step II fragment and the 5' end of the heavy chain Step II fragment, and contains either the kappa or lambda constant region, 2) a forward overlap primer containing an XbaI restriction site, and 3) a reverse primer containing an XhoI restriction site. This reaction results in an approximate 2400 bp or 2200 bp amplicon (i.e., cassette) for the kappa or lambda chains, respectively, consisting of the light chain variable region, linker, and heavy chain variable region. Following amplification, the overlap extension PCR reaction product was PCR-purified according to manufacturer's instructions (Qiagen PCR Purification Kit).

Linker preparation

The linker fragment was amplified using pCB-IgG, a dual-CMV promoter vector generated in house and used to express both heavy and light chain genes, as template and the primers listed in **Table 7**. The linker fragment is 1765 or 1536 base pairs in length for kappa or lambda linker, respectively. The kappa linker contains from 5' to 3' an intron sequence followed by the kappa constant region, poly(A) termination sequence, and cytomegalovirus promoter sequence, allowing for one vector expression of the recombinant antibodies. The lambda linker contains the lambda constant region, poly(A) termination sequence, and cytomegalovirus promoter sequence. A common reverse primer (Linker_VH_HAVT20_pCB-IgG-R) and kappa-specific forward primer (Linker_CK_intron_pCB-IgG-F) were used (**Table 7**). The amplified fragment was separated on a 1% agarose gel and purified according to manufacturer's protocol (Qiagen Gel Extraction Kit).

Table 7 LINKER AND OVERLAP PRIMERS

Primer ID	SEQUENCE (5'-3')	SEQ ID NO:
Linker_VH_HAVT20_pCB-IgG-R	GGCCATGCTGAATTCCAGACAGG	78
Linker_CK_intron_pCB-IgG-F	AAACGTAAGTGCACTTTGCGGCCGCTAGG	79
Linker_CL_intron_pCB-IgG-F_opt	ACTCTGTTCCCRCCCTCCTCTGAGG	80
pCB-overlap F	CCGGTCTAGAGTTTTCCATGGCG	81
pCB-overlap R	TCGGGCCTCGAGACTCACC	82

Cloning into mammalian expression vector

Following purification of the overlap extension PCR product, the fragment was digested with XhoI and XbaI and subsequently separated on a 1% agarose gel. The band corresponding to the overlap cassette (~2.4 kb) was purified and ligated into an IgG1 expression vector, pCB-IgG. Antibody variable genes were subcloned into this vector and antibodies were recombinantly expressed as IgG1 regardless of their original (native) isotype. (An example of an IgG1 heavy chain constant region amino acid sequence is shown in SEQ ID NO:83 and light chain kappa constant region amino acid sequence is shown in SEQ ID NO:84). All transformations were carried out using DH5a Max Efficiency cells (Invitrogen Corp.) and recovered in 250 µl of SOC for 1 hr at 37° C. Approximately 100 µl of recovered cells were plated onto a carbenicillin plate supplemented with 20 mM glucose. Plates were incubated overnight at 37° C to allow for colony growth. The remaining recovered cell mixture was cultured with 4 ml of Super Broth (SB) media supplemented with 50 µg/ml carbenicillin and incubated overnight at 37° C with shaking at 250 rpm. The following day, 5 colonies were picked per plate and grown in 3 ml of SB media supplemented with 50 µg/ml carbenicillin overnight at 37° C. Overnight cultures were used for DNA plasmid preparation (Qiagen).

EXAMPLE 4

Antibody sequencing, germline identification and confirmation of anti-tau peptide reactivity in transfection supernatant

To express IgG1s, DNA plasmid minipreps of the aforementioned 4 ml cultures were prepared (Qiagen) and used to transfect 293Expi cells using ExpiFectamine according to manufacturer's instructions (Invitrogen, Corp.). Transfections were carried out for a minimum of 72 hr in 10 ml cultures to allow for sufficient Ig1G expression. Cell media was harvested post-transfection and
 5 centrifuged to remove the cells and debris. Supernatants were quantified using Protein A sensor tips on an Octet Red system (ForteBio). Each supernatant was subsequently tested by ELISA with the bait peptide in order to confirm the presence of anti-tau reactive antibodies. Plasmid miniprep DNA (Qiagen) from the four individually picked cultures in Example 3 was prepared and heavy and light chains were sequenced with primers pC9_seq_HC-R (5'CATGTCACCGGGGTGTGG 3')(SEQ ID NO: 85) and pC9_seq_LC-R (5'TCACAGGGGATGTTAGGGACA3')(SEQ ID NO:86). One clone
 10 of the four was selected for subsequent experiments.

Heavy and Light chain variable region protein and nucleic acid sequences of antibody clones CBTAU-7.1(SEQ ID NOS:87, 88, 89, 90), CBTAU-8.1(SEQ ID NOS:91, 92, 93, 94), CBTAU-16.1(SEQ ID NOS:95, 96, 97, 98), CBTAU-18.1(SEQ ID NOS: 99, 100, 101, 102), CBTAU-
 15 20.1(SEQ ID NOS:103, 104, 105, 106), CBTAU-22.1(SEQ ID NOS:107, 108, 109, 110), CBTAU-24.1(SEQ ID NO:111, 112, 113, 114), CBTAU-27.1(SEQ ID NOS:115, 116, 117, 118,), CBTAU-28.1(SEQ ID NOS:119, 120, 121, 122), CBTAU-41.1(SEQ ID NOS:123, 124, 125, 126), CBTAU-41.2(SEQ ID NOS:127, 128, 129, 130) , CBTAU-42.1 (SEQ ID NOS:131, 132, 133, 134) , CBTAU-43.1(SEQ ID NOS:135, 136, 137, 138) , CBTAU-44.1(SEQ ID NOS:139, 140, 141, 142) ,
 20 CBTAU-45.1(SEQ ID NOS:143, 144, 145, 146) , CBTAU-46.1(SEQ ID NOS:147, 148, 149, 150) , CBTAU-47.1(SEQ ID NOS:151, 152, 153, 154) , CBTAU-47.2(SEQ ID NOS:155, 156, 157, 158) and CBTAU-49.1(SEQ ID NOS:159, 160, 161, 162) define novel CDRs for the selected anti-tau antibodies (**Table 8**).

An anti-tau antibody CBTAU-7.1 was generated comprising the VH of SEQ ID NO: 87 and
 25 the VL of SEQ ID NO: 88 and a human IgG1 constant region. An anti-tau antibody CBTAU-8.1 was generated comprising the VH of SEQ ID NO: 91 and the VL of SEQ ID NO: 92 and a human IgG1 constant region. An anti-tau antibody CBTAU-16.1 was generated comprising the VH of SEQ ID NO: 95 and the VL of SEQ ID NO: 96 and a human IgG1 constant region. An anti-tau antibody CBTAU-18.1 was generated comprising the VH of SEQ ID NO: 99 and the VL of SEQ ID NO: 100
 30 and a human IgG1 constant region. An anti-tau antibody CBTAU-20.1 was generated comprising the VH of SEQ ID NO: 103 and the VL of SEQ ID NO: 104 and a human IgG1 constant region. An

anti-tau antibody CBTAU-22.1 was generated comprising the VH of SEQ ID NO: 107 and the VL of SEQ ID NO: 108 and a human IgG1 constant region. An anti-tau antibody CBTAU-24.1 was generated comprising the VH of SEQ ID NO: 111 and the VL of SEQ ID NO: 112 and a human IgG1 constant region. An anti-tau antibody CBTAU-27.1 was generated comprising the VH of SEQ ID NO: 115 and the VL of SEQ ID NO: 116 and a human IgG1 constant region. An anti-tau antibody CBTAU 28.1 was generated comprising the VH of SEQ ID NO: 119 and the VL of SEQ ID NO: 120 and a human IgG1 constant region. An anti-tau antibody CBTAU -41.1 was generated comprising the VH of SEQ ID NO: 123 and the VL of SEQ ID NO: 124 and a human IgG1 constant region. An anti-tau antibody CBTAU -41.2 was generated comprising the VH of SEQ ID NO: 127 and the VL of SEQ ID NO: 128 and a human IgG1 constant region. An anti-tau antibody CBTAU -42.1 was generated comprising the VH of SEQ ID NO: 131 and the VL of SEQ ID NO: 132 and a human IgG1 constant region. An anti-tau antibody CBTAU 43.1 was generated comprising the VH of SEQ ID NO: 135 and the VL of SEQ ID NO: 136 and a human IgG1 constant region. An anti-tau antibody CBTAU 44.1 was generated comprising the VH of SEQ ID NO: 139 and the VL of SEQ ID NO: 140 and a human IgG1 constant region. An anti-tau antibody CBTAU 45.1 was generated comprising the VH of SEQ ID NO: 143 and the VL of SEQ ID NO: 144 and a human IgG1 constant region. An anti-tau antibody CBTAU 46.1 was generated comprising the VH of SEQ ID NO: 147 and the VL of SEQ ID NO: 148 and a human IgG1 constant region. An anti-tau antibody CBTAU 47.1 was generated comprising the VH of SEQ ID NO: 151 and the VL of SEQ ID NO: 152 and a human IgG1 constant region. An anti-tau antibody CBTAU 47.2 was generated comprising the VH of SEQ ID NO: 155 and the VL of SEQ ID NO: 156 and a human IgG1 constant region. An anti-tau antibody CBTAU 49.1 was generated comprising the VH of SEQ ID NO: 159 and the VL of SEQ ID NO: 160 and a human IgG1 constant region.

Table 8: Amino acid sequences of heavy and light chain variable region CDRs

Clone	Tau aa region	CDR1 (SEQ ID NO:)	CDR2 (SEQ ID NO:)	CDR3 (SEQ ID NO:)
CBTAU 7.1	194-212	SYWMH (163)	RINSDGSDTNYSVKG (164)	GRSYGFFDY (165)
		RASQIISNYLA (166)	GASSRAT (167)	QQYGTSPRT (168)

CBTAU 8.1	194-212	TYGMH (169)	VIWFDGNKYYADSVKG (170)	DWWEAGCRPCYFFDY (171)
		KSSQSVLYSSNNKNYLA (172)	WASTRES (173)	QQYYSPPLT (174)
CBTAU 16.1	204-221	DYWMS (175)	NINQDGSAAYYVDSVRG (176)	DAHYYDRNRNNYYYYFDF (177)
		RASQSVGANLA (178)	SASTRAT (179)	QQYNNWPRT (180)
CBTAU 18.1	200-217	SGNYYWS (181)	RMSSSGSTNYNPSLKS (182)	ESGSSWQNHYYYYGMDV (183)
		KSSQSVLYSSNNKNYLA (172)	WASTRES (173)	QQYYSTPLT (184)
CBTAU 20.1	58-78	NYAMS (185)	GISSDGNTFYADSVKG (186)	ESGRWGGGTLYGAHY (187)
		KSSQSLLYNSNNKNYLT (188)	WASTRES (173)	QQYYSSPLT (189)
CBTAU 22.1	406-429	DYNVH (190)	RISPNSGGTKYAQKFQG (191)	GHCDGTTCSTRAY (192)
		RSSQSLLHRSGHKYLH (193)	LGSNRAS (194)	MQTLQTPWT (195)
CBTAU 24.1	221-245	GYYLH (196)	WVNPRSGGTSYPPKFQG (197)	GRIPDVTAFDI (198)
		KSSESLLYDSNNKNYLA (199)	WASTRES (173)	QQYFSTPWT (200)
CBTAU 27.1	299-328	DYWTA (201)	IISGSDSDTRYHPSVQG (202)	LDARVDAGWQLDS (203)
		KSSQSVFSRDNNKNYLA (204)	WASSRES (205)	QHYFNTPHN (206)
CBTAU 28.1	52-71	NYWIG (207)	IIYPGSDSDTRYSPPFQG (208)	VGRPSKGGWFDP (209)
		ESSQTLLYSSNEKNYLA (210)	WASTPES (211)	QQYYNSPYT (212)
CBTAU 41.1	406-429	DSYMS (213)	YISRSSHTNYADSVKG (214)	VQTTMIEGKTKLNYFDY (215)

		ESSHSLLYRSNNRNYLA (216)	WASTRES (173)	QQFYTTPYT (217)
CBTAU 41.2	406-429	DSYMS (213)	YISRSSHTNYADSVKG (214)	VQTTMIEGKTKLNYFDY (215)
		ESSHSLLYRSNNKNYLA (218)	WASTRES (173)	QQFYTTPYT (217)
CBTAU 42.1	406-429	KAWMS (219)	RIKSKVDGETTDYAAPV RG (220)	LIHCDLSACLPHF (221)
		ESSHSLLYRSNNKNYLA (218)	WASTRES (173)	QQFYTTPYT (217)
CBTAU 43.1	299-328	NYWIA (222)	IIPGSDDTTYSFSFQG (223)	LPRTDGDNSIGYFEY (224)
		KSSQSVLYSSNSENLYA (225)	WASTRES (173)	QQYYSTPFT (226)
CBTAU 44.1	406-429	SYSMN (227)	YISSSTTTIYYADSVKG (228)	VPAPRLGGSYTY (229)
		RASQSVSSSYLA (230)	GASSRAT (167)	QQYGTSPLT (231)
CBTAU 45.1	406-429	DAWMS (232)	RIKSKNVGETTDYAEHV RG (233)	GLGGGTYG (234)
		RSSAGLRNNDGDILLS (235)	RVSRRDS (236)	MRGPY (237)
CBTAU 46.1	82-103	IYEMN (238)	YITNRGSTIYYADSVKG (239)	PRIGARVFDV (240)
		KSSQTLLYKSNNENLYA (241)	WASTRES (173)	QQYFTTALT (242)
CBTAU 47.1	52-71	DHWIG (243)	IIFPEDSDTRYSGSFEG (244)	VSVVRKGGWFDP (245)
		KSSQSLLYTSNNKNYLA (246)	WASTRES (173)	QQYYNSPYT (212)
CBTAU 47.2	52-71	DHWIG (243)	IIFPGSDIRYSPSFEG (247)	VAVVRKGGWFDS (248)
		KSTQSLLSANNKNYLA (249)	WASTRES (173)	QQYYNSPYT (212)

CBTAU 49.1	52-71	SYWIG (250)	IYPDDSDTRYNASLEG (251)	RDRNCSGTTTCYPRWFDS (252)
		KSSQSLFYSGNSKDFLA (253)	WASTRDS (254)	HQYHSTPLS (255)

CBTAU-7.1 antibody comprises a heavy chain CDR1 region of SEQ ID NO:163, a heavy chain CDR2 region of SEQ ID NO:164, and a heavy chain CDR3 region of SEQ ID NO:165, a light chain CDR1 region of SEQ ID NO:166, a light chain CDR2 region of SEQ ID NO:167 and a light chain CDR3 region of SEQ ID NO:168. CBTAU-8.1 antibody comprises a heavy chain CDR1 region of SEQ ID NO:169, a heavy chain CDR2 region of SEQ ID NO:170, and a heavy chain CDR3 region of SEQ ID NO:171, a light chain CDR1 region of SEQ ID NO:172, a light chain CDR2 region of SEQ ID NO:173 and a light chain CDR3 region of SEQ ID NO:174. CBTAU-16.1 antibody comprises a heavy chain CDR1 region of SEQ ID NO:175, a heavy chain CDR2 region of SEQ ID NO:176, and a heavy chain CDR3 region of SEQ ID NO:177, a light chain CDR1 region of SEQ ID NO:178, a light chain CDR2 region of SEQ ID NO:179 and a light chain CDR3 region of SEQ ID NO:180. CBTAU-18.1 antibody comprises a heavy chain CDR1 region of SEQ ID NO:181, a heavy chain CDR2 region of SEQ ID NO:182, and a heavy chain CDR3 region of SEQ ID NO:183, a light chain CDR1 region of SEQ ID NO:172, a light chain CDR2 region of SEQ ID NO:173 and a light chain CDR3 region of SEQ ID NO:184. CBTAU-20.1 antibody comprises a heavy chain CDR1 region of SEQ ID NO:185, a heavy chain CDR2 region of SEQ ID NO:186, and a heavy chain CDR3 region of SEQ ID NO:187, a light chain CDR1 region of SEQ ID NO:188, a light chain CDR2 region of SEQ ID NO:173 and a light chain CDR3 region of SEQ ID NO:189. CBTAU-22.1 antibody comprises a heavy chain CDR1 region of SEQ ID NO:190, a heavy chain CDR2 region of SEQ ID NO:191, and a heavy chain CDR3 region of SEQ ID NO:192, a light chain CDR1 region of SEQ ID NO:193, a light chain CDR2 region of SEQ ID NO:194 and a light chain CDR3 region of SEQ ID NO:195. CBTAU-24.1 antibody comprises a heavy chain CDR1 region of SEQ ID NO:196, a heavy chain CDR2 region of SEQ ID NO:197, and a heavy chain CDR3 region of SEQ ID NO:198, a light chain CDR1 region of SEQ ID NO:199, a light chain CDR2 region of SEQ ID NO:173 and a light chain CDR3 region of SEQ ID NO:200. CBTAU-27.1 antibody comprises a heavy chain CDR1 region of SEQ ID NO:201, a heavy chain CDR2 region of SEQ ID NO:202, and a heavy chain CDR3 region of SEQ ID NO:203, a light chain CDR1 region of SEQ ID

NO:204, a light chain CDR2 region of SEQ ID NO:205 and a light chain CDR3 region of SEQ ID NO:206. CBTAU-28.1 antibody comprises a heavy chain CDR1 region of SEQ ID NO:207, a heavy chain CDR2 region of SEQ ID NO:208, and a heavy chain CDR3 region of SEQ ID NO:209, a light chain CDR1 region of SEQ ID NO:210, a light chain CDR2 region of SEQ ID NO:211 and a light chain CDR3 region of SEQ ID NO:212. CBTAU-41.1 antibody comprises a heavy chain CDR1 region of SEQ ID NO:213, a heavy chain CDR2 region of SEQ ID NO:214, and a heavy chain CDR3 region of SEQ ID NO:215, a light chain CDR1 region of SEQ ID NO:216, a light chain CDR2 region of SEQ ID NO:173 and a light chain CDR3 region of SEQ ID NO:217. CBTAU-41.2 antibody comprises a heavy chain CDR1 region of SEQ ID NO:213, a heavy chain CDR2 region of SEQ ID NO:214, and a heavy chain CDR3 region of SEQ ID NO:215, a light chain CDR1 region of SEQ ID NO:218, a light chain CDR2 region of SEQ ID NO:174 and a light chain CDR3 region of SEQ ID NO:217. CBTAU-42.1 antibody comprises a heavy chain CDR1 region of SEQ ID NO:219, a heavy chain CDR2 region of SEQ ID NO:220, and a heavy chain CDR3 region of SEQ ID NO:221, a light chain CDR1 region of SEQ ID NO:218, a light chain CDR2 region of SEQ ID NO:173 and a light chain CDR3 region of SEQ ID NO:217. CBTAU-43.1 antibody comprises a heavy chain CDR1 region of SEQ ID NO:222, a heavy chain CDR2 region of SEQ ID NO:223, and a heavy chain CDR3 region of SEQ ID NO:224, a light chain CDR1 region of SEQ ID NO:225, a light chain CDR2 region of SEQ ID NO:173 and a light chain CDR3 region of SEQ ID NO:226. CBTAU-44.1 antibody comprises a heavy chain CDR1 region of SEQ ID NO:227, a heavy chain CDR2 region of SEQ ID NO:228, and a heavy chain CDR3 region of SEQ ID NO:229, a light chain CDR1 region of SEQ ID NO:230, a light chain CDR2 region of SEQ ID NO:167 and a light chain CDR3 region of SEQ ID NO:231. CBTAU-45.1 antibody comprises a heavy chain CDR1 region of SEQ ID NO:232, a heavy chain CDR2 region of SEQ ID NO:233, and a heavy chain CDR3 region of SEQ ID NO:234, a light chain CDR1 region of SEQ ID NO:235, a light chain CDR2 region of SEQ ID NO:236 and a light chain CDR3 region of SEQ ID NO:237. CBTAU-46.1 antibody comprises a heavy chain CDR1 region of SEQ ID NO:238, a heavy chain CDR2 region of SEQ ID NO:239, and a heavy chain CDR3 region of SEQ ID NO:240, a light chain CDR1 region of SEQ ID NO:241, a light chain CDR2 region of SEQ ID NO:173 and a light chain CDR3 region of SEQ ID NO:242. CBTAU-47.1 antibody comprises a heavy chain CDR1 region of SEQ ID NO:243, a heavy chain CDR2 region of SEQ ID NO:244, and a heavy chain CDR3 region of SEQ ID NO:245, a light chain CDR1 region of SEQ ID NO:246, a light chain CDR2 region of SEQ ID NO:173 and a light

chain CDR3 region of SEQ ID NO:212. CBTAU-47.2 antibody comprises a heavy chain CDR1 region of SEQ ID NO:243, a heavy chain CDR2 region of SEQ ID NO:247, and a heavy chain CDR3 region of SEQ ID NO:248, a light chain CDR1 region of SEQ ID NO:249 a light chain CDR2 region of SEQ ID NO:173 and a light chain CDR3 region of SEQ ID NO:212. CBTAU-49.1 antibody comprises a heavy chain CDR1 region of SEQ ID NO:250, a heavy chain CDR2 region of SEQ ID NO:251, and a heavy chain CDR3 region of SEQ ID NO:252, a light chain CDR1 region of SEQ ID NO:254, a light chain CDR2 region of SEQ ID NO:254 and a light chain CDR3 region of SEQ ID NO:255.

Nucleic acid sequences of heavy and light chain variable regions of the anti-tau monoclonal antibodies were compared to known germline sequences using IgBLAST, an immunoglobulin variable domain sequence analysis tool, available at the NCBI. (Nucleic Acids Res. 2013 Jul;41(Web Server issue):W34-40). Sequence alignment of heavy and light chain framework H1 and L1 regions aligned with their respective proposed germline sequence and PCR primer are shown in **Table 9**. Confirmed sequences were scaled up for expression and purification (detailed in Example 5). Selected clones were expanded into a 50 ml culture and plasmid midiprep DNA was prepared (Machery Nagel Midi Prep kit). Plasmid DNA was then used to transfect a 30 ml culture of 293Expi cells as detailed in Example 5.

Table 9: Framework nucleic acids of H1 and L1 aligned with germline and primer

(Amino acids above) Differences marked in lower case lettering. Native refers to the antibody

mAb	SEQ ID NO	Amino Terminal Protein and N-terminal Nucleic Acid Sequences
CBTAU-7.1		
VH	87	(Q V Q L V E S)
pCB-IgG-VH1b	37	CAGGTCCAGCTGGTGCAGTC
CBTAU-7.1VH	89	CAGGTCCAGCTGGTGGAGTCCGGGGGAGGCTTAGTTTCAGCCTGGGGGGTCCCTGAGACTCTCCT
IGHV3-74*01.	256	gAGGTgCAGCTGGTGGAGTCCGGGGGAGGCTTAGTTTCAGCCTGGGGGGTCCCTGAGACTCTCCT
Native 7.1 VH	257	(e V Q L V E)
VL	88	(D I V M T Q S P)
pCB-IgG-Vk4	61	GACATCGTGATGACCCAGTCTCC
CBTAU-7.1 VL	90	GACATCGTGATGACCCAGTCTCCAGACACCTGTCTTTGTCTCCAGGGGAGAGGCCACCTCT

IGKV3-NL5*01	258	GAaATtGTGtTGACgCAGTCTCCAGcCACCCGTGCTTTGTCTCCAGGGGAaAGAGCCACCCTCT
Native 7.1 VL	259	(e I V L T Q S P)

CBTAU-8.1		
VH	91	(Q V A L V E S)
pCB-IgG-VH3a	42	CAGGTGCAGCTGGTGGAGTC
CBTAU-8.1 VH	93	CAGGTGCAGCTGGTGGAGTCGAGGGGAGGCGTGGTCCAGCCTGGGACGTCCCTGAGACTCTCCT
IGHV3-33*01	260	CAGGTGCAGCTGGTGGAGTctgGGGGAGGCGTGGTCCAGCCTGGGACGTCCCTGAGACTCTCCT
Native 8.1 VH	261	(Q V A L V E S)
VL	92	(E T T L T Q S P)
pCB-IgG-Vk5	62	GAAACGACACTCACGCAGTCTCC
CBTAU-8.1 VL	94	GAAACGACACTCACGCAGTCTCCAGACTCCCTGGCTGTGTCTCTGGGCGAGAGGGCCACCATCA
IGKV4-1*01	262	GAcAtcgtgaTgACcCAGTCTCCAGACTCCCTGGCTGTGTCTCTGGGCGAGAGGGCCACCATCA
Native 8.1 VL	263	(d i v m T Q S P)

CBTAU-16.1		
VH	95	(E V Q L V Q)
pCB-IgG-VH5	48	GAGGTGCAGCTGGTGCAGTC
CBTAU-16.1 VH	97	GAGGTGCAGCTGGTGCAGTCTGGGGGAGGCTTGGTCCAGCCTGGGGGGTCCCTGAGACTCTCCT
IGHV3-64*01	264	GAGGTGCAGCTGGTGgAGTCTGGGGGAGGCTTGGTCCAGCCTGGGGGGTCCCTGAGACTCTCCT
Native 16.1 VH	265	(E V Q L V e S)
VL	96	(E I V M T Q S P)
pCB-IgG-VK3c	60	GAAATAGTGATGACGCAGTCTCCGG
CBTAU-16.1VL	98	GAAATAGTGATGACGCAGTCTCCGGCCACCCTGTCTGTGTCTCCAGGGGAAAGAGCCACCCTCT
IGKV3-15*01	266	GAAATAGTGATGACGCAGTCTCCaGCCACCCTGTCTGTGTCTCCAGGGGAAAGAGCCACCCTCT
Native 16.1 VL	267	(E I V M T Q S P)

CBTAU-18.1		
VH	99	(Q V Q L L E S)
		No exact primer match
CBTAU-18.1 VH	101	CAGGTGCAGCTGTTGGAGTCGGGCCCAGGACTGGTGAACCCTTCACAGACCCTGTCCCTCACCT
IGHV4-31*05	268	CAGGTGCAGCTGcaGGAGTCGGGCCCAGGACTGGTGAAGCCTTCACAGACCCTGTCCCTCACCT
Native 18.1 VH	269	(Q V Q L q E S)
VL	100	(E I V L T Q S P)
pCB-IgG-VK3b	59	GAAATTGTGTTGACACAGTCTCCAG
CBTAU-18.1 VL	102	GAAATTGTGTTGACACAGTCTCCAGACTCCCTGGCTGTGTCTCTGGGCGAGAGGGCCAACATTA
IGKV4-1*01	262	GAcATcGTGaTGACcCAGTCTCCAGACTCCCTGGCTGTGTCTCTGGGCGAGAGGGCCAcCATcA
Native 18.1 VL	270	(d I V m T Q S P)

CBTAU-20.1		
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VH	103	(Q V Q L V E S)
pCB-IgG-VH3d	45	CAGGTACAGCTGGTGGAGTCTG
CBTAU-20.1 VH	105	CAGGTACAGCTGGTGGAGTCTGGGGGAGGCTTGGTACAGCCTGGGGGGTCCCTGAGACTCTCCT
IGHV3-23*04	271	gAGGTgCAGCTGGTGGAGTCTGGGGGAGGCTTGGTACAGCCTGGGGGGTCCCTGAGACTCTCCT
Native 20.1 VH	272	(e V Q L V E S)
VL	104	(D I Q M T Q S P)
pCB-IgG-VK1a	54	GACATCCAGATGACCCAGTCTCC
CBTAU-20.1 VL	106	GACATCCAGATGACCCAGTCTCCAGACTCCCTGGCTGTGTCTCTGGGCGAGAGGGCCACCATCA
IGKV4-1*01	262	GACATCgtGATGACCCAGTCTCCAGACTCCCTGGCTGTGTCTCTGGGCGAGAGGGCCACCATCA
Native 20.1 VL	273	(D I v M T Q S P)

CBTAU-22.1		
VH	107	(Q V Q L V Q S)
pCB-IgG-VH1a	36	CAGGTGCAGCTGGTGCAGTC
CBTAU-22.1 VH	109	CAGGTGCAGCTGGTGCAGTCTGGGGCTGAGGTGAAGAAGCCTGGGGCCCCAGTGAAGGTCTC
IGHV1-2*02	274	CAGGTGCAGCTGGTGCAGTCTGGGGCTGAGGTGAAGAAGCCTGGGGCCtCAGTGAAGGTCTC
Native 22.1 VH	275	(Q V Q L V Q S)
VL	108	(D V V M T Q S P L)
		No Exact Primer match
CBTAU-22.1 VL	110	GATGTGTGATGACGCAGTCTCCACTCTCCCTGCCCGTCACCCCTGGAGAGCCGGCCTCCATC
IGKV2-28*01	276	GATaTTGTGATGACtCAGTCTCCACTCTCCCTGCCCGTCACCCCTGGAGAGCCGGCCTCCATC
Native 22.1 VL	277	(D i V M T Q S P L)

CBTAU-24.1		
VH	111	(Q V Q L V S G)
pCB-IgG-VH1d	39	CAGGTCCAGCTTGTGCAGTC
CBTAU-24.1 VH	113	CAGGTCCAGCTTGTGCAGTCTGGGGCTGAGGTGAAGAAGCCTGGGGCCTCAGTGAAGGTCTCC
IGHV1-3*01	278	CAGGTCCAGCTTGTGCAGTCTGGGGCTGAGGTGAAGAAGCCTGGGGCCTCAGTGAAGGTtTCC
Native 24.1 VH	279	(Q V Q L V S G)
VL	112	(D I Q M T Q S P)
pCB-IgG-VK1a	54	GACATCCAGATGACCCAGTCTCC
CBTAU-24.1 VL	114	GACATCCAGATGACCCAGTCTCCAGACTCCCTGGCTGTGTCTCTGGGCGAGAGGGCCACCATC
IGKV4-1*01	262	GACATCgtGATGACCCAGTCTCCAGACTCCCTGGCTGTGTCTCTGGGCGAGAGGGCCACCATC
Native 24.1 VL	280	(D I v M T Q S P)

CBTAU27.1		
VH	115	(Q V Q L V E S)
pCB-IgG-VH3a	42	CAGGTGCAGCTGGTGGAGTC
CBTAU27.1 VH	117	CAGGTTcAGCTGGTGGAGTCTGGACCGGAGATGAGAAAGCCCGGGAGTCTCTGAAAATTTC

IGHV5-51*01	281	gAGGTgCAGCTGGTGcAGTCTGGAgCaGAGgTGAAaAAAGCCCGGGGAGTCTCTGAAGATcTCC
Native 27.1 VH	282	(e V Q L V q S)
VL	116	(D I Q L T Q S P)
pCB-IgG-VK1b	55	GACATCCAGTTGACCCAGTCTCC
CBTAU27.1 VL	118	GACATCCAGTTGACCCAGTCTCCAGATTCCCTGGCTGTGTCTCTGGGCGAGCGGGCCACCATC
IGKV4-1*01	262	GACATCgtGATGACCCAGTCTCCAGACTCCCTGGCTGTGTCTCTGGGCGAGAGGGCCACCATC
Native 27.1 VL	283	(D I v m T Q S) P

CBTAU28.1		
VH	119	(Q V Q L Q Q S)
pCB-IgG-VH6	49	CAGGTaCAGCTgCAGCAGTCAG
CBTAU28.1 VH	121	CAGGTGCAGCTACAGCAGTCAGGAGCAGAAGTGAAAAAGCCCGGGGAGTCTCTGAAGATCTCC
IGHV5-51*01	281	gAGGTGCAGCTggtGCAGTctGGAGCAGAAGTGAAAAAGCCCGGGGAGTCTCTGAAGATCTCC
Native 28.1 VH	284	(e V Q L v Q S)
VL	120	(D I Q M T Q S P)
pCB-IgG-VK1a	54	GACATCCAGATGACCCAGTCTCC
CBTAU28.1 VL	122	GACATCCAGATGACCCAGTCTCCAGACTCCCTGGCTGTGTCTCTGGGCGAGAGGGCCACCATC
IGKV4-1*01	262	GACATCgtGATGACCCAGTCTCCAGACTCCCTGGCTGTGTCTCTGGGCGAGAGGGCCACCATC
Native 28.1 VL	285	(D I v M T Q S P)

CBTAU41.1		
VH	123	(E V Q L L E S)
pCB-IgG-VH3b	43	GAGGTGCAGCTGTTGGAGTC
CBTAU41.1 VH	125	GAGGTGCAGCTGTTGGAGTCTGGGGGAGGCTTGGTCAAGCCTGGAGGGTCCCTGAGACTCTCC
IGHV3-11*06	286	cAGGTGCAGCTGgTGGAGTCTGGGGGAGGCTTGGTCAAGCCTGGAGGGTCCCTGAGACTCTCC
Native 41.1 VH	287	(q V Q L v E S)
VL	124	(D I Q M T Q S P)
pCB-IgG-VK1a	54	GACATCCAGATGACCCAGTCTCC
CBTAU41.1 VL	126	GACATCCAGATGACCCAGTCTCCAGACTCCCTGGCTGTGTCTCTGGGCGAGAGGGTCACCATC
IGKV4-1*01	262	GACATCgtGATGACCCAGTCTCCAGACTCCCTGGCTGTGTCTCTGGGCGAGAGGGTCACCATC
Native 41.1 VL	288	(D I v M T Q S P)

CBTAU41.2		
VH	127	(E V Q L V Q S)
pCB-IgG-VH3b	43	GAGGTGCAGCTGGTGCAGTC
CBTAU41.2 VH	129	GAGGTGCAGCTGGTGCAGTCTGGGGGAGGCTTGGTCAAGCCTGGAGGGTCCCTGAGACTCTCC
IGHV3-11*06	286	cAGGTGCAGCTGGTgGAGTCTGGGGGAGGCTTGGTCAAGCCTGGAGGGTCCCTGAGACTCTCC
Native 41.2 VH	289	(q V Q L v E S)
VL	128	(A I Q L T Q S P)

pCB-IgG-VK1c	56	GCCATCCAGTTGACCCAGTCTCC
CBTAU41.2 VL	130	GCCATCCAGTTGACCCAGTCTCCAGACTCCCTGGCTGTGTCTCTGGGCGAGAGGGTCACCATC
IGKV4-1*01	262	gaCATCgtGaTGACCCAGTCTCCAGACTCCCTGGCTGTGTCTCTGGGCGAGAGGGTCACCATC
Native 41.2 VL	290	(d I v m T Q S P)

CBTAU42.1		
VH	131	(Q L V Q S E G)
pCB-IgG-VH1a-c	36	CAGCTGGTGCAGTC
CBTAU42.1 VH	133	CAGCTGGTGCAGTCTGAGGGAGGCCTGGCAGAGCCTGGGGGGTCCCTTAGACTC
IGHV3-15*01	291	CAGCTGGTGgAGTCTGgGGGAGGCCTGGCAGAGCCTGGGGGGTCCCTTAGACTC
Native 42.1 VH	292	(Q L V e S g g)
VL	132	(E I V L T Q S P)
pCB-IgG-VK3a	58	GAAATTGTGTTGACGCAGTCTCCAG
CBTAU41.1 VL	134	GAAATTGTGTTGACGCAGTCTCCAGACTCCCTGGCTGTGTCTCTGGGCGAGAGGGTCACCATC
IGKV4-1*01	262	GAcATcGTGaTGACcCAGTCTCCAGACTCCCTGGCTGTGTCTCTGGGCGAGAGGGTCACCATC
Native 42.1 VL	293	(d I V m T Q S P)

CBTAU43.1		
VH	135	(Q V Q L V Q S)
pCB-IgG-VH1a	36	CAGGTGCAGCTGGTGCAGTC
CBTAU43.1 VH	137	CAGGTGCAGCTGGTGCAGTCTGGAGGAGAGGTGAAAAAGCCGGGGGAGTCTCTGAAGATCTCC
IGHV5-51*03	294	gAGGTGCAGCTGGTGCAGTCTGGAGGAGAGGTGAAAAAGCCGGGGGAGTCTCTGAAGATCTCC
Native 43.1 VH	295	(e V Q L V Q S)
VL	136	(E I V L T Q S P)
pCB-IgG-VK3b	59	GAAATTGTGTTGACACAGTCTCCAG
CBTAU43.1 VL	138	GAAATTGTGTTGACACAGTCTCCAGCCTCCCTGGCTGTGTCTCTGGGCGAGAGGGCCACCATC
IGKV4-1*01	262	GAcATcGTGaTGACcCAGTCTCCAGCCTCCCTGGCTGTGTCTCTGGGCGAGAGGGCCACCATC
Native 43.1 VL	296	(d I V m T Q S P)

CBTAU44.1		
VH	139	(E V Q L V E S)
pCB-IgG-VH3c	44	GAGGTGCAGCTGGTGGAGTC
CBTAU44.1 VH	141	GAGGTGCAGCTGGTGGAGTCTGGGGGAGGCTTGGTACAGCCTGGGGGGTCCCTGAGACTCTCC
IGHV3-48*01	297	GAGGTGCAGCTGGTGGAGTCTGGGGGAGGCTTGGTACAGCCTGGGGGGTCCCTGAGACTCTCC
Native 44.1 VH	298	(E V Q L V E S)
VL	140	(D I Q M T Q S)
pCB-IgG-VK1a	54	GACATCCAGATGACCCAGTCTCC
CBTAU44.1 VL	142	GACATCCAGATGACCCAGTCTCCAGGCACCCTGTCTTTGTCTCCAGGGGAAAGAGCCACCCTC

IGKV3-20*01	299	GAaATtgtGtTGACgCAGTCTCCAGGCACCCTGTCTTTGTCTCCAGGGGAAAGAGCCACCCTC
Native 44.1 VL	300	(e I v L T Q S)

CBTAU45.1		
VH	143	(E V Q L V E S)
pCB-IgG-VH3c	44	GAGGTGCAGCTGGTGGAGTC
CBTAU45.1 VH	145	GAAATTGTGTTGACACAGTCTCCACTCTCCCTGCCCCGCCACCCTTGGACAGCCGGCCTCCATC
IGHV3-15*02	301	GAGGTGCAGCTGGTGGAGTCTGGGGGAGACTTGGTAAAGCCTGGGGGGTCCCTTAGACTCTCC
Native 45.1 VH	302	(E V Q L V E S)
VL	144	(E I V L T Q S P)
pCB-IgG-VK3b	59	GAAATTGTGTTGACACAGTCTCCAG
CBTAU45.1 VL	146	GAAATTGTGTTGACACAGTCTCCACTCTCCCTGCCCCGCCACCCTTGGACAGCCGGCCTCCATC
IGKV2-30*01	303	GAtgTTGTGaTGACtCAGTCTCCACTCTCCCTGCCCCGCCACCCTTGGACAGCCGGCCTCCATC
Native 45.1 VL	304	(d v V m T Q S P)

CBTAU46.1		
VH	147	(Q V Q L V E S)
pCB-IgG-VH3d	45	CAGGTACAGCTGGTGGAGTCTG
CBTAU46.1 VH	149	CAGGTACAGCTGGTGGAGTCTGGGGGAGGCTTGGTACAGCCTGGAGAGTCCCTGAGACTCTCC
IGHV3-48*03	305	gAGGTgCAGCTGGTGGAGTCTGGGGGAGGCTTGGTACAGCCTGGAGAGTCCCTGAGACTCTCC
Native 46.1 VH	306	(E V Q L V E S)
VL	148	(D I Q L T Q S P)
pCB-IgG-VK1b	55	GACATCCAGTTGACCCAGTCTCC
CBTAU46.1 VL	150	GACATCCAGTTGACCCAGTCTCCAGACTCCCTGGCTGTGTCTCTGGGCGAGAGGGCCACCATC
IGKV4-1*01	262	GACATCgtGaTGACCCAGTCTCCAGACTCCCTGGCTGTGTCTCTGGGCGAGAGGGCCACCATC
Native 46.1 VL	307	(D I v m T Q S P)

CBTAU47.1		
VH	151	(Q V Q L V Q S)
pCB-IgG-VH1a	36	CAGGTGCAGCTGGTGCAGTC
CBTAU47.1 VH	153	CAGGTGCAGCTGGTGCAGTCTGGAGCAGTGGTGAAAAAGCCCGGGGAGTCTCTGAAGATCTC
IGHV5-51*01	308	gAGGTGCAGCTGGTGCAGTCTGGAGCAGTGGTGAAAAAGCCCGGGGAGTCTCTGAAGATCTC
Native 47.1 VH	309	(E V Q L V Q S)
VL	152	(A I Q L T Q S P)
pCB-IgG-VK1c	56	GCCATCCAGTTGACCCAGTCTCC
CBTAU44.1 VL	154	GCCATCCAGTTGACCCAGTCTCCAGACTCCCTGGCTGTGTCTCTGGGCGAGAGGGCCACCATC
IGKV4-1*01	262	GaCATCgtGaTGACCCAGTCTCCAGACTCCCTGGCTGTGTCTCTGGGCGAGAGGGCCACCATC
Native 47.1 VL	310	(d I v m T Q S P)

CBTAU47.2		
VH	155	(Q V Q L V E S)
pCB-IgG-VH3d	45	CAGGTACAGCTGGTGGAGTCTG
CBTAU47.1 VH	157	CAGGTACAGCTGGTGGAGTCTGGAGCAGAACTGAAAAAGCCCGGGGAGTCTCTGAAGATCTCC
IGHV5-51*01	281	gAGGTgCAGCTGGTgCAGTCTGGAGCAGAACTGAAAAAGCCCGGGGAGTCTCTGAAGATCTCC
Native 47.2 VH	311	(e V Q L V q S)
VL	156	(E I V M T Q S P)
pCB-IgG-VK3c	60	GAAATAGTGATGACGCAGTCTCCAG
CBTAU44.1 VL	158	GAAATTGTGATGACCCAGTCTCCAGAGTCCCTGGCTGTGTCTCTGGGCGAGAGGGCCACCATC
IGKV4-1*01	262	GAcATcGTGATGACCCAGTCTCCAGAGTCCCTGGCTGTGTCTCTGGGCGAGAGGGCCACCATC
Native 47.2 VL	312	(d I V M T Q S P)

CBTAU49.1		
VH	159	(Q V Q L V Q S)
pCB-IgG-VH1a	36	CAGGTGCAGCTGGTGCAGTC
CBTAU49.1 VH	161	CAGGTGCAGCTGGTGCAGTCTGGGGCAGAGGTGAAAAAGCCGTGGGAGTCTCTGAAGATCTCC
IGHV5-51*03	294	gAGGTGCAGCTGGTGCAGTCTGGGGCAGAGGTGAAAAAGCCGTGGGAGTCTCTGAAGATCTCC
Native 49.1 VH	313	(e V Q L V Q S)
VL	160	(E I V L T Q S P)
pCB-IgG-VK6	63	GAAATTGTGCTGACTCAGTCTCCAG
CBTAU49.1 VL	162	GAAATTGTGCTGACTCAGTCTCCAGACTTCCTGGCTGTGTCTCTGGGCGAGAGGGCCACCATC
IGKV4-1*01	262	GAcATcGTGaTGACcCAGTCTCCAGACTTCCTGGCTGTGTCTCTGGGCGAGAGGGCCACCATC
Native 49.1 VL	314	(d I V m T Q S P)

Transfected IgG1 supernatants were assayed for reactivity to tau peptides by ELISA. First, 96 half-well ELISA plates (Costar) were coated with 50 µl of bovine actin (1 µg/ml, Sigma) as a negative control and Affinipure goat anti-human F(ab)₂ (2 µg/ml, Jackson ImmunoResearch) to confirm antibody production. Plates were coated in TBS overnight at 4°C. The following day, plates were washed 5-times with TBS/0.05% Tween (TBS-T) and blocked with 150 µl of TBS-T plus 2.5% BSA (blocking buffer) for 2 hr. Tau peptides were captured on streptavidin coated plates (Pierce) at a concentration of 0.43 µM in 100 µl of TBS. Tau peptides used to set up ELISA assays were the same used as baits in corresponding sorting experiment. Tau peptide coated plates were then incubated at RT for 1.5 hr. All plates were then washed 5-times with TBS/0.05% Tween and blocked with 150 µl and 300 µl (tau peptide plates only) of blocking buffer and incubated at RT for 2 hr. IgG transfection supernatants were diluted to 5 µg/ml (based on quantitation by Octet Red) and

titrated 5-fold in TBS/0.25% BSA. Mouse anti-actin (Sigma, Cat. No. A3853) was used at 1.25 µg/ml as a positive control for bovine actin coated plates. Commercial grade antibodies were used at 1 µg/ml as positive controls for ELISA assays, including AT8 monoclonal antibody (Thermo, MN1020), AT100 monoclonal antibody (Thermo, MN1060) and AT180 monoclonal antibody (Thermo, MN1040). Primary antibodies were incubated for 2 hr at RT and washed 5-times in TBS-T. Finally, goat-anti human IgG Fab or goat anti-mouse HRP (Jackson Labs) was used at 1:2000 and 1:4000, respectively, and incubated for 1 hr at RT. Plates were washed 5-times in TBS-T and developed with SureBlue Reserve TMB Microwell Peroxidase Substrate (KPL). The reaction was halted by the addition of 50 µl and 100 µl (peptide plates) of TMB Stop Solution (KPL) and the absorbance at 450 nm was measured using an ELISA plate reader. Supernatants with the aforementioned binding activities were subsequently reconfirmed in an independent ELISA experiment. Once reconfirmed, a clone was selected for downstream IgG expression and purification (Example 5).

EXAMPLE 5

IgG1 expression and purification of cloned anti-tau chimeric mAbs

After ELISA screening and confirmation of antibody reactivity, selected clones were expressed as IgG1s as indicated in Example 4. Cell culture media was harvested and centrifuged to remove the cells after a minimum of 72 hr and up to a maximum of 168 hr. Clarified supernatants were subsequently passed twice through a Protein A Sepharose column (GE Healthcare Life Sciences) and washed with 50 ml of PBS. IgGs were subsequently eluted with 10 ml of IgG elution buffer (Pierce) and neutralized with Tris pH 8.0 and subsequently dialyzed overnight against PBS. Dialyzed samples were concentrated using a 10,000 MWCO ultra-centrifugal unit (Amicon) to a final volume of about 1 mL, and antibody concentrations were determined with Protein A sensor tips using a human IgG standard on the Octet Red384 (ForteBio). Purified antibodies were further quality-controlled by performing SDS-PAGE under non-reducing and reducing conditions and by size exclusion chromatography.

EXAMPLE 6

IgG bindingReactivity to tau peptides

IgG1s generated and quality controlled as described above were tested by ELISA for their ability to bind to specified cognate peptide(s), as well as non-cognate peptide (Table 10). 96-well ELISA plates (Costar) or streptavidin coated plates (Pierce) were coated with antigen (bovine actin and affinipure goat anti-human F(ab)₂) or tau peptides, respectively, as detailed in Example 4. Purified anti-tau IgGs were diluted to 5 µg/ml in TBS containing 0.25% BSA, and titrated 5-fold. Antibody controls and secondary antibodies were used as detailed in Example 4. antibody reactivity at 1 µg/mL was determined by ELISA and scored as no binding (-), weak (-/+), moderate (+), or strong (++) . (-) for average of two O.D.450 nm readings <0.3; (-/+) for >0.5 and <1.0; (+) for > 1.0 and <1.5; (++) for >1.5.

Table 10 **Cognate and non-cognate peptides used in ELISAs**

mAb	Peptide	Peptide sequence (pX) denotes phosphorylated amino acid	SEQ ID NO	Results
CBTAU-7.1	ptau 194-212 (pS202,pT205)	RSGYSSPG(pS)PG(pT)PGSRsRT	315	+
	tau 194-212	RSGYSSPGSPGTPGSRsRT	316	-
CBTAU-8.1	ptau 194-212 (pS202,pT205)	RSGYSSPG(pS)PG(pT)PGSRsRT	315	-/+
	tau 194-212	RSGYSSPGSPGTPGSRsRT	316	-
CBTAU-16.1	ptau 204-221 (pT212,pS214)	GTPGSRsR(pT)P(pS)LPTPPTR	317	++
	tau 204-221	GTPGSRsRTPSLPTPPTR	318	++
CBTAU-18.1	ptau 200-217 (pS210)	PGSPGTPGSR(pS)RTPSLPT	319	-/+

	tau 200-217	PGSPGTPGSRSRTPSLPT	320	-
	tau 186-253	GEPPKSGDRSGYSSPGSPGTPGSRSRTPSLPT PPTREPKKVA VVRTPPKSPSSAKSRLQTAPV PMPDL	321	-
CBTAU- 20.1	ptau 58-76 (pS68,pT69)	EPGSETSDAK(pS)(pT)PTAEDVT	322	++
	ptau 59-78 (pT69, pT71)	PGSETSDAKS(pT)P(pT)AEDVTAP	323	++
	ptau 61-78 (pT71)	SETSDAKSTP(pT)AEDVTAP	324	-/+
	tau 42-103	GLKESPLQTPTEDGSEEPGSETSDAKSTPTAE DVTAPLVDEGAPGKQAAAQPHTEIPEGTTA	325	-
CBTAU- 22.1	ptau 406-429 (pS416,pS422)	RHLSNVSSSTG(pS)IDMVD(pS)PQLATLA	326	++
	tau 389-441	GAEIVYKSPVVSGDTSRHLNVSSSTGSIDM VDSPQLATLADEV SASLAKQGL	327	-
CBTAU- 24.1	ptau 221-245 (pT231,pS238)	REPKKVA VVR(pT)PPKSPS(pS)AKSRLQT	328	++
	ptau 228-245 (pS238)	VVRTPPKSPS(pS)AKSRLQT	329	++
	ptau 225-245 (pS235,pS238)	KVA VVRTPPK(pS)PS(pS)AKSRLQT	330	++
	tau 186-253	GEPPKSGDRSGYSSPGSPGTPGSRSRTPSLPT PPTREPKKVA VVRTPPKSPSSAKSRLQTAPV PMPDL	321	++
CBTAU- 27.1	tau 299-369	HVPGGGSVQIVYKPVDSLKVTSKCGSLGNIH HKPGGGQVEVKSEKLDKDRVQSKIGSLDN ITHVPGGGNK	331	+
	ptau 194-212 (pS202,pT205)	RSGYSSPG(pS)PG(pT)PGSRSRRT	315	-
CBTAU- 28.1	tau 42-103	GLKESPLQTPTEDGSEEPGSETSDAKSTPTAE DVTAPLVDEGAPGKQAAAQPHTEIPEGTTA	325	++
	ptau 257-272	KSKIG(pS)TENLKHQPGG	332	-

CBTAU-41.1	ptau 406-429 (p416, p422)	RHLSNVSSSTG(pS)IDMVD(pS)PQLATLA	326	+
	tau 389-441	GAEIVYKSPVVSGDTSRHLNSVSSTGSIDM VDSPQLATLADEV SASLAKQGL	327	-
CBTAU-41.2	ptau 406-429 (p416, p422)	RHLSNVSSSTG(pS)IDMVD(pS)PQLATLA	326	+
	tau 389-441	GAEIVYKSPVVSGDTSRHLNSVSSTGSIDM VDSPQLATLADEV SASLAKQGL	327	-
CBTAU-42.1	ptau 406-429 (p416, p422)	RHLSNVSSSTG(pS)IDMVD(pS)PQLATLA	326	++
	tau 389-441	GAEIVYKSPVVSGDTSRHLNSVSSTGSIDM VDSPQLATLADEV SASLAKQGL	327	-
CBTAU-43.1	tau 299-369	HVPGGGSVQIVYKPVDSLKVTSKCGSLGNIH HKPGGGQVEVKSEKLDKDRVQSKIGSLDN ITHVPGGGNK	331	+
	ptau 194-212 (pS202, pT205)	RSGYSSPG(pS)PG(pT)PGSRRT	315	-
CBTAU-44.1	ptau 406-429 (p416, p422)	RHLSNVSSSTG(pS)IDMVD(pS)PQLATLA	326	-/+
	tau 389-441	GAEIVYKSPVVSGDTSRHLNSVSSTGSIDM VDSPQLATLADEV SASLAKQGL	327	-
CBTAU-45.1	ptau 406-429 (p416, p422)	RHLSNVSSSTG(pS)IDMVD(pS)PQLATLA	326	++
	tau 389-441	GAEIVYKSPVVSGDTSRHLNSVSSTGSIDM VDSPQLATLADEV SASLAKQGL	327	-
CBTAU-46.1	tau 42-103	GLKESPLQTPTEGSEEPGSETSDAKSTPTAE DVTAPLVDEGAPGKQAAAQPHTEIPEGTTA	325	+
	ptau 224-241 (pT231, pS235)	KKVAVVR(pT)PPK(pS)PSSAKS	333	-
CBTAU-47.1	tau 42-103	GLKESPLQTPTEGSEEPGSETSDAKSTPTAE DVTAPLVDEGAPGKQAAAQPHTEIPEGTTA	325	++
	ptau 257-272 (pS262)	KSKIG(pS)TENLKHQPGG	332	-

CBTAU-47.2	tau 42-103	GLKESPLQTPTEDGSEEPGSETSDAKSTPTAE DVTAPLVDEGAPGKQAAQPHTEIPEGTTA	325	++
	ptau 257-272 (pS262)	KSKIG(pS)TENLKHQPGG	332	-
CBTAU-49.1	tau 42-103	GLKESPLQTPTEDGSEEPGSETSDAKSTPTAE DVTAPLVDEGAPGKQAAQPHTEIPEGTTA	325	++
	ptau 224-241 (pT231, pS235)	KKVAVVR(pT)PPK(pS)PSSAKS	333	-
AT8	ptau 194-212 (pS202,pT205)	RSGYSSPG(pS)PG(pT)PGSRRT	315	++
	tau 194-212	RSGYSSPGSPGTPGSRRT	316	-

**Amino acid region on human tau441 isoform*

Results are shown in **Figure 1 a-t**. The anti-tau mAbs described herein can be classified into two main groups: those that react only to phosphorylated peptides (phospho-dependent mAbs) and those that react to both phosphorylated and non-phosphorylated peptides (phospho-independent mAbs). Anti-tau mAbs CBTAU-7.1, CBTAU-8.1, CBTAU-18.1, and CBTAU-22.1 were recovered from non-AD individuals using the approach detailed in Example 3. These mAbs are phospho-dependent and, as shown by ELISA (**Fig. 1**), react only with the phosphorylated peptide but not to either a non-phosphorylated peptide spanning that region or a non-phosphorylated version of the peptide. Anti-tau mAbs CBTAU-7.1 and CBTAU-8.1 react specifically to a phosphorylated peptide containing the AT8 binding epitope. This peptide spans amino acids 194 to 212 and contains phosphorylated residues at positions 202 and 205. CBTAU-18.1 reacts to a phosphorylated peptide spanning amino acids 200-217 with a phosphorylated serine residue at position 210. Lastly, CBTAU-22.1 reacts to a peptide spanning amino acids 406-429, with two phosphorylated serines at positions 416 and 422.

Similarly, CBTAU-20.1 was identified from a non-AD individual and is predominately phospho-dependent as it reacts to three different phosphorylated peptides spanning amino acids 59-77. Two of these peptides are dually phosphorylated, one at positions 68 and 69, and the second at positions 69 and 71. CBTAU-20.1 also reacts to a third peptide that is singly phosphorylated at position 71, suggesting that phosphorylation at threonine 71 is sufficient and important for CBTAU-

20.1 reactivity. CBTAU-20.1 shows weak reactivity to a non-phosphorylated peptide spanning region 42-103.

Like the aforementioned mAbs, CBTAU-16.1 and CBTAU-24.1 were also recovered from non-AD individuals; however, both mAbs are phospho-independent and, as observed by ELISA, react to both a phosphorylated and non-phosphorylated peptide spanning the specified region. CBTAU-16.1 reacts to amino acid region 204-221, whereas CBTAU-24.1 reacts to three different peptides spanning amino acids 221-245. In addition, two additional anti-tau mAbs (CBTAU-27.1 and CBTAU-28.1) were identified from screens conducted with non-AD donor samples using 60-70 amino acid-length non-phosphorylated peptides corresponding to amino acid regions 42-103 and 299-369, respectively; therefore, both mAbs are specific to non-phosphorylated tau.

Finally, CBTAU mAbs 41.1, 41.2, 42.1, 43.1, 44.1, 45.1, 46.1, 47.1, 47.2, and 49.1 were identified from a small study where 25 young non-AD (18-27 y.o.), 25 non-AD (55+ y.o.), and 25 AD (55+ y.o.) individuals were screened. The peptide set that was used for this study included 8 phosphorylated peptides (including CBTAU-22.1 cognate peptide) and 2 non-phosphorylated peptides (CBTAU-27.1 and CBTAU-28.1 cognate peptides). CBTAU mAbs 41.1, 41.2, and 42.1 were recovered from AD donors and react with the CBTAU-22.1 cognate peptide. Similar to CBTAU-22.1, these mAbs are phospho-dependent as shown in **Fig. 1j-l**. Two additional mAbs (CBTAU-44.1 and CBTAU-45.1) were identified from non-AD (55+ y.o.) individuals with reactivity to the CBTAU-22.1 cognate peptide. As expected, these two were also phospho-dependent (**Fig. 1 n-o**). CBTAU-43.1 was also identified from screens conducted in non-AD (55+ y.o.) individuals; however, the mAb was recovered with the CBTAU-27.1 cognate peptide and is specific to non-phosphorylated tau (**Fig. 1 m**). Lastly, CBTAU-46.1, 47.1, 47.2, and 49.1 were recovered from non-AD (18-27 y.o.) individuals with reactivity to the CBTAU-28.1 peptide and, similar to CBTAU-28.1, are specific to non-phosphorylated tau (**Fig.1 p-s**).

EXAMPLE 7

Reactivity to paired helical filaments and recombinant tau by ELISA

To further characterize the specificity of some of the chimeric antibodies, their reactivity to recombinant tau, enriched and immunopurified paired helical filaments was tested by ELISA.

PHF-tau was immunopurified according to the protocol of Greenberg and Davies. Briefly, cortical tissues corresponding to Alzheimer's disease individuals were homogenized with 10 volumes of cold buffer (10mM Tris, pH7.4, 1mM EGTA, 0.8 M NaCL and 10% sucrose) and centrifuged at 27,200 x g for 20 min at 4 C. N-lauroylsarcosine and 2-mercaptoethanol were added to the supernatant to reach a final concentration of 1% (wt/vol) and 1% (vol/vol), respectively. The mixture was incubated at 37°C for 2-2.5 hr with constant rocking, followed by centrifugation at 108,000 x g for 30 min at room temperature. The pellet containing PHF-tau was washed 3-times with PBS and dissolved in PBS without protein inhibitors and further centrifuged at 12,000 x g for 5 min. The recovered supernatant containing enriched PHF-tau (ePHF-tau) was immunoaffinity purified over an hTau10 affinity column, and eluted with 3M or 4 M KSCN overnight at 4°C, followed by dialysis against 1L PBS at 4°C. with 3 changes of buffer. hTau10 is an antibody generated in house by immunizing with recombinant tau. It binds to both recombinant and PHF-tau at an amino-terminal epitope. The immunopurified PHF-tau (iPHF-tau) was concentrated with a Sartorius centrifugal filtered device.

For the ELISA, half-area 96-well binding plates (Costar) were coated with 50 µl of antigen in TBS (2 µg/ml recombinant tau, 2 µg/ml bovine action affinipure goat anti-human F(ab)₂, 1 µg/ml of affinity purified paired helical filaments, and 1 µg/ml of monoclonal anti-tau antibody, HT7 (Thermo Scientific, MN1000). The next day, plates were washed with TBS-T and subsequently blocked with 150 µl of TBS plus 2.5% BSA for 2 hr at RT. Following blocking, ePHF-tau was captured for 2 hr at RT on the anti-tau antibody coated plate. Purified anti-tau IgGs were diluted to 10 µg/ml in TBS plus 0.25% BSA, and IgGs were titrated 5-fold at RT for 2 hr. AT8 (10 µg/ml) was used as a positive control for iPHF-tau and captured ePHF-tau. Plates were washed 5-times with TBS-T and secondary antibodies, diluted in TBS plus 0.25% BSA, were added and incubated at RT for 1 hr. Goat Anti-Human IgG F(ab')₂ (Jackson Labs) was used at a 1:2000 dilution and goat anti-mouse HRP (Jackson Labs) was used at 1:4000 (used for anti-actin control). Following incubation, plates were washed 4-times in TBS-T and developed with SureBlue Reserve TMB Microwell Peroxidase Substrate (KPL) for approximately 2 min. The reaction was immediately halted by the addition of TMB Stop Solution (KPL) and the absorbance at 450 nm was measured using an ELISA plate reader.

Results are shown in **Figure 2 a-j**. As expected, phospho-dependent mAbs CBTAU-7.1, CBTAU-8.1, and CBTAU-18.1 do not react to recombinant tau by ELISA (**Fig. 2 a, b, d**). CBTAU-20.1 shows minor reactivity to recombinant tau consistent with its weak reactivity to a non-phosphorylated peptide spanning region 42-103. Interestingly, these phospho-dependent mAbs do not show any reactivity to paired helical filaments (i.e., ePHF-tau and iPHF-tau) with the exception of CBTAU-7.1, which shows minor reactivity to ePHF-tau at higher antibody concentrations. Lastly, phospho-dependent CBTAU-22.1 shows no reactivity to recombinant tau, but does react to both iPHF-tau and ePHF-tau (**Fig. 2f**).

Phospho-independent anti-tau mAbs, CBTAU-16.1 and CBTAU-24.1 react to both recombinant tau and both formats of paired helical filaments (i.e., iPHF-tau and ePHF-tau; **Fig. 2c and g**). CBTAU-28.1 shows strong binding to recombinant tau, with weak immunoreactivity to both PHF-tau formats (**Fig. 2i**). Finally, CBTAU-27.1 shows weak immunoreactivity to both recombinant tau and PHF-tau (**Fig. 1h**).

EXAMPLE 8

Reactivity to paired helical filaments and recombinant tau by Western Blot Analysis

To extend the observations of the rTau and PHF binding ELISAs and to examine if secondary structure plays role in reactivity, recombinant tau, enriched and immunopurified paired helical filaments were tested by Western blot analysis. Approximately 0.5 µg of iPHF, ePHF, and 1 µg of rTau at a final concentration of 1X NuPAGE LDS Sample buffer (0.5% LDS final) (Novex, NP0007) was heated at 70°C for 10 minutes. Samples were loaded onto a 26-well, 4-12% Bis-Tris Novex NuPAGE gel (Invitrogen with MOPS SDS running buffer (Novagen, NP0001), and subsequently transferred onto a nitrocellulose membrane. Membrane was blocked overnight in 1X Tris Buffered Saline (TBS) with 0.05% Tween20 and 4% non-fat dry milk. CBTAU mAbs were used as primary at 25 µg/mL in 1X TBS with 0.05% Tween20 and 4% non-fat dry milk and incubated for 2 hr at room temperature. The membrane was then washed 3 times for 5 min each in 1X TBS with 0.05% Tween20. Peroxidase AffiniPure goat anti-human IgG, Fcγ fragment specific (Jackson ImmunoResearch) was then used as secondary at a 1:2000 dilution in 1X TBS with

0.05% Tween20 and 4% non-fat dry milk and incubated for 45 min at RT. The membrane was washed 3-times for 5 min each and developed using the Supersignal West Pico kit (Pierce).

The results for the western blot analysis are shown in **Figure 3**. The figure shows reactivity of three control antibodies, AT8, AT100, and HT7 (two phospho-tau specific and total tau specific, respectively). Both AT8 and AT100 show the triple bands characteristic of PHF-tau, which correspond to approximately 68, 64, and 60 kDa. Contrary to the ELISA results, phospho-dependent mAbs, CBTAU-7.1 and CBTAU-18.1 react to both iPHF-tau and ePHF-tau by western blot, suggesting that the epitopes for these mAbs are not accessible when tau adopts higher order conformations present in PHF-tau. However, these epitopes become accessible under the strong denaturing conditions of SDS-PAGE. CBTAU-27.1 shows binding to recombinant tau and PHF by western blot yet weak reactivity to each by ELISA, suggesting that the epitope for this antibody is only exposed under strong denaturing conditions. CBTAU-28.1 reacts strongly to recombinant tau by both western blot and ELISA, and also shows reactivity to PHF-tau by both assays. CBTAU-28.1 reacts to the E1/E2 region of tau (amino acids 42-103) which is not present in all tau isoforms; therefore, only the 68 and 64 kDa bands on PHF-tau are detected by CBTAU-28.1. Finally, CBTAU-22.1 and CBTAU-24.1 show similar results to the ELISA assay, reacting to either PHF-tau but not recombinant tau and to both PHF-tau and recombinant tau, respectively.

EXAMPLE 9

Reactivity to tau fragment peptides by ELISA

To characterize the specificity of the recovered antibodies, their reactivity to tau phosphorylated and non-phosphorylated peptides (**Table 11-21, Figure 4 a-g**) was tested by ELISA. Biotinylated tau peptides were synthesized commercially and dissolved in water at 1 mg/ml and frozen at -80 °C. Briefly, 96-well streptavidin binding plates (Thermo-Fisher) were coated with 2 µg/ml of tau peptides diluted in TBS and incubated overnight at 4 °C. The following day, plates were washed with TBS-T and subsequently blocked with 2.5% BSA in TBS for 2 hr at RT. Following blocking, purified anti-tau IgGs were diluted to 2 µg/ml (or to 5 µg/ml and titrated 5-fold for finer mapping of CBTAU-27.1, 28.1, 43.1, 46.1, 47.1, 47.2, and 49.1 using peptide sequences in **Tables 15-20**) in TBS plus 0.25% BSA and incubated at RT for 2 hr. The human chimerized version of AT8 IgG (at 2 µg/ml) described in Example 11 was used as a positive control in each of the

mapping experiments. Plates were washed 5-times with TBS-T followed by the addition of secondary antibody [goat Anti-Human IgG F(ab')₂ (Jackson Labs) at 1:2000 dilution], diluted in TBS plus 0.25% BSA, and incubated at RT for 1 hr. Following incubation, plates were washed 4-times in TBS-T and developed with SureBlue Reserve TMB Microwell Peroxidase Substrate (KPL) for approximately 90 sec. The reaction was immediately halted by the addition of TMB Stop Solution (KPL) and the absorbance at 450 nm was measured using an ELISA plate reader. Each experiment was conducted in triplicate across three different days. Reactivity was considered positive when values were equal to or higher than an OD of 0.4 in the ELISA assay. For determining the reactivity of each mAb to tau phosphorylated and non-phosphorylated peptides, antibody reactivity at 2 µg/mL was determined by ELISA and scored as no binding (-), weak (-/+), moderate (+), or strong (++). (-) for average of two O.D.450 nm readings <0.3; (-/+) for >0.5 and <1.0; (+) for > 1.0 and <1.5; (++) for >1.5. For finer mapping of CBTAU-27.1, 28.1, 43.1, 46.1, 47.1, 47.2, and 49.1 (detailed on **Table 15-20**), antibody reactivity at 1 µg/mL was determined by ELISA and scored as no binding (-), weak (-/+), moderate (+), or strong (++). (-) for average of three O.D.450 nm readings <0.3; (-/+) for >0.5 and <1.0; (+) for > 1.0 and <1.5; (++) for >1.5.

Table 11. CBTAU-7.1: Peptides for reactivity by ELISA

Peptide	SEQ ID NO.	Peptide sequence (pX) denotes phosphorylated amino acid	Results
tau 186-253	321	GEPPKSGDRSGYSSPGSPGTPGSRSRTPSLPTPPTREPK KVAVVVRTPPKSPSSAKSRLQTAPVPMPL	-
ptau 187-212	334	EPPKSGDRSG(pY)SSPGSPG(pT)PGSRSR	-/+
ptau 188-205	335	PPKSGDRSGY(pS)SPGSPG	-
ptau 188-206	336	PPKSGDRSGY(pS(pS)PGSPGTP	-
ptau 188-209	337	PPKSGDRSGY(pS)SPG(pS)PGTPGSR	++
ptau 188-212	338	PPKSGDRSGY(pS)SPGSPG(pT)PGSRSR	-/+
ptau 189-206	339	PKSGDRSGYS(pS)PGSPGTP	-
ptau 189-209	340	PKSGDRSGYS(pS)PG(pS)PGTPGSR	-
ptau 189-212	341	PKSGDRSGYS(pS)PGSPG(pT)PGSRSR	+
tau 190-209	342	KSGDRSGYSSPGSPGTPGSR	-

ptau 192-209	343	GDRSGYSSPG(pS)PGTPGSR	-
ptau 192-212	344	GDRSGYSSPG(pS)PG(pT)PGSRRT	+
ptau 192-215	345	GDRSGYSSPG(pS)PGTPG(pS)RSRTPSL	-
ptau 192-217	346	GDRSGYSSPG(pS)PGTPGSR(pS)RTPSLPT	-
ptau 194-212	315	RSGYSSPG(pS)PG(pT)PGSRRT	+
tau 194-212	316	RSGYSSPGSPGTPGSRRT	-

Table 12. CBTAU-18.1: Peptides for reactivity by ELISA

Peptide	SEQ ID NO.	Peptide sequence (pX) denotes phosphorylated amino acid	Results
ptau 186-253	321	GEPPKSGDRSGYSSPGSPGTPGSRRTPSLPTPPTREPKKV AVVRTPPKSPSSAKSRLQTAPVMPDL	-
ptau 192-217	346	GDRSGYSSPG(pS)PGTPGSR(pS)RTPSLPT	++
ptau 194-212	315	RSGYSSPG(pS)PG(pT)GSRRT	-
tau 194-212*	316	RSGYSSPGSPGTPGSRRT	-
ptau 195-212	347	SGYSSPGSPG(pT)PGSRRT	-
ptau 195-215	348	SGYSSPGSPG(pT)PG(pS)RSRTPSL	-
ptau 195-217	349	SGYSSPGSPG(pT)PGSR(pS)RTPSLPT	++
ptau 195-219	350	SGYSSPGSPG(pT)PGSR(pT)PSLPTPP	-
tau 195-214	351	SGYSSPGSPGTPGSRRTPS	-
ptau 198-215	352	SSPGSPGTPG(pS)RSRTPSL	-
ptau 198-217	353	SSPGSPGTPG(pS)R(pS)RTPSLPT	++
ptau 198-219	354	SSPGSPGTPG(pS)RSR(pT)PSLPTPP	-/+
ptau 198-221	355	SSPGSPGTPG(pS)RSRTP(pS)LTPPTR	-
tau 198-217	356	SSPGSPGTPGSRRTPSLPT	-
ptau 200-217	319	PGSPGTPGSR(pS)RTPSLPT	+
tau 200-217	320	PGSPGTPGSRRTPSLPT	-
ptau 200-219	357	PGSPGTPGSR(pS)R(pT)PSLPTPP	-/+
ptau 200-221	358	PGSPGTPGSR(pS)RTP(pS)LTPPTR	-
ptau 200-224	359	PGSPGTPGSR(pS)RTPSLP(pT)PPTREPK	-

Table 13. CBTAU-22.1: Peptides for reactivity by ELISA

Peptide	SEQ ID NO.	Peptide sequence (pX) denotes phosphorylated amino acid	Results
ptau 404-421	360	SPRHLSNVSS(pT)GSIDMVD	-
ptau 404-429	361	SPRHLSNVSS(pT)GSIDMVD(pS)PQLATLA	++
tau 405-423	362	PRHLSNVSSSTGSIDMVDSP	-
ptau 406-423	363	RHLSNVSSSTG(pS)IDMVDSP	-
ptau 406-429	326	RHLSNVSSSTG(pS)IDMVD(pS)PQLATLA	++
tau 409-428	364	SNVSSTGSIDMVDSPQLATL	-
ptau 412-429	365	SSTGSIDMVD(pS)PQLATLA	++
ptau 412-434	366	SSTGSIDMVD(pS)PQLA(pT)LADEVSA	++

Table 14. CBTAU-24.1: Peptides for reactivity by ELISA

Peptide	SEQ ID NO.	Peptide sequence (pX) denotes phosphorylated amino acid	Results
tau 221-253	367	GEPPKSGDRSGYSSPGSPGTPGSRRTPSLPTPPTR EPKKVAVVRTPPKSPSSAKSRLQTAPVMPDL	++
ptau 221-238	368	REPKKVAVVR(pT)PPKSPSS	-
ptau 221-242	369	REPKKVAVVR(pT)PPK(pS)PSSAKSR	-
ptau 221-244	370	REPKKVAVVR(pT)PPKSP(pS)SAKSRLQ	-
ptau 221-245	328	REPKKVAVVR(pT)PPKSPS(pS)AKSRLQT	++
ptau 225-242	371	KVAVVRTPPK(pS)PSSAKSR	-
ptau 225-244	372	KVAVVRTPPK(pS)P(pS)SAKSRLQ	-/+
ptau 225-245	330	KVAVVRTPPK(pS)PS(pS)AKSRLQT	++
ptau 227-244	373	AVVRTPPKSP(pS)SAKSRLQ	++
ptau 227-245	374	AVVRTPPKSP(pS)(pS)AKSRLQT	++
ptau 228-245	329	VVRTPPKSPS(pS)AKSRLQT	++

Table 15. CBTAU-27.1: Peptides for reactivity by ELISA

Peptide	SEQ ID NO.	Peptide sequence (pX) denotes phosphorylated amino acid	Results
		Cluster 1	
tau 299-369	331	HVPGGGSVQIVYKPVDSLKVTSKCGSLGNIHHKP GGGQVEVKSEKLDFKDRVQSKIGSLDNITHVPGG GNK	++
		Cluster 2	
ptau 404-421 p414	360	SPRHLSNVSS(pT)GSIDMVD	-
ptau 404-429 p414, 422	361	SPRHLSNVSS(pT)GSIDMVD(pS)PQLATLA	-/+
ptau 406-423 p416	363	RHLSNVSSTG(pS)IDMVDSP	-
ptau 406-429 p416, 422	326	RHLSNVSSTG(pS)IDMVD(pS)PQLATLA	-/+
ptau 412-429 p422	365	SSTGSIDMVD(pS)PQLATLA	-/+
ptau 412-434 p422, 427	366	SSTGSIDMVD(pS)PQLA(pT)LADEVSA	-/+
tau 299-318	375	HVPGGGSVQIVYKPVDSLKV	+
tau 309-328	376	VYKPVDSLKVTSKCGSLGNI	-/+
tau 319-338	377	TSKCGSLGNIHHKPGGGQVE	-
tau 329-348	378	HHKPGGGQVEVKSEKLDFKD	-
tau 339-358	379	VKSEKLDFKDRVQSKIGSLD	-
tau 349-369	380	RVQSKIGSLDNITHVPGGGNK	-

Table 16. CBTAU-28.1: Peptides for reactivity by ELISA

Peptide	SEQ ID NO.	Peptide sequence	Results
tau 42-103	325	GLKESPLQTPTEDGSEEPGSETSDAKSTPTAEDVT APLVDEGAPGKQAAAQPHT EIPEGTTA	++
tau 42-61	381	GLKESPLQTPTEDGSEEPGS	-
tau 52-71	382	TEDGSEEPGSETSDAKSTPT	++

ptau 58-75	383	EPGSETSDAK(pS)TPTAEDV	-
tau 62-81	384	ETSDAKSTPTAEDVTAPLVD	-
tau 72-91	385	AEDVTAPLVDEGAPGKQAAA	-
tau 82-103	386	EGAPGKQAAAQPHTEIPEGTTA	-

Table 17. CBTAU-43.1: Peptides for reactivity by ELISA

Peptide	SEQ ID NO.	Peptide sequence	Results
tau 299-369	331	HVPGGGSVQIVYKPVDLSKVTSTKCGSLGNIHHP GGGQVEVKSEKLDKDRVQSKIGSLDNITHVPGG GNK	++
tau 299-318	375	HVPGGGSVQIVYKPVDLSKV	++
tau 309-328	376	VYKPVDLSKVTSTKCGSLGNI	++
tau 319-338	377	TSKCGSLGNIHHPGGGQVE	-
tau 329-348	378	HHKPGGGQVEVKSEKLDKFD	-
tau 339-358	379	VKSEKLDKDRVQSKIGSLD	-
tau 349-369	380	RVQSKIGSLDNITHVPGGGNK	-

Table 18. CBTAU-46.1: Peptides for reactivity by ELISA

Peptide	SEQ ID NO.	Peptide sequence	Results
tau 42-103	325	GLKESPLQPTEDGSEEPGSETSDAKSTPTAEDVT APLVDEGAPGKQAAAQPHTEIPEGTTA	++
tau 42-61	381	GLKESPLQPTEDGSEEPS	-
tau 52-71	382	TEDGSEEPGSETSDAKSTPT	-
tau 62-81	384	ETSDAKSTPTAEDVTAPLVD	-
tau 72-91	385	AEDVTAPLVDEGAPGKQAAA	-
tau 82-103	386	EGAPGKQAAAQPHTEIPEGTTA	++

Table 19. CBTAU-47.1 and CBTAU-47.2: Peptides for reactivity by ELISA

Peptide	SEQ ID NO.	Peptide sequence	Results
tau 42-103	325	GLKESPLQTPTEDGSEEPGSETSDAKSTPTAEDVT APLVDEGAPGKQAAAQPHTEIPEGTTA	++
tau 42-61	381	GLKESPLQTPTEDGSEEPGS	-
tau 52-71	382	TEDGSEEPGSETSDAKSTPT	++
tau 62-81	384	ETSDAKSTPTAEDVTAPLVD	-
tau 72-91	385	AEDVTAPLVDEGAPGKQAAA	-
tau 82-103	386	EGAPGKQAAAQPHTEIPEGTTA	-

Table 20. CBTAU-49.1: Peptides for reactivity by ELISA

Peptide	SEQ ID NO.	Peptide sequence	Results
tau 42-103	325	GLKESPLQTPTEDGSEEPGSETSDAKSTPTAEDVT APLVDEGAPGKQAAAQPHTEIPEGTTA	++
tau 42-61	381	GLKESPLQTPTEDGSEEPGS	-
tau 52-71	382	TEDGSEEPGSETSDAKSTPT	++
tau 62-81	384	ETSDAKSTPTAEDVTAPLVD	-
tau 72-91	385	AEDVTAPLVDEGAPGKQAAA	-
tau 82-103	386	EGAPGKQAAAQPHTEIPEGTTA	-

Table 21. AT8 Peptides for reactivity by ELISA

Peptide	SEQ ID NO.	Peptide sequence (pX) denotes phosphorylated amino acid	Result
ptau 189-212	341	PKSGDRSGYS(pS)PGSPG(pT)PGSRsRT	-

tau 192-211	387	GDRSGYSSPGSPGTPGSRSR	-
ptau 192-209	343	GDRSGYSSPG(pS)PGTPGSR	-
ptau 192-212	344	GDRSGYSSPG(pS)PG(pT)PGSRRT	++
ptau 192-215	345	GDRSGYSSPG(pS)PGTPG(pS)RSRTPSL	-
ptau 192-217	346	GDRSGYSSPG(pS)PGTPGSR(pS)RTPSLPT	--
ptau 194-212	315	RSGYSSPG(pS)PG(pT)PGSRRT	++
tau 194-212*	316	RSGYSSPGSPGTPGSRRT	-
ptau 195-212	347	SGYSSPGSPG(pT)PGSRRT	-

Although CBTAU-7.1 was recovered using a peptide that contains the AT8 epitope (Table 21; 192-212; pS202, pT205), the phospho-residues contributing to binding for CBTAU-7.1 appeared to be promiscuous involving positions S202+T205, but also combinations of S198+S202, S198+T205, S199+T205 and possibly Y197+T205. Unphosphorylated peptides showed no reactivity to CBTAU-7.1. For CBTAU-18.1, the minimal epitope was found to consist of amino acids 198-217 and dependent on pS210 but not when T212, S214 or T217 were also phosphorylated. CBTAU-22.1 reactivity was found to be dependent on pS422, while antibody CBTAU-24.1 revealed strong binding to its corresponding unphosphorylated peptide and thus unaffected by phosphorylation.

CBTAU-27.1 and CBTAU-43.1 were recovered using an unphosphorylated peptide spanning amino acids 299-369. Interestingly, overlapping peptides within this region revealed similar binding requirements for both mAbs (i.e. CBTAU-27.1 and CBTAU-43.1 reacted to peptides spanning amino acids 299-318 and 309-328, respectively), suggesting that the epitope for both mAbs is within region 299-328 on tau441 (**Fig. 4a and c**).

CBTAU-28.1, 46.1, 47.1, 47.2, and 49.1 were recovered from human donor samples using a peptide spanning region 42-103 on tau441. Testing the reactivity of each mAb against a smaller overlapping set of peptides showed similar binding for CBTAU-47.1, 47.2, and 49.1 as CBTAU-28.1 (i.e., reactivity to a peptide spanning region 52-71), suggesting comparable binding requirements; however, CBTAU-46.1 bound to a region C-terminal to the aforementioned mAbs (i.e., 82-103); **Fig. 4b and d-g**

EXAMPLE 10

Alanine scanning of peptide epitopes

To further characterize the specificity and amino acid contribution to binding of each of the recover mAbs, their reactivity to tau peptides with each position replaced with Alanine was tested by ELISA. All experimental protocols were identical to Example 9. Antibody reactivity at 1 µg/mL was determined by ELISA and scored as no binding (-), weak (-/+), moderate (+), or strong (++) . (-) for average of two O.D.450 nm readings <0.3; (-/+) for >0.5 and <1.0; (+) for > 1.0 and <1.5; (++) for >1.5. Results for each antibody are shown in **Tables 22 – 29**.

Table 22. Alanine scanning results for CBTAU-7.1 and CBTAU-8.1

Region (Tau441)	SEQ ID NO	Peptide sequence (pX) denotes phosphorylated amino acid	Results 7.1	Results 8.1
ptau 187-212	334	EPPKSGDRSGYSSPG(pS)PG(pT)PGSRsRT	++	++
ptau 187-212 (A187)	388	APPKSGDRSGYSSPG(pS)PG(pT)PGSRsRT	+	++
ptau 187-212 (A188)	389	EAPKSGDRSGYSSPG(pS)PG(pT)PGSRsRT	+	++
ptau 187-212 (A189)	390	EPAKSGDRSGYSSPG(pS)PG(pT)PGSRsRT	+	++
ptau 187-212 (A190)	391	EPPASGDRSGYSSPG(pS)PG(pT)PGSRsRT	++	++
ptau 187-212 (A191)	392	EPPKAGDRSGYSSPG(pS)PG(pT)PGSRsRT	++	++
ptau 187-212 (A192)	393	EPPKSADRSYSSPG(pS)PG(pT)PGSRsRT	++	++
ptau 187-212 (A193)	394	EPPKSGARSGYSSPG(pS)PG(pT)PGSRsRT	++	++
ptau 187-212 (A194)	395	EPPKSGDASGYSSPG(pS)PG(pT)PGSRsRT	++	++

ptau 187-212 (A195)	396	EPPKSGDRAGYSSPG(pS)PG(pT)PGSRsRT	++	++
ptau 187-212 (A196)	397	EPPKSGDRSAYSSPG(pS)PG(pT)PGSRsRT	++	++
ptau 187-212 (A197)	398	EPPKSGDRSGASSPG(pS)PG(pT)PGSRsRT	++	++
ptau 187-212 (A198)	399	EPPKSGDRSGYASPG(pS)PG(pT)PGSRsRT	++	++
ptau 187-212 (A199)	400	EPPKSGDRSGYSAPG(pS)PG(pT)PGSRsRT	+	++
ptau 187-212 (A200)	401	EPPKSGDRSGYSSAG(pS)PG(pT)PGSRsRT	+	++
ptau 187-212 (A201)	402	EPPKSGDRSGYSSPA(pS)PG(pT)PGSRsRT	++	++
ptau 187-212 (A202)	403	EPPKSGDRSGYSSPGAPG(pT)PGSRsRT	+	++
ptau 187-212 (A203)	404	EPPKSGDRSGYSSPG(pS)AG(pT)PGSRsRT	++	++
ptau 187-212 (A204)	405	EPPKSGDRSGYSSPG(pS)PA(pT)PGSRsRT	-/+	+
ptau 187-212 (A205)	406	EPPKSGDRSGYSSPG(pS)PGAPSRsRT	-/+	-/+
ptau 187-212 (A206)	407	EPPKSGDRSGYSSPG(pS)PG(pT)AGSRsRT	-/+	-
ptau 187-212 (A207)	408	EPPKSGDRSGYSSPG(pS)PG(pT)PASRsRT	++	-/+
ptau 187-212 (A208)	409	EPPKSGDRSGYSSPG(pS)PG(pT)PGARsRT	++	++
ptau 187-212 (A209)	410	EPPKSGDRSGYSSPG(pS)PG(pT)PGSAsRT	++	-
ptau 187-212	411	EPPKSGDRSGYSSPG(pS)PG(pT)PGSRART	++	++

(A210)				
ptau 187-212 (A211)	412	EPPKSGDRSGYSSPG(pS)PG(pT)PGSRSAT	++	++
ptau 187-212 (A212)	413	EPPKSGDRSGYSSPG(pS)PG(pT)PGSRSRA	++	++

Table 23. Alanine scanning results for CBTAU-22.1

Region (Tau441)	SEQ ID NO	Peptide sequence (pX) denotes phosphorylated amino acid	Results
ptau 406-429	326	RHLSNVSSTG(pS)IDMVD(pS)PQLATLA	++
ptau 406-429 (A406)	414	AHLSNVSSTG(pS)IDMVD(pS)PQLATLA	++
ptau 406-429 (A407)	415	RALSNVSSTG(pS)IDMVD(pS)PQLATLA	++
ptau 406-429 (A408)	416	RHASNVSSTG(pS)IDMVD(pS)PQLATLA	++
ptau 406-429 (A409)	417	RHLANVSSTG(pS)IDMVD(pS)PQLATLA	++
ptau 406-429 (A410)	418	RHLSAVSSTG(pS)IDMVD(pS)PQLATLA	++
ptau 406-429 (A411)	419	RHLSNASSTG(pS)IDMVD(pS)PQLATLA	++
ptau 406-429 (A412)	420	RHLSNVASTG(pS)IDMVD(pS)PQLATLA	++
ptau 406-429 (A413)	421	RHLSNVSATG(pS)IDMVD(pS)PQLATLA	++
ptau 406-429 (A414)	422	RHLSNVSSAG(pS)IDMVD(pS)PQLATLA	++
ptau 406-429 (A415)	423	RHLSNVSSTA(pS)IDMVD(pS)PQLATLA	++
ptau 406-429 (A416)	424	RHLSNVSSTGAIDMVD(pS)PQLATLA	++
ptau 406-429 (A417)	425	RHLSNVSSTG(pS)ADMVD(pS)PQLATLA	++
ptau 406-429 (A418)	426	RHLSNVSSTG(pS)IAMVD(pS)PQLATLA	++
ptau 406-429 (A419)	427	RHLSNVSSTG(pS)IDAVD(pS)PQLATLA	++
ptau 406-429 (A420)	428	RHLSNVSSTG(pS)IDMAD(pS)PQLATLA	++
ptau 406-429 (A421)	429	RHLSNVSSTG(pS)IDMVA(pS)PQLATLA	-/+
ptau 406-429 (A422)	430	RHLSNVSSTG(pS)IDMVDAPQLATLA	-
ptau 406-429(A423)	431	RHLSNVSSTG(pS)IDMVD(pS)AQLATLA	++

ptau 406-429 (A424)	432	RHLSNVSSTG(pS)IDMVD(pS)PALATLA	++
ptau 406-429 (A425)	433	RHLSNVSSTG(pS)IDMVD(pS)PQAATLA	++
ptau 406-429 (A427)	434	RHLSNVSSTG(pS)IDMVD(pS)PQLAALA	++
ptau 406-429 (A428)	435	RHLSNVSSTG(pS)IDMVD(pS)PQLATAA	++

Table 24. CBTAU-24.1 Alanine Scan Results

Region (Tau441)	SEQ ID NO	Peptide sequence (pX) denotes phosphorylated amino acid	Results
ptau 221-245	328	REPKKVAVVR(pT)PPKSPS(pS)AKSRLQT	++
ptau 221-245 (A222)	436	RAPKKVAVVR(pT)PPKSPS(pS)AKSRLQT	++
ptau 221-245 (A223)	437	REAKKVAVVR(pT)PPKSPS(pS)AKSRLQT	++
ptau 221-245 (A224)	438	REPAKVAVVR(pT)PPKSPS(pS)AKSRLQT	++
ptau 221-245 (A225)	439	REPKAVAVVR(pT)PPKSPS(pS)AKSRLQT	++
ptau 221-245 (A226)	440	REPKKAAVVR(pT)PPKSPS(pS)AKSRLQT	++
ptau 221-245 (A228)	441	REPKKVAAVR(pT)PPKSPS(pS)AKSRLQT	++
ptau 221-245 (A229)	442	REPKKVAVAR(pT)PPKSPS(pS)AKSRLQT	++
ptau 221-245 (A230)	443	REPKKVAVVA(pT)PPKSPS(pS)AKSRLQT	++
ptau 221-245 (A231)	444	REPKKVAVVRAPPKSPS(pS)AKSRLQT	++
ptau 221-245 (A232)	445	REPKKVAVVR(pT)APKSPS(pS)AKSRLQT	++
ptau 221-245 (A233)	446	REPKKVAVVR(pT)PAKSPS(pS)AKSRLQT	++
ptau 221-245 (A234)	447	REPKKVAVVR(pT)PPASPS(pS)AKSRLQT	++
ptau 221-245 (A235)	448	REPKKVAVVR(pT)PPKAPS(pS)AKSRLQT	++
ptau 221-245 (A236)	449	REPKKVAVVR(pT)PPKSAS(pS)AKSRLQT	-
ptau 221-245 (A237)	450	REPKKVAVVR(pT)PPKSPA(pS)AKSRLQT	++
ptau 221-245 (A238)	451	REPKKVAVVR(pT)PPKSPSAKSRLQT	++
ptau 221-245 (A240)	452	REPKKVAVVR(pT)PPKSPS(pS)AASRLQT	++
ptau 221-245 (A241)	453	REPKKVAVVR(pT)PPKSPS(pS)AKARLQT	++
ptau 221-245 (A242)	454	REPKKVAVVR(pT)PPKSPS(pS)AKSALQT	++
ptau 221-245 (A243)	455	REPKKVAVVR(pT)PPKSPS(pS)AKSRAQT	++

ptau 221-245 (A244)	456	REPKKVAVVR(pT)PPKSPS(pS)AKSRLAT	++
ptau 221-245 (A245)	457	REPKKVAVVR(pT)PPKSPS(pS)AKSRLQA	++

Table 25. CBTAU-27.1 Alanine Scan Results

Region (Tau441)	SEQ ID NO:	Peptide sequence	Results
tau 299-323	458	HVPGGGSVQIVYKPVDLSKVTSKCG	++
tau 299-323(A299)	459	AVPGGGSVQIVYKPVDLSKVTSKCG	++
tau 299-323(A300)	460	HAPGGGSVQIVYKPVDLSKVTSKCG	++
tau 299-323(A301)	461	HVAGGGSVQIVYKPVDLSKVTSKCG	++
tau 299-323(A302)	462	HVPAGGSVQIVYKPVDLSKVTSKCG	++
tau 299-323(A303)	463	HVPGAGSVQIVYKPVDLSKVTSKCG	++
tau 299-323(A304)	464	HVPGGASVQIVYKPVDLSKVTSKCG	++
tau 299-323(A305)	465	HVPGGGAIVQIVYKPVDLSKVTSKCG	++
tau 299-323(A306)	466	HVPGGGSAQIVYKPVDLSKVTSKCG	++
tau 299-323(A307)	467	HVPGGGSVAIVYKPVDLSKVTSKCG	++
tau 299-323(A308)	468	HVPGGGSVQAVYKPVDLSKVTSKCG	++
tau 299-323(A309)	469	HVPGGGSVQIAYKPVDLSKVTSKCG	++
tau 299-323(A310)	470	HVPGGGSVQIVAKPVDLSKVTSKCG	++
tau 299-323(A311)	471	HVPGGGSVQIVYAPVDLSKVTSKCG	++
tau 299-323(A312)	472	HVPGGGSVQIVYKAVDLSKVTSKCG	++
tau 299-323(A313)	473	HVPGGGSVQIVYKPADLSKVTSKCG	++
tau 299-323(A314)	474	HVPGGGSVQIVYKPVALSKVTSKCG	-/+
tau 299-323(A315)	475	HVPGGGSVQIVYKPDASKVTSKCG	-
tau 299-323(A316)	476	HVPGGGSVQIVYKPD LAKVTSKCG	++
tau 299-323(A317)	477	HVPGGGSVQIVYKPDLSAVTSKCG	-
tau 299-323(A318)	478	HVPGGGSVQIVYKPDLSKATSKCG	++
tau 299-323(A319)	479	HVPGGGSVQIVYKPDLSKVASKCG	++

tau 299-323(A320)	480	HVPGGGSVQIVYKPVDLSKVTAKCG	++
tau 299-323(A321)	481	HVPGGGSVQIVYKPVDLSKVTSAACG	++
tau 299-323(A322)	482	HVPGGGSVQIVYKPVDLSKVTSKAG	++
tau 299-323(A323)	483	HVPGGGSVQIVYKPVDLSKVTSKCA	++

Table 26. CBTAU-28.1 Alanine Scan Results

Region (Tau441)	SEQ ID NO	Peptide sequence	Results
tau 52-71	382	TEDGSEEPGSETSDAKSTPT	++
tau 52-71 (A52)	484	AEDGSEEPGSETSDAKSTPT	++
tau 52-71 (A53)	485	TADGSEEPGSETSDAKSTPT	++
tau 52-71 (A54)	486	TEAGSEEPGSETSDAKSTPT	++
tau 52-71 (A55)	487	TEDASEEPGSETSDAKSTPT	++
tau 52-71 (A56)	488	TEDGAEEPGSETSDAKSTPT	++
tau 52-71 (A57)	489	TEDGSAEPGSETSDAKSTPT	++
tau 52-71 (A58)	490	TEDGSEAPGSETSDAKSTPT	++
tau 52-71 (A59)	491	TEDGSEEAGSETSDAKSTPT	-/+
tau 52-71 (A60)	492	TEDGSEEPASETSDAKSTPT	++
tau 52-71 (A61)	493	TEDGSEEPGAETSDAKSTPT	++
tau 52-71 (A62)	494	TEDGSEEPGSATSDAKSTPT	-
tau 52-71 (A63)	495	TEDGSEEPGSEASDAKSTPT	-/+
tau 52-71 (A64)	496	TEDGSEEPGSETADAKSTPT	++
tau 52-71 (A65)	497	TEDGSEEPGSETSAAKSTPT	-
tau 52-71 (A67)	498	TEDGSEEPGSETSDAASTPT	-
tau 52-71 (A68)	499	TEDGSEEPGSETSDAKATPT	++
tau 52-71 (A69)	500	TEDGSEEPGSETSDAKSAPT	++
tau 52-71 (A70)	501	TEDGSEEPGSETSDAKSTAT	++
tau 52-71 (A71)	502	TEDGSEEPGSETSDAKSTPA	++

Table 27. CBTAU-43.1 Alanine Scan Results

Region (Tau441)	SEQ ID NO:	Peptide sequence	Results
tau 299-323	458	HVPGGGSVQIVYKPVDLSKVTSKCG	++
tau 299-323(A299)	459	AVPGGGSVQIVYKPVDLSKVTSKCG	++
tau 299-323(A300)	460	HAPGGGSVQIVYKPVDLSKVTSKCG	++
tau 299-323(A301)	461	HVAGGGSVQIVYKPVDLSKVTSKCG	++
tau 299-323(A302)	462	HVPAGGSVQIVYKPVDLSKVTSKCG	++
tau 299-323(A303)	463	HVPGAGSVQIVYKPVDLSKVTSKCG	++
tau 299-323(A304)	464	HVPGGASVQIVYKPVDLSKVTSKCG	++
tau 299-323(A305)	465	HVPGGGAVQIVYKPVDLSKVTSKCG	++
tau 299-323(A306)	466	HVPGGGSAQIVYKPVDLSKVTSKCG	++
tau 299-323(A307)	467	HVPGGGSVAIVYKPVDLSKVTSKCG	++
tau 299-323(A308)	468	HVPGGGSVQAVYKPVDLSKVTSKCG	++
tau 299-323(A309)	469	HVPGGGSVQIAYKPVDLSKVTSKCG	++
tau 299-323(A310)	470	HVPGGGSVQIVAKPVDLSKVTSKCG	++
tau 299-323(A311)	471	HVPGGGSVQIVYAPVDLSKVTSKCG	++
tau 299-323(A312)	472	HVPGGGSVQIVYKAVDLSKVTSKCG	-/+
tau 299-323(A313)	473	HVPGGGSVQIVYKPADLSKVTSKCG	++
tau 299-323(A314)	474	HVPGGGSVQIVYKPVALSKVTSKCG	++
tau 299-323(A315)	475	HVPGGGSVQIVYKPVDASKVTSKCG	-
tau 299-323(A316)	476	HVPGGGSVQIVYKPVDLAKVTSKCG	++
tau 299-323(A317)	477	HVPGGGSVQIVYKPVDLSAVTSKCG	-
tau 299-323(A318)	478	HVPGGGSVQIVYKPVDLSKATSKCG	++
tau 299-323(A319)	479	HVPGGGSVQIVYKPVDLSKVASKCG	++
tau 299-323(A320)	480	HVPGGGSVQIVYKPVDLSKVTAACKG	++
tau 299-323(A321)	481	HVPGGGSVQIVYKPVDLSKVTSACKG	++
tau 299-323(A322)	482	HVPGGGSVQIVYKPVDLSKVTSKAG	++
tau 299-323(A323)	483	HVPGGGSVQIVYKPVDLSKVTSKCA	++

Table 28. CBTAU-47.1 and 47.2 Alanine Scan Results

Region (Tau441)	SEQ ID NO	Peptide sequence	Results CBTAU-47.1	Results CBTAU-47.2
tau 52-71	382	TEDGSEEPGSETSDAKSTPT	++	++
tau 52-71 (A52)	484	AEDGSEEPGSETSDAKSTPT	++	++
tau 52-71 (A53)	485	TADGSEEPGSETSDAKSTPT	++	++
tau 52-71 (A54)	486	TEAGSEEPGSETSDAKSTPT	++	++
tau 52-71 (A55)	487	TEDASEEPGSETSDAKSTPT	++	++
tau 52-71 (A56)	488	TEDGAEPPGSETSDAKSTPT	++	++
tau 52-71 (A57)	489	TEDGSAEPGSETSDAKSTPT	++	++
tau 52-71 (A58)	490	TEDGSEAPGSETSDAKSTPT	++	++
tau 52-71 (A59)	491	TEDGSEEAGSETSDAKSTPT	-	-
tau 52-71 (A60)	492	TEDGSEEPASETSDAKSTPT	++	++
tau 52-71 (A61)	493	TEDGSEEPGAETSDAKSTPT	-/+	++
tau 52-71 (A62)	494	TEDGSEEPGSATSDAKSTPT	-	-
tau 52-71 (A63)	495	TEDGSEEPGSEASDAKSTPT	-	-
tau 52-71 (A64)	496	TEDGSEEPGSETADAKSTPT	++	++
tau 52-71 (A65)	497	TEDGSEEPGSETSAAKSTPT	-	-
tau 52-71 (A67)	498	TEDGSEEPGSETSDAASTPT	-	-
tau 52-71 (A68)	499	TEDGSEEPGSETSDAKATPT	++	++
tau 52-71 (A69)	500	TEDGSEEPGSETSDAKSAPT	++	++
tau 52-71 (A70)	501	TEDGSEEPGSETSDAKSTAT	++	++
tau 52-71 (A71)	502	TEDGSEEPGSETSDAKSTPA	++	++

Table 29. CBTAU-49.1 Alanine Scan Results

Region (Tau441)	SEQ ID NO	Peptide sequence	Results
tau 52-71	382	TEDGSEEPGSETSDAKSTPT	++
tau 52-71 (A52)	484	AEDGSEEPGSETSDAKSTPT	++

tau 52-71 (A53)	485	TADGSEEPGSETSDAKSTPT	++
tau 52-71 (A54)	486	TEAGSEEPGSETSDAKSTPT	++
tau 52-71 (A55)	487	TEDASEEPGSETSDAKSTPT	++
tau 52-71 (A56)	488	TEDGAEPEGSETSDAKSTPT	++
tau 52-71 (A57)	489	TEDGSAEPGSETSDAKSTPT	++
tau 52-71 (A58)	490	TEDGSEAPGSETSDAKSTPT	++
tau 52-71 (A59)	491	TEDGSEEAGSETSDAKSTPT	-
tau 52-71 (A60)	492	TEDGSEEPASETSDAKSTPT	++
tau 52-71 (A61)	493	TEDGSEEPGAETSDAKSTPT	-
tau 52-71 (A62)	494	TEDGSEEPGSATSDAKSTPT	-
tau 52-71 (A63)	495	TEDGSEEPGSEASDAKSTPT	+
tau 52-71 (A64)	496	TEDGSEEPGSETADAKSTPT	+
tau 52-71 (A65)	497	TEDGSEEPGSETSAAKSTPT	-
tau 52-71 (A67)	498	TEDGSEEPGSETSDAASTPT	-
tau 52-71 (A68)	499	TEDGSEEPGSETSDAKATPT	+
tau 52-71 (A69)	500	TEDGSEEPGSETSDAKSAPT	+
tau 52-71 (A70)	501	TEDGSEEPGSETSDAKSTAT	+
tau 52-71 (A71)	502	TEDGSEEPGSETSDAKSTPA	+

Although CBTAU-7.1 and CBTAU-8.1 were recovered using a tau phosphopeptide containing the AT8 epitope (i.e., pS202, pT205), both mAbs exhibited different epitope requirements according to the alanine scan results (**Table 22**). In addition to S202 and T205, substitutions at positions G204 and P206 resulted in reduced binding for CBTAU-7.1. In contrast, alanine substitutions at positions G204, T205, P206, and R209 reduced the reactivity of CBTAU-8.1 to the peptide, yet the S202A substitution had no effect. Like AT8, both mAbs are phospho-dependent, but require additional (non-phosphorylated residues) for binding. The alanine scan results for CBTAU-22.1 showed a dependency on phosphorylation at S422 (**Table 23**), as substitution at this position completely inhibited binding. Substitution at D421 resulted in a reduction but not a complete inhibition in binding. Finally, alanine scan results for CBTAU-24.1 showed P236 to be the only critical residue for binding (**Table 24**).

To map the critical contact residues for CBTAU-27.1 and 43.1, alanine scanning was also conducted within the region 299-323 of tau (**Table 25** and **Table 27**, respectively). The critical contact residues for CBTAU-27.1 binding were shown to be D314, L315, and K317. The results suggest that residues D314 and K317 may form salt bridge interactions between the epitope and CDR residues on the mAb. While CBTAU-43.1 was recovered using the cognate peptide for CBAU-27.1, the critical residues according to the alanine scan were different. In addition to L315 and K317, the proline at position 312 was shown to be an important contact for CBTAU-43.1 binding. Lastly, alanine scanning was also conducted for CBTAU-28.1 as well as to CBTAU-47.1, 47.1, and 49.1 (**Table 26, 28, 29**). As shown in Example 9, CBTAU mAbs 47.1, 47.2, 49.1 mapped to the same peptide region as CBTAU-28.1 (i.e., 52-71). Interestingly, all mAbs shared identical binding requirements as to CBTAU-28.1. The critical contact residues were shown to be P59, S61, E62, T63, D65, and K67. Several of these residues were found to be charged, implicating important salt bridge interactions between the epitope and the mAbs.

EXAMPLE 11

Immunohistochemistry

Tau pathology is believed to initiate within the entorhinal cortex (EC) and spread along connected neuronal pathways in the hippocampus before progressing into the cortex. To determine the reactivity of the recovered IgGs to pathogenic deposits of tau along these neuronal pathways, hippocampal tissues were obtained from an 82-year-old, non-diseased (non-AD; Abcam, Cat No. ab4305) male and an 88-year-old Alzheimer's disease (AD; Abcam, Cat. No. ab4583) male (Abcam). Cortical tissues were obtained from a 71-year-old non-diseased (non-AD) and 71-year-old Alzheimer's disease (AD) individual (Banner Sun Health). In addition to AD, there are many neurological disorders that are characterized by tau pathology, also known as tauopathies. To extent our findings, we tested the recovered mAbs in tissues obtained from progressive supranuclear palsy (PSP) and non-progressive supranuclear palsy (non-PSP) frontal lobes obtained from a 73-year-old male and 81-year-old female, respectively (Biochain). Brain tissues were de-paraffinized and rehydrated by washing twice for 10 min in xylene (VWR International), followed by washing twice for 3 min in 100% ethanol, twice for 3 min in 95% ethanol, twice for 3 min in 70% ethanol, and

once for 30 sec in distilled H₂O using Tissue-Tek® Slide Staining Set (VWR International). Tissue sections underwent heat mediated antigen retrieval using citrate buffer (10 mM citric acid, pH.6.0) to expose antigenic sites. Sections were then incubated with blocking buffer [10% normal goat serum (Jackson ImmunoResearch, Inc.), 1% BSA and 0.3% Triton-X100 in PBS)] at RT for 1 hr.

5 Excess water was removed and tissue sections were circled with an ImmEdge Hydrophobic Barrier Pen (Vector Labs). A humidified chamber was prepared by covering the bottom of a staining tray with H₂O, and sections were then washed with PBS 3-times for 5 min by aspiration. Endogenous peroxidase activity was quenched in 10% H₂O₂ for 30 min at RT. Following quenching, slides were washed with PBS 3-times for 5 min by aspiration. Slides were then blocked for 1 hr at RT with a
10 solution of 10% normal goat serum, 0.3% TritonX-100, 1% BSA in 1X PBS. Primary antibodies were labeled with biotin using the Zenon Human IgG Labeling Kit (Life Technologies) per manufacturer's instructions. As a negative control, a human anti-RSV specific antibody was used. An Fc-region human chimerized version of AT8 IgG was used as a positive control. After labeling, primary antibodies were diluted separately in blocking buffer at concentrations of 5µg/ml and 20
15 µg/ml. For peptide competition experiments, 13.3 µM of cognate peptide (i.e., peptide used to recover the mAb in sorting experiments) was pre-incubated with the primary antibody for 30 min at RT prior to incubation with tissue sections. Tissue sections were incubated at RT for two hr with 100 µl of diluted biotin labeled primary antibody or peptide competed antibody. After antibody was removed by aspiration, a second fixation of the tissue section was performed in 4% formaldehyde in
20 PBS and incubated for 15 min at RT. The section was washed with PBS 3-times for 5 min by aspiration. Sections were then incubated for 30 min with streptavidin substrate Vectastain ABC Reagent (Vector Labs) before washing with PBS. Tissues were then developed with DAB substrate (Vector Labs) in the presence of nickel. Sections were then washed 2-times with ddH₂O and allowed to completely dry at RT before mounting with 50 µl of VectaMount Permanent Mounting Medium
25 (Vector Labs). Finally, tissue sections were counterstained with hematoxylin (Vector Labs). Representative images were acquired with Olympus BX-41 upright microscope using MetaMorph software.

Results from the immunohistochemistry are shown in **Figures 5a-d**. CBTAU-7.1 and CBTAU-8.1 showed positive immunoreactivity on AD brain tissues specifically, and not to healthy
30 brain tissues, which suggests binding to pathogenic tau deposits present in diseased brain tissues. These antibodies recognize AT8 positive tau tangles and neurophil threads in subregions of the

hippocampus (**Fig. 5a**; entorhinal cortex) and cerebral cortex (**Fig. 5b**). Furthermore, the positive immunoreactivity was consistently found across multiple experiments in the neuronal cytoplasm and processes. In addition, CBTAU-18.1, 22.1, and 24.1 were also tested against hippocampal and cortical tissue sections (**Fig. 5a-b**). Similar to CBTAU-7.1 and CBTAU-8.1, all mAbs reacted specifically to tau on AD tissue sections but not to tau on non-AD tissue sections. Of interest, CBTAU-24.1, which is not specific to phosphorylated tau, reacts specifically to diseased tau on AD tissue sections but not to tau on non-AD sections. Finally, CBTAU-16.1 and CBTAU-20.1 show reactivity to tau on both non-AD and AD tissue sections.

In addition, CBTAU-7.1, 8.1, 16.1, 18.1, 20.1, 22.1, and 24.1 were tested on cortical tissue sections corresponding to progressive supranuclear palsy (**Fig. 5c**). Unlike AT8, CBTAU-7.1 and CBTAU-8.1 failed to detect tau tangles in the human PSP brain, suggesting that the epitope for both mAbs is not present on PSP. CBTAU-16.1 and CBTAU-20.1 showed positive immunoreactivity to tau on non-PSP and PSP cortical brain sections, suggesting binding to both normal tau and pathogenic forms of tau. In non-AD brain sections, these antibodies showed positive immunostaining of tau in neuronal cytoplasm and processes (**Fig. 5a and 5b**), yet both mAbs detected tangles and neurofibrillary threads in AD brain sections similar to AT8. Similar immunoreactivity as AT8 to tau tangles was also detected in PSP brain tissue sections, suggesting that both CBTAU-16.1 and CBTAU-20.1 recognize common pathogenic tau forms in other non-AD tauopathies. Furthermore, CBTAU-22.1 and CBTAU-24.1 showed immunoreactivity exclusively in AD brain tissues, with positive immunoreactivity to tangles and neurofibrillary threads. CBTAU-18.1 showed weak immunoreactivity in non-AD brain tissues, yet reacted stronger to AD tissue samples. CBTAU-18.1, CBTAU-22.1 and CBTAU-24.1 were also positive for tau tangles in PSP brain tissue sections (**Fig. 5c**).

CBTAU-27.1 and CBTAU-28.1 showed selective immunostaining in non-AD tissue sections with diffuse immunostaining in neuronal cytoplasm and processes. Interestingly, both antibodies failed to show immunoreactivity in AD tissue sections (both hippocampal and cortical), defining a novel epitope that is lost during disease progression. Unlike the majority of the human anti-tau mAbs we identified, CBTAU-27.1 and CBTAU-28.1 were recovered by screening donor samples using an unphosphorylated peptide set spanning the entire region of human tau441. These antibodies do not require phosphorylation for binding (**Fig. 1**) and, as shown in **Fig. 2**, do not react to PHF by ELISA. Therefore, the diffuse immunostaining pattern observed for these two mAbs was expected.

In addition, CBTAU -43.1, which was originally recovered using the CBTAU-27.1 cognate peptide, was tested against cortical tissue sections. CBTAU-43.1 reacted similar to CBTAU-27.1, staining tau on non-AD but not tau on AD tissue sections. Likewise, CBTAU-46.1, 47.2 (only one variant tested), and 49.1, which were recovered using the CBTAU-28.1 cognate peptide, reacted specifically to tau on non-AD but not AD tissue sections (**Fig. 5d**). It is interesting to note that these mAbs all share common heavy and light chain germlines (i.e., VH5-51 and VK4-1), bind to the same regions on tau, and, as shown in **Fig. 5d**, share similar immunohistochemical properties.

The immunohistochemistry results presented here for CBTAU-7.1, 8.1, 18.1, 22.1, 24.1, 27.1, and 28.1 have been confirmed on multiple regions of the brain and tissue samples corresponding to several non-AD and AD individuals. Immunoreactivity of CBTAU mAbs 43.1, 46.1, 47.2, and 49.1 has been confirmed once using the same tissue sample and has not yet been confirmed on samples corresponding to other AD and non-AD individuals.

EXAMPLE 12

Desphosphorylation IHC

Given that the results of the IHC for CBTAU-28.1 showed immunoreactivity to tau on non-AD tissue sections but not to tau on AD tissue sections, we hypothesized that the loss of this epitope during disease progression was a result modification(s) (i.e., phosphorylation). To test this hypothesis, human brain tissue sections were dephosphorylated prior to assessing immunoreactivity of CBTAU-28.1. Paraffin embedded human brain tissue sections (Abcam, cat#:ab4305, 54 old male, no clinical symptom vs. Abcam, cat#:ab4583, 93 yr old Hispanic female, Alzheimer disease) were deparaffinized and rehydrated by washing twice for 10 min in xylene (VWR International), followed by washing twice for 3 minutes in 100% ethanol, twice for 3 min in 95% ethanol, twice for 3 min in 70% ethanol, and once for 30 sec in distilled H₂O using Tissue-Tek® Slide Staining Set (VWR International). To minimize non-specific antibody binding, tissues were never allowed to dry during washes. Tissue sections underwent heat mediated antigen retrieval using citrate buffer (citric acid, pH.6.0) to expose antigenic sites. Excess water was removed and tissue sections were circled with an ImmEdge Hydrophobic Barrier Pen (Vector Labs). A humidified chamber was prepared by covering the bottom of a staining tray with H₂O, and sections were then washed with PBS 3-times for 5 min by aspiration. Endogenous peroxidase activity was quenched in H₂O₂ for 15 minutes at

RT. Following quenching, slides were washed with PBS 3-times for 5 min by aspiration. Sections were subsequently treated with 130 units/mL of calf intestinal alkaline phosphatase (CIAP) for 2.5 hrs at 32 deg. Slides were then blocked for 1 hr at RT with a solution of 10% normal goat serum, 0.3% TritonX-100, 1% BSA in 1X PBS. Murinized CBTAU-28.1 (Fc region murinized), and
5 control mAbs AT8 and isotype control (anti-RSV mAb 4.1) were incubated overnight on hippocampal sections at a final concentration of 1 ug/mL. Sections were washed and incubated with anti-mouse, Fcy fragment-specific antibody for 2 hrs at room temperature. Samples were developed with peroxidase substrate solution DAB in the presence of Nickel. Samples were counterstained with hematoxylin. (Vector Labs). Representative images were acquired with Olympus BX-41
10 upright microscope using MetaMorph software.

Results are shown in **Figure 6a and b**. As expected, CBTAU-28.1 reacts to tau present in the non-AD hippocampal tissue sections but does not react to tau in the AD tissue sections. In contrast, control mAb, AT8, does not react to tau in non-AD sections, but clearly reacts to pathogenic tau deposits present in the AD sections (**Fig. 6a**). However, pretreatment of AD tissue
15 sections with phosphatase restores reactivity of CBTAU-28.1, allowing it to stain pathogenic tau deposits present in these sections. As expected, the reactivity of AT8 was reduced with pretreatment of the AD tissue sections with phosphatase (**Fig. 6b**).

EXAMPLE 13

20 Dephosphorylation ELISA

To confirm the results of Example 12, dephosphorylation of paired helical filaments was tested for reactivity to CBTAU-28.1 by ELISA. Half-area 96-well binding plates (Costar) were coated with 50 µl of antigen in TBS (2 µg/ml bovine action affinipure goat anti-human F(ab)₂, and 1 µg/ml of affinity purified paired helical filaments, iPHF, pretreated with and without calf intestinal
25 phosphatase, CIP). Phosphatase treated iPHF was prepared as follows. iPHF samples were resuspended in 1XNEB buffer 4 (50 mM potassium acetate, 20 mM Tris-acetate, 10 mM magnesium acetate, and 1 mM DTT) at a final concentration of 0.05 µg/ml. One unit of CIP per µg of iPHF was added (CIP, NEB Cat. No. M0290S). iPHF samples were incubated with CIP for 90 min at 37°C prior to coating on ELISA binding plates. Following antigen binding overnight, the
30 plates were washed with TBS-T and subsequently blocked with 150 µl of TBS plus 2.5% BSA for 2

hr at RT. Purified control and anti-tau IgG, CBTAU-28.1), were titrated in 5-fold dilutions starting at 25 µg/ml in TBS/0.25% BSA, and IgGs and incubated for 1.5 hrs. Plates were washed 4-times with TBS-T and secondary antibody (anti-human Fab HRP, Jackson ImmunoResearch, Cat. No. 109-036-097) was added and incubated at RT for 45 min. Following incubation, plates were washed 4-
 5 times in TBS-T and developed with SureBlue Reserve TMB Microwell Peroxidase Substrate (KPL) for approximately 2 min. The reaction was immediately halted by the addition of TMB Stop Solution (KPL) and the absorbance at 450 nm was measured using an ELISA plate reader. Each experimental point was performed in triplicates.

Results are shown in **Figure 7**. As previously shown in Example 7, CBTAU-28.1 reacts
 10 poorly to iPHF by ELISA in contrast to AT8. However, dephosphorylation of iPHF with CIP restores the reactivity of CBTAU-28.1 to the filamentous sample. As expected, the reactivity of the phospho-tau control mAb, AT8, is abolished after desphosphorylation of iPHF with CIP.

EXAMPLE 14

15 **Reactivity of CBTAU-27.1, 28.1, 43.1, 47.1, 47.2 and 49.1 to phosphopeptides**

The immunohistochemical results for CBTAU-27.1 (and CBTAU-43.1) and CBTAU-28.1 (and CBTAU-46.1, 47.2, 49.1) suggest that these mAbs react with an epitope on tau that is present in normal, non-AD, tissue sections but is lost or masked during the disease setting (**Figure 5**). We hypothesized that this was a result of a phosphorylation event within the region that results in
 20 masking of the epitope(s). The experiments highlighted in Examples 12 and 13 showed that this was indeed true for 28.1. Therefore, we wanted to specifically identify the site(s) that could potentially be targeted for phosphorylation and account for loss of reactivity for CBTAU-28.1. Because 47.1, 47.2, and 49.1 bound to the same region on tau as CBTAU-28.1 (i.e., 52-71), we decided to test these mAbs as well in these experiments. In addition, we also carried out the same exercise for
 25 CBTAU-43.1 and CBTAU-27.1 as they behave similarly to CBTAU-28.1 by IHC.

Singly and dually phosphorylated tau peptides were designed to cover all potential phosphorylation sites with regions 52-71 and 299-323 (CBTAU-28.1 and CBTAU-27.1 binding region, respectively). CBTAU-27.1 and CBTAU-43.1 mAbs were tested against the peptides listed on **Table 30 and 32**. 96-well Streptavidin coated ELISA plates (Pierce) were coated with
 30 phosphorylated tau peptides as detailed in Example 9. Purified anti-tau IgGs were diluted to 5 µg/ml

in TBS containing 0.25% BSA, and titrated 5-fold. Antibody control and secondary antibodies were used as detailed in Example 9. Antibody reactivity at 1 µg/mL was determined by ELISA and scored as no binding (-), weak (-/+), moderate (+), or strong (++). (-) for average of two O.D.450 nm readings <0.3; (-/+) for >0.5 and <1.0; (+) for > 1.0 and <1.5; (++) for >1.5. Results for each antibody are shown in **Tables 30 – 34 and Figure 8**.

Table 30. CBTAU-27.1: Peptides for reactivity by ELISA

Peptide	SEQ ID NO.		Result
tau 299-369	331	HVPGGGSVQIVYKPDLSKVTSSKCGSLGNI HHKPGGGQVEVKSEKLDKDRVQSKIGSL DNITHVPGGGNK	++
tau 299-323	458	HVPGGGSVQIVYKPDLSKVTSSKCG	++
ptau 299-323 p305	503	HVPGGG(pS)VQIVYKPDLSKVTSSKCG	++
ptau 299-323 p310	504	HVPGGGSVQIV(pY)KPDLSKVTSSKCG	++
ptau 299-323 p316	505	HVPGGGSVQIVYKPD(pS)KVTSSKCG	-
ptau 299-323 p319	506	HVPGGGSVQIVYKPDLSKV(pT)SKCG	++
ptau 299-323 p320	507	HVPGGGSVQIVYKPDLSKVT(pS)KCG	++
ptau 299-323 p305, 310	508	HVPGGG(pS)VQIV(pY)KPDLSKVTSSKCG	++
ptau 299-323 p305, 316	509	HVPGGG(pS)VQIVYKPD(pS)KVTSSKCG	-
ptau 299-323 p305, 320	510	HVPGGG(pS)VQIVYKPDLSKV(pT)SKCG	++
ptau 299-323 p305, 321	511	HVPGGG(pS)VQIVYKPDLSKVT(pS)KCG	++
ptau 299-323 p310, 316	512	HVPGGGSVQIV(pY)KPD(pS)KVTSSKCG	-
ptau 299-323 p310, 320	513	HVPGGGSVQIV(pY)KPDLSKV(pT)SKCG	++
ptau 299-323 p310, 321	514	HVPGGGSVQIV(pY)KPDLSKVT(pS)KCG	++
ptau 299-323 p316, 320	515	HVPGGGSVQIVYKPD(pS)KV(pT)SKCG	-
ptau 299-323 p316, 321	516	HVPGGGSVQIVYKPD(pS)KVT(pS)KCG	-
ptau 299-323 p320, 321	517	HVPGGGSVQIVYKPDLSKV(pT)(pS)KCG	-/+

Table 31. CBTAU-28.1: Peptides for reactivity by ELISA

Peptide	SEQ ID NO.		Result
tau 42-103	325	GLKESPLQTPTEGSEEPGSETSDAKSTPTA EDVTAPLVDEGAPGKQAAAQPHT EIPEGTTA	++
tau 48-71	518	LQTPTEGSEEPGSETSDAKSTPT	++
ptau 48-71 (p71)	519	LQTPTEGSEEPGSETSDAKSTP(pT)	++
ptau 48-71 (p63)	520	LQTPTEGSEEPGSE(pT)SDAKSTPT	-
ptau 48-71 (p61)	521	LQTPTEGSEEPG(pS)ETSDAKSTPT	-
ptau 48-71 (p56)	522	LQTPTEG(pS)EEPGSETSDAKSTPT	++
ptau 48-71 (p52)	523	LQTP(pT)EDGSEEPGSETSDAKSTPT	++
ptau 48-71 (p68)	524	LQTPTEGSEEPGSETSDAK(pS)TPT	++
ptau 48-71 (p69)	525	LQTPTEGSEEPGSETSDAKS(pT)PT	++
ptau 48-71 (p64)	526	LQTPTEGSEEPGSET(pS)DAKSTPT	++
ptau 48-71 (p61,p64)	527	LQTPTEGSEEPG(pS)ET(pS)DAKSTPT	-
ptau 48-71 (p61,p63)	528	LQTPTEGSEEPG(pS)E(pT)SDAKSTPT	-
ptau 48-71 (p63,p64)	529	LQTPTEGSEEPGSE(pT)(pS)DAKSTPT	-

Table 32. CBTAU-43.1: Peptides for reactivity by ELISA

Peptide	SEQ ID NO.		Result
tau 299-369	331	HVPGGGSVQIVYKPVDSLKVTSKCGSLGNI HHKPGGGQVEVKSEKLDFKDRVQSKIGSL DNITHVPGGGNK	++
tau 299-323	458	HVPGGGSVQIVYKPVDSLKVTSKCG	++
ptau 299-323 p305	503	HVPGGG(pS)VQIVYKPVDSLKVTSKCG	++
ptau 299-323 p310	504	HVPGGGSVQIV(pY)KPVDSLKVTSKCG	++
ptau 299-323 p316	505	HVPGGGSVQIVYKPVDL(pS)KVTSKCG	-

ptau 299-323 p319	506	HVPGGGSVQIVYKPVDLSKV(pT)SKCG	++
ptau 299-323 p320	507	HVPGGGSVQIVYKPVDLSKVT(pS)KCG	++
ptau 299-323 p305, 310	508	HVPGGG(pS)VQIV(pY)KPVDLSKVTSKCG	++
ptau 299-323 p305, 316	509	HVPGGG(pS)VQIVYKPVDL(pS)KVTSKCG	-
ptau 299-323 p305, 320	510	HVPGGG(pS)VQIVYKPVDLSKV(pT)SKCG	++
ptau 299-323 p305, 321	511	HVPGGG(pS)VQIVYKPVDLSKVT(pS)KCG	++
ptau 299-323 p310, 316	512	HVPGGGSVQIV(pY)KPVDL(pS)KVTSKCG	-
ptau 299-323 p310, 320	513	HVPGGGSVQIV(pY)KPVDLSKV(pT)SKCG	++
ptau 299-323 p310, 321	514	HVPGGGSVQIV(pY)KPVDLSKVT(pS)KCG	++
ptau 299-323 p316, 320	515	HVPGGGSVQIVYKPVDL(pS)KV(pT)SKCG	-/+
ptau 299-323 p316, 321	516	HVPGGGSVQIVYKPVDL(pS)KVT(pS)KCG	-
ptau 299-323 p320, 321	517	HVPGGGSVQIVYKPVDLSKV(pT)(pS)KCG	++

Table 33. CBTAU-47.1 and CBTAU-47.2: Peptides for reactivity by ELISA

Peptide	SEQ ID NO.		Result CBTAU-47.1	Result CBTAU-47.2
tau 42-103	325	GLKESPLQTPTEGSEEPGSETSDAKST PTAEDVTAPLVDEGAPGKQAAAQPHTI PEGTTA	++	++
tau 48-71	518	LQTPTEGSEEPGSETSDAKSTPT	++	++
ptau 48-71 (p71)	519	LQTPTEGSEEPGSETSDAKSTP(pT)	++	++
ptau 48-71 (p63)	520	LQTPTEGSEEPGSE(pT)SDAKSTPT	-	-
ptau 48-71 (p61)	521	LQTPTEGSEEPG(pS)ETSDAKSTPT	-	-
ptau 48-71 (p56)	522	LQTPTEG(pS)EEPGSETSDAKSTPT	++	++
ptau 48-71 (p52)	523	LQTP(pT)EDGSEEPGSETSDAKSTPT	++	++
ptau 48-71 (p68)	524	LQTPTEGSEEPGSETSDAK(pS)TPT	++	++
ptau 48-71 (p69)	525	LQTPTEGSEEPGSETSDAKS(pT)PT	++	++
ptau 48-71 (Ser64)	526	LQTPTEGSEEPGSET(pS)DAKSTPT	++	++
ptau 48-71 (p61,Ser64)	527	LQTPTEGSEEPG(pS)ET(pS)DAKSTPT	-	-
ptau 48-71 (p61,p63)	528	LQTPTEGSEEPG(pS)E(pT)SDAKSTPT	-	-
ptau 48-71 (p63,p64)	529	LQTPTEGSEEPGSE(pT)(pS)DAKSTPT	-	-

Table 34. CBTAU-49.1: Peptides for reactivity by ELISA

Peptide	SEQ ID NO.		Result
tau 42-103	325	GLKESPLQTPTEDGSEEPGSETSDAKST PTAEDVTAPLVDEGAPGKQAAAQPH EIPGTTA	++
tau 48-71	518	LQTPTEDGSEEPGSETSDAKSTPT	++
ptau 48-71 (p71)	519	LQTPTEDGSEEPGSETSDAKSTP(pT)	++
ptau 48-71 (p63)	520	LQTPTEDGSEEPGSE(pT)SDAKSTPT	-
ptau 48-71 (p61)	521	LQTPTEDGSEEPG(pS)ETSDAKSTPT	-
ptau 48-71 (p56)	522	LQTPTEDG(pS)EEPGSETSDAKSTPT	++
ptau 48-71 (p52)	523	LQTP(pT)EDGSEEPGSETSDAKSTPT	++
ptau 48-71 (p68)	524	LQTPTEDGSEEPGSETSDAK(pS)TPT	++
ptau 48-71 (p69)	525	LQTPTEDGSEEPGSETSDAKS(pT)PT	-/+
ptau 48-71 (Ser64)	526	LQTPTEDGSEEPGSET(pS)DAKSTPT	++
ptau 48-71 (p61,Ser64)	527	LQTPTEDGSEEPG(pS)ET(pS)DAKSTPT	-
ptau 48-71 (p61,p63)	528	LQTPTEDGSEEPG(pS)E(pT)SDAKSTPT	-
ptau 48-71 (p63,p64)	529	LQTPTEDGSEEPGSE(pT)(pS)DAKSTPT	-

The results for CBTAU-27.1 and CBTAU-43.1 show that phosphorylation at S316 is
 5 sufficient to completely inhibit reactivity (**Table 30** and **Table 32**). This suggests that the loss in
 reactivity to tau on AD tissue sections (Example 11) may be due to phosphorylation at S316, an
 event that may occur early during the course of the disease. For CBTAU-28.1, 47.1, 47.2, 49.1,
 phosphorylation at either S61 or T63 is sufficient to completely inhibit reactivity. Taken together,
 the results for CBTAU-28.1, 47.1, 47.2, and 49.1 suggest that phosphorylation at S61 and/or T63 is
 10 a mechanism that accounts for loss of the this epitope during the course of the disease.

EXAMPLE 15

Generation of anti-tau mAb mouse-human chimeras and human isotypes

To test the efficacy of the human anti-tau mAbs in a mouse model of tauopathy, mouse-human antibody chimeras were generated by replacing the human Fc region with a mouse IgG1 Fc. Briefly, the human IgG1 CH1 region was amplified from the pCB-IgG vector using primers Step1HMchim-Fwd and Step1HMchim-Rev (**Table 35**) to generate a 0.95 kb fragment containing a 5'-XhoI site (frag.1). Mouse IgG1 CH2 and CH3 domains (Fc region) are amplified from a gene-synthesized construct using primers Step2HMchim-Fwd and Step2HMchim-Rev (**Table 30**) to generate a 0.82 kb fragment (frag. 2). A third fragment (frag. 3) was generated by amplifying the polyA region of the pCB-IgG vector using primers Step3HMchim-Fwd and Step3HMchim-Rev (**Table 30**), which includes a 3'-DraIII site. The three fragments were linked into a single cassette by overlap extension PCR to generate a 2.3 kb overlap fragment harboring the human CH1 followed by the mouse CH2-CH3 domains. The overlap fragment was subsequently cloned via the XhoI and DraIII sites into the pCB-IgG CBTAU-7.1 vector to generate a mouse-human chimera of CBTAU-7.1, containing the human variable, CH1, hinge and Ck regions followed by the mouse CH2 and CH3 regions. CBTAU-22.1, 24.1, 27.1, 28.1, 47.1, 47.2, 46.1, 49.1, and 43.1 chimeras were then generated by digesting the CBTAU-7.1 chimera construct and pCB-IgG CBTAU human mAb constructs with XhoI and XbaI, and the corresponding fragments were subcloned. Nucleotide sequences for all constructs are verified according to standard techniques known to the skilled artisan. Chimeric antibodies were subsequently expressed and purified as detailed in Example 5 using Protein G agarose instead of Protein A.

Table 35 PRIMERS FOR MOUSE-HUMAN CHIMERIZATION

Primer ID	DNA SEQUENCE (5'-3')	SEQ ID NO:
Step1HMchim-Fwd	TCTCCGCCGGTGAGTCTCGAGGC	530
Step1HMchim-Rev	TGTCCCTGGATGCAGGCTACTCTAGG	531
Step2HMchim-Fwd	AGAGTAGCCTGCATCCAGGGACAG	532
Step2HMchim-Rev	TCTAGATCATTTACCAGGAGAGTGGGAGAG	533
Step3HMchim-Fwd	TCTCCTGGTAAATGATCTAGAGTTTAAACCGCTG	534
Step3HMchim-Rev	ATGGCCCACTACGTGAACCATCACC	535

EXAMPLE 16:

Preparation of IgG2, 3 and 4 isotypes

As mentioned in Example 3, all CBTAU mAbs were cloned and expressed as chimeric human IgG1 regardless of their native isotype. To generate additional human isotype versions (i.e., IgG2/3/4), the CH1 through CH3 region corresponding to each of the human IgG isotypes is PCR amplified from gene-synthesized constructs containing the corresponding constant regions, hinge, and intron sequences. The PCR amplicons contain 5'-XhoI and 3'-DraIII sites, which are used to subclone the fragments into the corresponding pCB-IgG CBTAU antibody construct. In this manner, human IgG2, 3 and 4 isotype versions are generated for each of the anti-tau mAbs.

EXAMPLE 17

Generation of de-risked and Fc engineered anti-tau chimeric monoclonal antibody variants

The heavy and light chain variable regions (VH and VL) for each anti-tau antibody clone isolated in Example 3 are analyzed for the presence of free cysteines and potential post-translational modification sites, which include glycosylation, deamidation and oxidation sites. Amino acid mutations consisting of structurally conserved and/or germline-based substitutions are used to change these sites. Non-conserved cysteines in the variable regions are mutated to serine. For glycosylation sites, several mutations are used, including replacement of asparagine for the conservative glutamine or germline mutations. Modifications to the deamidation sites include replacement of aspartic acid for asparagine and serine or alanine for glycine. Sites of potential oxidation are not modified. To increase the binding affinity to FcRn and thus increase the half-life of IgG1 mAbs in vivo, several mutations located at the boundary between the CH2 and CH3 region are generated. These mutations included M252Y/S254T/T256E plus H433K/N434F (Vaccaro C. et al., 2005) or T250Q/M428L (Hinton PR. et al., 2004), which have been shown to increase IgG1 binding to FcRn. All substitutions are generated by site-directed mutagenesis per manufacturer's instructions (QuickChange II, Agilent Technologies, cat. no. 200521). Nucleotide sequences for all constructs are verified according to standard techniques known to the skilled artisan.

Claims

1. A monoclonal antibody or antigen-binding fragment thereof, wherein the antibody or antigen-binding fragment: a) binds tau in normal human brain tissue and b) does not binds tau in human Alzheimer's disease (AD) brain tissue, wherein the antibody is selected from the group consisting of
 - a) an antibody comprising a heavy chain CDR1 region of SEQ ID NO:201, a heavy chain CDR2 region of SEQ ID NO:202, and a heavy chain CDR3 region of SEQ ID NO:203, a light chain CDR1 region of SEQ ID NO:204, a light chain CDR2 region of SEQ ID NO:205 and a light chain CDR3 region of SEQ ID NO:206,
 - b) an antibody comprising a heavy chain CDR1 region of SEQ ID NO:207, a heavy chain CDR2 region of SEQ ID NO:208, and a heavy chain CDR3 region of SEQ ID NO:209, a light chain CDR1 region of SEQ ID NO:210, a light chain CDR2 region of SEQ ID NO:211 and a light chain CDR3 region of SEQ ID NO:212,
 - c) an antibody comprising a heavy chain CDR1 region of SEQ ID NO:222, a heavy chain CDR2 region of SEQ ID NO:223, and a heavy chain CDR3 region of SEQ ID NO:224, a light chain CDR1 region of SEQ ID NO:225, a light chain CDR2 region of SEQ ID NO:173 and a light chain CDR3 region of SEQ ID NO:226,
 - d) an antibody comprising a heavy chain CDR1 region of SEQ ID NO:238, a heavy chain CDR2 region of SEQ ID NO:239, and a heavy chain CDR3 region of SEQ ID NO:240, a light chain CDR1 region of SEQ ID NO:241, a light chain CDR2 region of SEQ ID NO:173 and a light chain CDR3 region of SEQ ID NO:242,
 - e) an antibody comprising a heavy chain CDR1 region of SEQ ID NO:243, a heavy chain CDR2 region of SEQ ID NO:244, and a heavy chain CDR3 region of SEQ ID NO:245, a light chain CDR1 region of SEQ ID NO:246, a light chain CDR2 region of SEQ ID NO:173 and a light chain CDR3 region of SEQ ID NO:212,
 - f) an antibody comprising a heavy chain CDR1 region of SEQ ID NO:243, a heavy chain CDR2 region of SEQ ID NO:247, and a heavy chain CDR3 region of SEQ ID NO:248, a

light chain CDR1 region of SEQ ID NO:249 a light chain CDR2 region of SEQ ID NO:173 and a light chain CDR3 region of SEQ ID NO:212, and

g) an antibody comprising a heavy chain CDR1 region of SEQ ID NO:250, a heavy chain CDR2 region of SEQ ID NO:251, and a heavy chain CDR3 region of SEQ ID NO:252, a light chain CDR1 region of SEQ ID NO:254, a light chain CDR2 region of SEQ ID NO:254 and a light chain CDR3 region of SEQ ID NO:255.

2. The antibody or antigen-binding fragment thereof of claim 1 comprising an antigen-binding site of a VH of SEQ ID NO: 115 and the VL of SEQ ID NO: 116.
3. The antibody or antigen-binding fragment thereof of claim 1 comprising an antigen-binding site of a VH of SEQ ID NO: 119 and the VL of SEQ ID NO: 120.
4. The antibody or antigen-binding fragment thereof of claim 1 comprising an antigen-binding site of a VH of SEQ ID NO: 135 and the VL of SEQ ID NO: 136.
5. The antibody or antigen-binding fragment thereof of claim 1 comprising an antigen-binding site of a VH of SEQ ID NO: 147 and the VL of SEQ ID NO: 148
6. The antibody or antigen-binding fragment thereof of claim 1 comprising an antigen-binding site of a VH of SEQ ID NO: 151 and the VL of SEQ ID NO: 152
7. The antibody or antigen-binding fragment thereof of claim 1 comprising an antigen-binding site of a VH of SEQ ID NO: 155 and the VL of SEQ ID NO: 156
8. The antibody or antigen-binding fragment thereof of claim 1 comprising an antigen-binding site of a VH of SEQ ID NO: 159 and the VL of SEQ ID NO: 160
9. An isolated nucleic acid encoding the antibody or antigen-binding fragment according to any one of claims 1-8.
10. A vector comprising a nucleic acid according to claim 9.
11. A host cell comprising the vector according to claim 10.

12. A method of producing an antibody or antigen-binding fragment according to any one of claims 1-8, comprising culturing the host cell of claim 11 and recovering the antibody or fragment thereof produced by the host cell.
13. A pharmaceutical composition comprising an antibody according to any one of claims 1-8, the pharmaceutical composition further comprising at least one pharmaceutically acceptable excipient.