TAU IMMUNOTHERAPY

CROSS-REFERENCE TO RELATED APPLICATIONS

[0001] The present application is a non-provisional of 61/780,624 filed March 13, 2013 and 61/800,382, filed March 15, 2013, each incorporated by reference in its entirety for all purposes.

BACKGROUND

[0002] Tau is a well-known human protein that can exist in phosphorylated forms (see, *e.g.*, Goedert, Proc. Natl. Acad. Sci. U.S.A. 85:4051-4055(1988); Goedert, EMBO J. 8:393-399(1989); Lee, Neuron 2:1615-1624(1989); Goedert, Neuron 3:519-526(1989); Andreadis, Biochemistry 31:10626-10633(1992). Tau has been reported to have a role in stabilizing microtubules, particularly in the central nervous system. Total tau (t-tau, *i.e.*, phosphorylated and unphosphorylated forms) and phospho-tau (p-tau, *i.e.*, phosphorylated tau) are released by the brain in response to neuronal injury and neurodegeneration and have been reported to occur at increased levels in the CSF of Alzheimer's patients relative to the general population (Jack et al., Lancet Neurol 9: 119–28 (2010)).

[0003] Tau is the principal constituent of neurofibrillary tangles, which together with plaques are a hallmark characteristic of Alzheimer's disease. The tangles constitute abnormal fibrils measuring 10 nm in diameter occurring in pairs wound in a helical fashion with a regular periodicity of 80 nm. The tau within neurofibrillary tangles is abnormally phosphorylated (hyperphosphorylated) with phosphate groups attached to specific sites on the molecule. Severe involvement of neurofibrillary tangles is seen in the layer II neurons of the entorhinal cortex, the CA1 and subicular regions of the hippocampus, the amygdala, and the deeper layers (layers III, V, and superficial VI) of the neocortex in Alzheimer's disease. Hyperphosphorylated tau has also been reported to interfere with microtubule assembly, which may promote neuronal network breakdown.

[0004] Tau inclusions are part of the defining neurophathology of several neurodegenerative diseases including Alzheimer's disease, frontotemporal lobar degeneration, progressive supranuclear palsy and Pick's disease.

SUMMARY OF THE CLAIMED INVENTION

[0005] The invention provides monoclonal antibodies that compete for binding to tau with monoclonal antibody 16B5. Some antibodies are humanized, chimeric, veneered or human antibodies. Some antibodies are of human IgG isotype (*e.g.*, IgG1, IgG2, or IgG4). Some such antibodies have a human IgG1 constant region having the sequence of SEQ ID NO: 29. Some antibodies have a human kappa constant region having the sequence of SEQ ID NO: 32. Some antibodies have at least one mutation in the constant region.

[0006] Some of the antibodies are a humanized, chimeric or veneered form of monoclonal antibody 16B5. Some antibodies have the three light chain CDRs as defined by Kabat and three heavy chain CDRs as defined by Kabat of monoclonal antibody 16B5. Some antibodies bind to tau in phosphorylated and unphosphorylated forms.

[0007] The invention further provides monoclonal antibodies that specifically bind to an epitope within residues 24-46 of SEQ ID NO:1 (Swiss-Prot No. P10636-8). Some such antibodies are human, humanized, chimeric, or veneered antibodies. Some antibodies specifically bind to an epitope within residues 25-44 of SEQ ID NO:1. Some antibodies specifically bind to an epitope within residues 30-39 of SEQ ID NO:1. Some antibodies bind to tau in phosphorylated and unphosphorylated forms.

[0008] The invention further provides monoclonal antibodies comprising a mature heavy chain variable region having an amino acid sequence at least 90% (*e.g.*, at least 95%, at least 98%, at least 99%) identical to SEQ ID NO:15 and a mature light chain variable region at least 90% (*e.g.*, at least 95%, at least 98%, at least 99%) identical to SEQ ID NO:22. In certain embodiments, the monoclonal antibody comprises three Kabat CDRs of SEQ ID NO:15 and three Kabat CDRs of SEQ ID NO:22. In some antibodies, the mature heavy chain variable region has an amino acid sequence designated SEQ ID NO:15 and the mature light chain variable region has an amino acid sequence designated SEQ ID NO:21, 22, or 23.

[0009] In some antibodies, at least one of positions H13, H48 and H91 is occupied by K, M and F, respectively, and at least one of positions L1, L4, L36 and L43 is occupied by N, L, F and S, respectively. In some antibodies, positions H13, H48 and H91 are occupied by K, M and F, respectively, and at least two of positions L1, L4, L36 and L43 are occupied by N, L, F and S,

respectively. In some antibodies, positions H13, H48 and H91 are occupied by K, M and F, respectively, and at least three of positions L1, L4, L36 and L43 are occupied by N, L, F and S, respectively. In some antibodies, positions H13, H48 and H91 are occupied by K, M and F, respectively, and positions L1, L4, L36 and L43 are occupied by N, L, F and S, respectively.

[0010] In some antibodies, the mature heavy chain variable region is fused to a heavy chain constant region and the mature light chain variable region is fused to a light chain constant region. In some antibodies, the heavy chain constant region is a mutant form of a natural human constant region which has reduced binding to an Fcγ receptor relative to the natural human constant region. In some antibodies, the heavy chain constant region is of IgG1 isotype.

[0011] In some antibodies, differences in the CDRs of the mature heavy chain variable region and mature light variable region from SEQ ID NOS: 15 and 22, respectively, reside in positions H60-H65.

[0012] The antibodies can be intact antibodies or fragments, such as a Fab fragment.

[0013] Any of the monoclonal antibodies or fragments can be conjugated to a cytotoxic or cytostatic agent.

[0014] The invention further provides methods of humanizing an antibody. Some methods comprise determining the sequences of the heavy and light chain variable regions of a mouse antibody; synthesizing a nucleic acid encoding a humanized heavy chain comprising CDRs of the mouse antibody heavy chain and a nucleic acid encoding a humanized light chain comprising CDRs of the mouse antibody light chain; and expressing the nucleic acids in a host cell to produce a humanized antibody, wherein the mouse antibody is 16B5.

[0015] The invention further provides methods of producing a humanized, chimeric or veneered antibody. Some methods comprise culturing cells transformed with nucleic acids encoding the heavy and light chains of the antibody, so that the cell secretes the antibody; and purifying the antibody from cell culture media, wherein the antibody is a humanized, chimeric or veneered form of 16B5.

[0016] The invention further provides methods of producing a cell line producing a humanized, chimeric or veneered antibody. Some methods comprise introducing a vector encoding heavy and light chains of an antibody and a selectable marker into cells; propagating the cells under conditions to select for cells having increased copy number of the vector;

isolating single cells from the selected cells; and banking cells cloned from a single cell selected based on yield of antibody; wherein the antibody is a humanized, chimeric or veneered form of 16B5.

[0017] The invention further provides pharmaceutical compositions comprising any antibody disclosed herein and a pharmaceutically acceptable carrier.

[0018] The invention further provides nucleic acids comprising a segment encoding a heavy chain variable region having the sequence of SEQ ID NO: 10.

[0019] The invention further provides nucleic acids comprising a segment encoding a heavy chain variable region having the sequence of SEQ ID NO: 15. In some nucleic acids, the segment has a nucleotide sequence of SEQ ID NO: 25. Some nucleic acids further comprise a segment encoding an IgG1 constant region, optionally a human IgG1 constant region, for example, having a sequence of SEQ ID NO: 29 provided the C-terminal lysine can be omitted. In some nucleic acids, the segment encoding the IgG1 constant region has a nucleotide sequence of SEQ ID NO: 30. Some such nucleic acids further comprise an intron linking the segments encoding the heavy chain variable region and the IgG1 constant region. For example, the intron can have the sequence of the intron found in SEQ ID NO: 31. Thus, the intron and the segment encoding the IgG1 constant region can have a nucleotide sequence of SEQ ID NO: 31.

[0020] The invention further provides a nucleic acid comprising a segment encoding a light chain variable region having the sequence of SEQ ID NO: 16.

[0021] The invention further provides a nucleic acid comprising a segment encoding a light chain variable region having a sequence of SEQ ID NO: 21, 22, or 23. In some nucleic acids, the segment encoding the light chain variable region has the sequence of SEQ ID NO: 26, 27 or 28. Some such nucleic acids further comprise a segment encoding a kappa constant region. The kappa constant region can be a human kappa constant region and can have the sequence of SEQ ID NO: 32. Optionally, the nucleic acid encoding the kappa constant region has the sequence of SEQ ID NO: 33. Some such nucleic acids further comprise an intron linking the segment encoding the light chain variable region to the segment encoding the kappa constant region. For example, the intron can have the sequence of the intron found in SEQ ID NO: 34. Thus, the intron and the segment encoding the kappa constant region can have a nucleotide sequence of SEQ ID NO: 34.

[0022] Any of the above-mentioned antibodies can include a heavy chain comprising a human IgG1 constant region having the sequence of SEQ ID NO: 29 and/or a light chain comprising a human kappa constant region having the sequence of SEQ ID NO: 32.

[0023] The invention further provides an isolated fragment of tau including 3-10 contiguous residues of tau within residues 24-46 of SEQ ID NO:1 (Swiss-Prot No. P10636-8). Some fragments include 3-10 contiguous residues of tau within residues 30-39 of SEQ ID NO:1 (Swiss-Prot No. P10636-8). Some fragments include residues 33-37 of SEQ ID NO:1 (Swiss-Prot No. P10636-8). Some fragments are linked to a carrier molecule, optionally via a spacer that helps elicit antibodies against the fragment. Some fragments are part of a pharmaceutical composition comprising an adjuvant acceptable for administration to humans.

[0024] The invention further provides methods of treating or effecting prophylaxis of Alzheimer's disease. Some methods comprise administering an effective regime of an antibody that specifically binds to an epitope within residues 24-46 of SEQ ID NO:1 (Swiss-Prot No. P10636-8), or an agent that induces such an antibody, to a patient having or at risk of Alzheimer's disease and thereby treating or effecting prophylaxis of the disease. Preferably, the antibody is an antibody described herein. In some methods, the agent that induces the antibody is a fragment of tau that includes 3-10 contiguous residues of tau within residues 24-46 of SEQ ID NO: 1. In some methods, the patient is an ApoE4 carrier.

[0025] The invention further provides methods of treating or effecting prophylaxis of a disease associated with tau. Some methods comprise administering an effective regime of an antibody that specifically binds to an epitope within residues 24-46 of SEQ ID NO:1 (Swiss-Prot No. P10636-8), or an agent that induces such an antibody, to a patient having or at risk of the disease and thereby treating or effecting prophylaxis of the disease. Preferably, the antibody is an antibody described herein (*e.g.*, a humanized 16B5 antibody). In some methods, the agent that induces the antibody is a fragment of tau that includes 3-10 contiguous residues of tau within residues 24-46 of SEQ ID NO:1. In some methods, the disease is a neurological disease.

[0026] The invention further provides methods of reducing aberrant transmission of tau. Some methods comprise administering an effective regime of an antibody that specifically binds to an epitope within residues 24-46 of SEQ ID NO:1 (Swiss-Prot No. P10636-8), or an agent that induces such an antibody, to a patient having or at risk of a disease associated with aberrant transmission of tau, and thereby treating or effecting prophylaxis of the disease. Preferably the

antibody is an antibody described herein (*e.g.*, a humanized 16B5 antibody). In some methods, the agent that induces the antibody is a fragment of tau that includes 3-10 contiguous residues of tau within residues 24-46 of SEQ ID NO: 1.

[0027] The invention further provides methods of inducing phagocytosis of tau. Some methods comprise administering an effective regime of an antibody that specifically binds to an epitope within residues 24-46 of SEQ ID NO:1 (Swiss-Prot No. P10636-8), or an agent that induces such an antibody, to a patient having or at risk of a disease associated with the accumulation of tau. Preferably, the antibody is an antibody described herein (*e.g.*, a humanized 16B5 antibody). In some methods, the agent that induces the antibody is a fragment of tau that includes 3-10 contiguous residues of tau within residues 24-46 of SEQ ID NO: 1. In some methods, the disease is a neurological disease.

[0028] The invention further provides methods of inhibiting tau aggregation or deposition. In certain embodiments, the methods comprise administering an effective regime of an antibody that specifically binds to an epitope within residues 24-46 of SEQ ID NO:1 (Swiss-Prot No. P10636-8), or an agent that induces such an antibody, to a patient having or at risk of a disease associated with the aggregation or deposition of tau. Preferably, the antibody is an antibody described herein (*e.g.*, a humanized 16B5 antibody). In some methods, the agent that induces the antibody is a fragment of tau that includes 3-10 contiguous residues of tau within residues 24-46 of SEQ ID NO: 1. In some methods, the disease is a neurological disease.

[0029] The invention further provides methods of inhibiting formation of tau tangles. Some methods comprise administering an effective regime of an antibody that specifically binds to an epitope within residues 24-46 of SEQ ID NO:1 (Swiss-Prot No. P10636-8), or an agent that induces such an antibody, to a patient having or at risk of a disease associated with the formation of tau tangles. Preferably, the antibody is an antibody described herein (*e.g.*, a humanized 16B5 antibody). In some methods, the agent that induces the antibody is a fragment of tau that includes 3-10 contiguous residues of tau within residues 24-46 of SEQ ID NO: 1. In some methods, the disease is a neurological disease.

[0030] The invention further provides methods of screening an agent for activity against Alzheimer's disease. Some methods comprise administering the agent to a transgenic animal expressing a tau transgene, and determining whether the agent inhibits or delays at least one sign or symptom of Alzheimer's disease, wherein the agent is an antibody that specifically binds to an

epitope within residues 24-46 of SEQ ID NO:1 (Swiss-Prot No. P10636-8), or an agent that induces such an antibody.

BRIEF DESCRIPTION OF THE DRAWINGS

[0031] Figure 1 depicts the results of experiments designed to map the epitope(s) bound by the 16B5 monoclonal antibody. Western blots containing full-length Tau or deletion mutants of Tau (Δ5-24 or Δ25-44) were stained with 16B5 antibodies (left panel) or Tau46 antibodies (right panel). The Tau46 antibody binds to the C-terminal epitope of Tau.

[0032] Figure 2 depicts the results of experiments designed to map the epitope(s) bound by the 16B5 monoclonal antibody. Western blots containing full-length Tau or deletion mutants of Tau were stained with 16B5 antibodies (upper left panel) or Tau46 antibodies (right panel). A longer exposure of the blot stained with 16B5 antibodies is shown in the lower left panel. The deletion mutants of Tau analyzed in this experiment include Δ25-44, Δ5-24, Δ23-32, Δ30-39, and Δ37-46.

[0033] Figure 3 depicts the results of an alanine scanning experiment designed to map the epitope(s) bound by the 16B5 monoclonal antibody. Western blots containing wild-type Tau (WT) or alanine point mutants of Tau were stained with 16B5 antibodies (left panel) or Tau46 antibodies (right panel). The alanine mutants of Tau analyzed in this experiment include T30A, M31A, H32A, Q33A, D34A, Q35A, E36A, G37A, D38A, T39A, D40A, A41L, and G42A.

[0034] Figure 4 shows relative amounts of tau protein detected in a sarkosyl insoluble fraction of the brainstem of transgenic mice that express the human tau.P301L protein. The mice were passively immunized with either the 16B5 antibody or the 6F10 antibody, a non-immune IgG1 isotype control. Samples were analyzed by Western blotting, antibody staining, and quantification of the resulting signal. Antibodies used to detect tau included anti-phospho-tau specific antibodies (AT8, upper left panel; AT100, lower left panel; or 1F5, upper right panel) and a pan tau antibody (HT7, lower right panel).

[0035] Figure 5 shows the ratio of phospho-tau to total tau protein (left panel) and a normalized amount of total tau (right panel) detected in total brainstem homogenates of transgenic mice that express the human tau.P301L protein. The mice were passively immunized with either the 16B5 antibody or the 6F10 antibody, a non-immune IgG1 isotype control. Samples were analyzed by Western blotting, antibody staining, and quantification of the resulting signal. The AT8 antibody was used to detect phospho-tau and the HT7 antibody was used to detect total tau. An anti-

GAPDH antibody was used to normalize the amount of tau detected in mice treated with the 16B5 antibody versus the control 6F10 antibody.

[0036] Figure 6 depicts sections of cerebellar nuclei of transgenic mice that express the human tau.P301L protein, immunohistochemically stained using the AT8 anti-phospho-tau antibody. The mice were passively immunized with either the 16B5 antibody (upper left panel) or the 6F10 antibody (lower left panel), a non-immune IgG1 isotype control. Quantification of the amount of tau staining detected with the AT8 antibody in the interposed nucleus of the cerebellum, anterior and posterior part, annex lateral cerebellar nucleus (IntA/P/LAT) and the subthalamic nucleus annex zona incerta (STH/ZI) from mice passively immunized with 16B5 or 6F10 antibodies is shown in the upper bar graph panels. Quantification of the amount of phospho-tau staining detected using the AT100 anti-phospho-tau antibody on IntA/P/LAT and STH/ZI sections from mice passively immunized with 16B5 or 6F10 antibodies is shown in the lower bar graph panels. Statistical significance was assessed using the Student's t test, $p<0.05$.

[0037] Figure 7 depicts tau immunoprecipitation results obtained with chimeric 16B5 antibodies and humanized 16B5 antibodies (H1L2 and H1L3 versions). Tau was immunoprecipitated from both soluble and insoluble fractions of postmortem frontal cortex samples obtained from an Alzheimer disease patient. Tau present in blotted immuno-precipitates was detected using a polyclonal anti-tau antibody (tau pAb).

DEFINITIONS

[0038] Monoclonal antibodies and other therapeutic agents are typically provided in isolated form. This means that the agent is typically at least 50% w/w pure of interfering proteins and other contaminants arising from its production or purification but does not exclude the possibility that the agent is combined with an excess of pharmaceutical acceptable carrier(s) or other vehicle intended to facilitate its use. Sometimes monoclonal antibodies (or other therapeutic agents) are at least 60%, 70%, 80%, 90%, 95% or 99% w/w pure of interfering proteins and contaminants from production or purification.

[0039] Antibodies of the invention typically bind to their designated target with an association constant of at least 10^6, 10^7, 10^8, 10^9, or 10^{10} M^{-1}. Such binding is specific binding in that it is detectably higher in magnitude and distinguishable from non-specific binding occurring to at least one unrelated target. Specific binding can be the result of formation of bonds between

particular functional groups or particular spatial fit (*e.g.*, lock and key type) whereas nonspecific binding is usually the result of van der Waals forces. Specific binding does not however necessarily imply that a monoclonal antibody binds one and only one target.

[0040] The basic antibody structural unit is a tetramer of subunits. Each tetramer includes two identical pairs of polypeptide chains, each pair having one "light" (about 25 kDa) and one "heavy" chain (about 50-70 kDa). The amino-terminal portion of each chain includes a variable region of about 100 to 110 or more amino acids primarily responsible for antigen recognition. This variable region is initially expressed linked to a cleavable signal peptide. The variable region without the signal peptide is sometimes referred to as a mature variable region. Thus, for example, a light chain mature variable region, means a light chain variable region without the light chain signal peptide. The carboxy-terminal portion of each chain defines a constant region primarily responsible for effector function. A constant region can include any or all of a CH1 region, hinge region, CH2 region and CH3 region.

[0041] Light chains are classified as either kappa or lambda. Heavy chains are classified as gamma, mu, alpha, delta, or epsilon, and define the antibody's isotype as IgG, IgM, IgA, IgD and IgE, respectively. Within light and heavy chains, the variable and constant regions are joined by a "J" region of about 12 or more amino acids, with the heavy chain also including a "D" region of about 10 or more amino acids. (*See generally, Fundamental Immunology* (Paul, W., ed., 2nd ed. Raven Press, N.Y., 1989), Ch. 7) (incorporated by reference in its entirety for all purposes).

[0042] The mature variable regions of each light/heavy chain pair form the antibody binding site. Thus, an intact antibody has two binding sites. Except in bifunctional or bispecific antibodies, the two binding sites are the same. The chains all exhibit the same general structure of relatively conserved framework regions (FR) joined by three hypervariable regions, also called complementarity determining regions or CDRs. The CDRs from the two chains of each pair are aligned by the framework regions, enabling binding to a specific epitope. From N-terminal to C-terminal, both light and heavy chains comprise the domains FR1, CDR1, FR2, CDR2, FR3, CDR3 and FR4. The assignment of amino acids to each domain is in accordance with the definitions of Kabat, *Sequences of Proteins of Immunological Interest* (National Institutes of Health, Bethesda, MD, 1987 and 1991), or Chothia & Lesk, *J. Mol. Biol.* 196:901-917 (1987); Chothia et al., *Nature* 342:878-883 (1989). Kabat also provides a widely used numbering

convention (Kabat numbering) in which corresponding residues between different heavy chains or between different light chains are assigned the same number.

[0043] The term "antibody" includes intact antibodies and binding fragments thereof. Typically, fragments compete with the intact antibody from which they were derived for specific binding to the target. Fragments include separate heavy chains, light chains Fab, Fab', $F(ab')_2$, F(ab)c, Fv and single domain antibodies. Single (variable) domain antibodies include VH regions separated from their VL partners (or vice versa) in conventional antibodies (Ward et al., 1989, Nature 341: 544-546) as well as VH regions (sometimes known as VHH) from species such as Camelidae or cartilaginous fish (*e.g.*, a nurse shark) in which VH regions are not associated with VL regions (see, *e.g.*, WO 9404678). Single domain antibodies in which one chain is separated from its natural partners are sometimes known as Dabs and single domain antibodies from Caemelidae or cartilaginous fish are sometimes known as nanobodies. Constant regions or parts of constant regions may or may not be present in single domain antibodies. For example, natural single variable region antibodies from Camelidae include a VHH variable region, and CH2 and CH3 constant regions. Single domain antibodies can be subject of humanization by analogous approaches to conventional antibodies. The Dabs type of antibodies are usually obtained from antibodies of human origin. NANOBODY types of antibody are of Camelidae or shark origin and can be subject to humanization. Fragments can be produced by recombinant DNA techniques, or by enzymatic or chemical separation of intact immunoglobulins. The term "antibody" also includes a bispecific antibody. A bispecific or bifunctional antibody is an artificial hybrid antibody having two different heavy/light chain pairs and two different binding sites (see, *e.g.*, Songsivilai and Lachmann, Clin. Exp. Immunol., 79:315-321 (1990); Kostelny et al., J. Immunol., 148:1547-53 (1992)).

[0044] The term "epitope" refers to a site on an antigen to which an antibody binds. An epitope can be formed from contiguous amino acids or noncontiguous amino acids juxtaposed by tertiary folding of one or more proteins. Epitopes formed from contiguous amino acids are typically retained on exposure to denaturing solvents whereas epitopes formed by tertiary folding are typically lost on treatment with denaturing solvents. An epitope typically includes at least 3, and more usually, at least 5 or 8-10 amino acids in a unique spatial conformation. When an epitope is said to be within a range of amino acid residues in a protein (*e.g.*, within residues 25 to 44 of tau), the range is inclusive of the residues defining its borders. Certain residues within the range contribute to the epitope, whereas others may not. The residues that form the epitope may or

may not be contiguous with one another. Similarly, when an antibody binds to an epitope found within a particular range of amino acids, the antibody need not contact all the amino acids residues within the range, and the residues of the epitope that are contacted by the antibody may or may not be contiguous with one another. Methods of determining spatial conformation of epitopes include, for example, x-ray crystallography and 2-dimensional nuclear magnetic resonance. See, *e.g.*, Epitope Mapping Protocols, in Methods in Molecular Biology, Vol. 66, Glenn E. Morris, Ed. (1996).

[0045] Antibodies that recognize the same or overlapping epitopes can be identified in a simple immunoassay showing the ability of one antibody to compete with the binding of another antibody to a target antigen. The epitope of an antibody can also be defined by X-ray crystallography of the antibody bound to its antigen to identify contact residues. Alternatively, two antibodies have the same epitope if all amino acid mutations in the antigen that reduce or eliminate binding of one antibody reduce or eliminate binding of the other. Two antibodies have overlapping epitopes if some amino acid mutations that reduce or eliminate binding of one antibody reduce or eliminate binding of the other. The invention includes antibodies that compete with 16B5 and/or which bind to the same epitope on tau as 16B5.

[0046] Competition between antibodies is determined by an assay in which an antibody under test inhibits specific binding of a reference antibody (*e.g.* 16B5) to a common antigen (see, *e.g.*, Junghans et al., Cancer Res. 50:1495, 1990). A test antibody competes with a reference antibody if an excess of a test antibody (*e.g.*, at least 2x, 5x, 10x, 20x or 100x) inhibits binding of the reference antibody by at least 50% but preferably 75%, 90% or 99% as measured in a competitive binding assay. Antibodies identified by competition assay (competing antibodies) include antibodies binding to the same epitope as the reference antibody and antibodies binding to an adjacent epitope sufficiently proximal to the epitope bound by the reference antibody for steric hindrance to occur.

[0047] The term "patient" includes human and other mammalian subjects that receive either prophylactic or therapeutic treatment.

[0048] For purposes of classifying amino acids substitutions as conservative or nonconservative, amino acids are grouped as follows: Group I (hydrophobic side chains): met, ala, val, leu, ile; Group II (neutral hydrophilic side chains): cys, ser, thr; Group III (acidic side chains): asp, glu; Group IV (basic side chains): asn, gln, his, lys, arg; Group V (residues influencing chain

orientation): gly, pro; and Group VI (aromatic side chains): trp, tyr, phe. Conservative substitutions involve substitutions between amino acids in the same class. Non-conservative substitutions constitute exchanging a member of one of these classes for a member of another.

[0049] Percentage sequence identities are determined with antibody sequences maximally aligned by the Kabat numbering convention. After alignment, if a subject antibody region (*e.g.*, the entire mature variable region of a heavy or light chain) is being compared with the same region of a reference antibody, the percentage sequence identity between the subject and reference antibody regions is the number of positions occupied by the same amino acid in both the subject and reference antibody region divided by the total number of aligned positions of the two regions, with gaps not counted, multiplied by 100 to convert to percentage.

[0050] The term "adjuvant" refers to a compound that when administered in conjunction with an antigen augments and/or redirects the immune response to the antigen, but when administered alone does not generate an immune response to the antigen. Adjuvants can augment an immune response by several mechanisms including lymphocyte recruitment, stimulation of B and/or T cells, and stimulation of macrophages.

[0051] A disease is associated with tau if a population of patients with the disease have increased levels of tau in the brain, or increased deposition or inclusions of tau, or the presence of tau tangles in the brain, or increased phosphorylation of tau in the brain (average number of phosphate groups per molecule tau), or aberrant intercellular or intracellular transmission of tau compared with a population of subjects not known to have a neurological disease. A disease is also associated with tau if patients with a variant form of a tau gene have an increased risk of developing the disease relative to patients with a wildtype (most frequently occurring variant in a human population) tau gene.

[0052] An individual is at increased risk of a disease if the subject has at least one known risk-factor (*e.g.*, genetic, biochemical, family history, situational exposure) placing individuals with that risk factor at a statistically significant greater risk of developing the disease than individuals without the risk factor.

[0053] The term "symptom" refers to a subjective evidence of a disease, such as altered gait, as perceived by the patient. A "sign" refers to objective evidence of a disease as observed by a physician.

[0054] Statistical significance means $p \leq 0.05$.

DETAILED DESCRIPTION

I. General

[0055] The invention provides antibodies that bind to tau. Some antibodies specifically bind to an epitope within residues 23-46 of SEQ ID NO.1. Some antibodies bind to tau irrespective of phosphorylation state. Some antibodies of the invention serve to inhibit or delay tau-associated pathologies and associated symptomatic deterioration. Although an understanding of mechanism is not required for practice of the invention, a reduction in toxicity may occur as a result of the antibody inducing phagocytosis of tau, inhibiting tau from inter or intramolecular aggregation, or from binding to other molecules, by stabilizing a non-toxic conformation, or by inhibiting intercellular or intracellular transmission of pathogenic tau forms, among other mechanisms. The antibodies of the invention or agents that induce such antibodies can be used in methods of treating or effecting prophylaxis of Alzheimer's and other diseases associated with tau.

II. Tau

[0056] Unless otherwise apparent from the context, reference to tau means a natural human form of tau including all isoforms irrespective of whether posttranslational modification (*e.g.*, phosphorylation, glycation, or acetylation) is present. There are six major isoforms (splice variants) of tau occurring in the human brain. The longest of these variants has 441 amino acids, of which the initial met residue is cleaved. Residues are numbered according to the 441 isoform. Thus, for example, reference to a phosphorylation at position 404 means position 404 of the 441 isoform, or corresponding position of any other isoform when maximally aligned with the 441 isoform. The amino acid sequences of the isoforms and Swiss-Prot numbers are indicated below.

P10636-8 (SEQ ID NO:1)

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        10         20         30         40         50         60
MAEPRQEFEV MEDHAGTYGL GDRKDQGGYT MHQDQEGDTD AGLKESPLQT PTEDGSEEPG
        70         80         90        100        110        120
SETSDAKSTP TAEDVTAPLV DEGAPGKQAA AQPHTEIPEG TTAEEAGIGD TPSLEDEAAG
       130        140        150        160        170        180
HVTQARMVSK SKDGTGSDDK KAKGADGKTK IATPRGAAPP GQKGQANATR IPAKTPPAPK
       190        200        210        220        230        240
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TPPSSGEPPK SGDRSGYSSP GSPGTPGSRS RTPSLPTPPT REPKKVAVVR TPPKSPSSAK
       250        260        270        280        290        300
SRLQTAPVPM PDLKNVKSKI GSTENLKHQP GGGKVQIINK KLDLSNVQSK CGSKDNIKHV
       310        320        330        340        350        360
PGGGSVQIVY KPVDLSKVTS KCGSLGNIHH KPGGGQVEVK SEKLDFKDRV QSKIGSLDNI
       370        380        390        400        410        420
THVPGGGNKK IETHKLTFRE NAKAKTDHGA EIVYKSPVVS GDTSPRHLSN VSSTGSIDMV
       430        440
DSPQLATLAD EVSASLAKQG L
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P10636-7 (SEQ ID NO:2)

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        10         20         30         40         50         60
MAEPRQEFEV MEDHAGTYGL GDRKDQGGYT MHQDQEGDTD AGLKESPLQT PTEDGSEEPG
        70         80         90        100        110        120
SETSDAKSTP TAEAEEAGIG DTPSLEDEAA GHVTQARMVS KSKDGTGSDD KKAKGADGKT
       130        140        150        160        170        180
KIATPRGAAP PGQKGQANAT RIPAKTPPAP KTPPSSGEPP KSGDRSGYSS PGSPGTPGSR
       190        200        210        220        230        240
SRTPSLPTPP TREPKKVAVV RTPPKSPSSA KSRLQTAPVP MPDLKNVKSK IGSTENLKHQ
       250        260        270        280        290        300
PGGGKVQIIN KKLDLSNVQS KCGSKDNIKH VPGGGSVQIV YKPVDLSKVT SKCGSLGNIH
       310        320        330        340        350        360
HKPGGGQVEV KSEKLDFKDR VQSKIGSLDN ITHVPGGGNK KIETHKLTFR ENAKAKTDHG
       370        380        390        400        410
AEIVYKSPVV SGDTSPRHLS NVSSTGSIDM VDSPQLATLA DEVSASLAKQ GL
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P10636-6 (SEQ ID NO:3)

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        10         20         30         40         50         60
MAEPRQEFEV MEDHAGTYGL GDRKDQGGYT MHQDQEGDTD AGLKAEEAGI GDTPSLEDEA
        70         80         90        100        110        120
AGHVTQARMV SKSKDGTGSD DKKAKGADGK TKIATPRGAA PPGQKGQANA TRIPAKTPPA
       130        140        150        160        170        180
PKTPPSSGEP PKSGDRSGYS SPGSPGTPGS RSRTPSLPTP PTREPKKVAV VRTPPKSPSS
       190        200        210        220        230        240
AKSRLQTAPV PMPDLKNVKS KIGSTENLKH QPGGGKVQII NKKLDLSNVQ SKCGSKDNIK
       250        260        270        280        290        300
HVPGGGSVQI VYKPVDLSKV TSKCGSLGNI HHKPGGGQVE VKSEKLDFKD RVQSKIGSLD
       310        320        330        340        350        360
NITHVPGGGN KKIETHKLTF RENAKAKTDH GAEIVYKSPV VSGDTSPRHL SNVSSTGSID
       370        380
MVDSPQLATL ADEVSASLAK QGL
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P10636-5 (SEQ ID NO:4)

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        10         20         30         40         50         60
MAEPRQEFEV MEDHAGTYGL GDRKDQGGYT MHQDQEGDTD AGLKESPLQT PTEDGSEEPG
        70         80         90        100        110        120
SETSDAKSTP TAEDVTAPLV DEGAPGKQAA AQPHTEIPEG TTAEEAGIGD TPSLEDEAAG
       130        140        150        160        170        180
HVTQARMVSK SKDGTGSDDK KAKGADGKTK IATPRGAAPP GQKGQANATR IPAKTPPAPK
       190        200        210        220        230        240
TPPSSGEPPK SGDRSGYSSP GSPGTPGSRS RTPSLPTPPT REPKKVAVVR TPPKSPSSAK
       250        260        270        280        290        300
SRLQTAPVPM PDLKNVKSKI GSTENLKHQP GGGKVQIVYK PVDLSKVTSK CGSLGNIHHK
       310        320        330        340        350        360
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PGGGQVEVKS EKLDFKDRVQ SKIGSLDNIT HVPGGGNKKI ETHKLTFREN AKAKTDHGAE
       370        380        390        400        410
IVYKSPVVSG DTSPRHLSNV SSTGSIDMVD SPQLATLADE VSASLAKQGL
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P10636-4 (SEQ ID NO:5)

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        10         20         30         40         50         60
MAEPRQEFEV MEDHAGTYGL GDRKDQGGYT MHQDQEGDTD AGLKESPLQT PTEDGSEEPG
        70         80         90        100        110        120
SETSDAKSTP TAEAEEAGIG DTPSLEDEAA GHVTQARMVS KSKDGTGSDD KKAKGADGKT
       130        140        150        160        170        180
KIATPRGAAP PGQKGQANAT RIPAKTPPAP KTPPSSGEPP KSGDRSGYSS PGSPGTPGSR
       190        200        210        220        230        240
SRTPSLPTPP TREPKKVAVV RTPPKSPSSA KSRLQTAPVP MPDLKNVKSK IGSTENLKHQ
       250        260        270        280        290        300
PGGGKVQIVY KPVDLSKVTS KCGSLGNIHH KPGGGQVEVK SEKLDFKDRV QSKIGSLDNI
       310        320        330        340        350        360
THVPGGGNKK IETHKLTFRE NAKAKTDHGA EIVYKSPVVS GDTSPRHLSN VSSTGSIDMV
       370        380
DSPQLATLAD EVSASLAKQG L
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P10636-2 (SEQ ID NO:6)

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10         20         30         40         50         60
MAEPRQEFEV MEDHAGTYGL GDRKDQGGYT MHQDQEGDTD AGLKAEEAGI GDTPSLEDEA

        70         80         90        100        110        120
AGHVTQARMV SKSKDGTGSD DKKAKGADGK TKIATPRGAA PPGQKGQANA TRIPAKTPPA

       130        140        150        160        170        180
PKTPPSSGEP PKSGDRSGYS SPGSPGTPGS RSRTPSLPTP PTREPKKVAV VRTPPKSPSS

       190        200        210        220        230        240
AKSRLQTAPV PMPDLKNVKS KIGSTENLKH QPGGGKVQIV YKPVDLSKVT SKCGSLGNIH

       250        260        270        280        290        300
HKPGGGQVEV KSEKLDFKDR VQSKIGSLDN ITHVPGGGNK KIETHKLTFR ENAKAKTDHG

       310        320        330        340        350
AEIVYKSPVV SGDTSPRHLS NVSSTGSIDM VDSPQLATLA DEVSASLAKQ GL
```

[0057] Reference to tau includes known natural variations about 30 of which are listed in the Swiss-Pro database and permutations thereof, as well as mutations associated with tau pathologies, such as dementia, Pick's disease, supranuclear palsy, etc. (see, *e.g.*, Swiss-Pro database and Poorkaj, et al. Ann Neurol. 43:815-825 (1998)). Some examples of tau mutations numbered by the 441 isoform are a lysine to threonine mutation at amino acid residue 257 (K257T), an isoleucine to valine mutation at amino acid position 260 (I260V); a glycine to valine mutation at amino acid position 272 (G272V); an asparagine to lysine mutation at amino acid position 279 (N279K); an asparagine to histidine mutation at amino acid position 296 (N296H);

a proline to serine mutation at amino acid position 301 (P301S); a glycine to valine mutation at amino acid position 303 (G303V); a serine to asparagine mutation at position 305 (S305N); a glycine to serine mutation at amino acid position 335 (G335S); a valine to methionine mutation at position 337 (V337M); a glutamic acid to valine mutation at position 342 (E342V); a lysine to isoleucine mutation at amino acid position 369 (K3691); a glycine to arginine mutation at amino acid position 389 (G389R); and an arginine to tryptophan mutation at amino acid position 406 (R406W).

[0058] Tau can be phosphorylated at one or more amino acid residues including tyrosine at amino acid positions 18, 29, 97, 310, and 394 serine at amino acid positions 184, 185, 198, 199, 202, 208, 214, 235, 237, 238, 262, 293, 324, 356, 396, 400, 404, 409, 412, 413, and 422; and threonine at amino acids positions 175, 181, 205, 212, 217, 231, and 403.

III. Antibodies

A. Binding specificity and functional properties

[0059] The invention provides antibodies that bind to tau. Some antibodies specifically bind to an epitope within residues 23-46 of SEQ ID NO:1. Some antibodies specifically bind to an epitope within residues 25-44 of SEQ ID NO:1. Some antibodies specifically bind to an epitope within 28-41 of SEQ ID NO:1. Some antibodies specifically bind to an epitope within residues 30-39 of SEQ ID NO:1. Some antibodies specifically bind to an epitope within residues 30-36 of SEQ ID NO:1. Some antibodies specifically bind to an epitope within residues 33-39 of SEQ ID NO:1. Some antibodies specifically bind to an epitope within residues 33-36 of SEQ ID NO:1. Some antibodies specifically bind to an epitope including residues 28-30, 28-31, 28-32, 28-33, 28-34, 28-35, 28-36, 28-37, 28-38, 28-39, 28-40, 28-41, 29-31, 29-32, 29-33, 29-34, 29-35, 29-36, 29-37, 29-38, 29-39, 29-40, 29-41, 30-32, 30-33, 30-34, 30-35, 30-36, 30-37, 30-38, 30-39, 30-40, 30-41, 31-33, 31-34, 31-35, 31-36, 31-37, 31-38, 31-39, 31-40, 31-41, 32-34, 32-35, 32-36, 32-37, 32-38, 32-39, 32-40, 32-41, 33-35, 33-36, 33-37, 33-38, 33-39, 33-40, 33-41, 34-36, 34-37, 34-38, 34-39, 34-40, 34-41, 35-37, 35-38, 35-39, 35-40, 35-41, 36-38, 36-39, 36-40, 36-41 of SEQ ID NO:1. Some antibodies bind to tau irrespective of phosphorylation state. Some antibodies bind to an epitope not including a residue subject to phosphorylation. These antibodies can be obtained by immunizing with a tau polypeptide purified from a natural source or recombinantly expressed. Antibodies can be screened for binding tau in unphosphorylated form as well as a form in which one or more residues susceptible to phosphorylation are

phosphorylated. Such antibodies preferably bind with indistinguishable affinities or at least within a factor of 1.5, 2 or 3-fold to phosphorylated tau compared to non-phosphorylated tau (*i.e.*, are "pan-specific). 16B5 is an example of a pan-specific monoclonal antibody. The invention also provides antibodies binding to the same epitope as any of the foregoing antibodies, such as, for example, the epitope of 16B5. Also included are antibodies competing for binding to tau with any of the foregoing antibodies, such as, for example, competing with 16B5.

[0060] The above-mentioned antibodies can be generated de novo by immunizing with a peptide including residues 23-46, 25-44, 28-30, 28-31, 28-32, 28-33, 28-34, 28-35, 28-36, 28-37, 28-38, 28-39, 28-40, 28-41, 29-31, 29-32, 29-33, 29-34, 29-35, 29-36, 29-37, 29-38, 29-39, 29-40, 29-41, 30-32, 30-33, 30-34, 30-35, 30-36, 30-37, 30-38, 30-39, 30-40, 30-41, 31-33, 31-34, 31-35, 31-36, 31-37, 31-38, 31-39, 31-40, 31-41, 32-34, 32-35, 32-36, 32-37, 32-38, 32-39, 32-40, 32-41, 33-35, 33-36, 33-37, 33-38, 33-39, 33-40, 33-41, 34-36, 34-37, 34-38, 34-39, 34-40, 34-41, 35-37, 35-38, 35-39, 35-40, 35-41, 36-38, 36-39, 36-40, 36-41 of SEQ ID NO:1 or by immunizing with a full length tau polypeptide or fragment thereof comprising such residues and screening for specific binding to a peptide including such residues. Such peptides are preferably attached to a heterologous conjugate molecule that helps elicit an antibody response to the peptide. Attachment can be direct or via a spacer peptide or amino acid. Cysteine is used as a spacer amino acid because its free SH group facilitates attachment of a carrier molecule. A polyglycine linker (*e.g.*, 2-6 glycines), with or without a cysteine residue between the glycines and the peptide can also be used. The carrier molecule serves to provide a T-cell epitope that helps elicit an antibody response against the peptide. Several carriers are commonly used particularly keyhole limpet hemocyanin (KLH), ovalbumin and bovine serum albumin (BSA). Peptide spacers can be added to peptide immunogen as part of solid phase peptide synthesis. Carriers are typically added by chemical cross-linking. Some examples of chemical crosslinkers that can be used include cross-N-maleimido-6-aminocaproyl ester or m-maleimidobenzoyl-N-hydroxysuccinimide ester (MBS) (see for example, Harlow, E. et al., Antibodies: A Laboratory Manual, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y. 1988; Sinigaglia et al., Nature, 336:778-780 (1988); Chicz et al., J. Exp. Med., 178:27-47 (1993); Hammer et al., Cell 74:197-203 (1993); Falk K. et al., Immunogenetics, 39:230-242 (1994); WO 98/23635; and, Southwood et al. J. Immunology, 160:3363-3373 (1998)). The carrier and spacer if present can be attached to either end of the immunogen.

[0061] A peptide with optional spacer and carrier can be used to immunize laboratory animals or B-cells as described in more detail below. Hybridoma supernatants can be tested for ability to bind one or more peptides including residues 24-46, 25-44, 28-30, 28-31, 28-32, 28-33, 28-34, 28-35, 28-36, 28-37, 28-38, 28-39, 28-40, 28-41, 29-31, 29-32, 29-33, 29-34, 29-35, 29-36, 29-37, 29-38, 29-39, 29-40, 29-41, 30-32, 30-33, 30-34, 30-35, 30-36, 30-37, 30-38, 30-39, 30-40, 30-41, 31-33, 31-34, 31-35, 31-36, 31-37, 31-38, 31-39, 31-40, 31-41, 32-34, 32-35, 32-36, 32-37, 32-38, 32-39, 32-40, 32-41, 33-35, 33-36, 33-37, 33-38, 33-39, 33-40, 33-41, 34-36, 34-37, 34-38, 34-39, 34-40, 34-41, 35-37, 35-38, 35-39, 35-40, 35-41, 36-38, 36-39, 36-40, 36-41 of SEQ ID NO:1 and/or phosphorylated and non-phosphorylated forms of tau, such as, for example, a full-length isoform of tau with position 404 in phosphorylated form. The peptide can be attached to a carrier or other tag to facilitate the screening assay. In this case, the carrier or tag is preferentially different than the combination of spacer and carrier molecule used for immunization to eliminate antibodies specific for the spacer or carrier rather than the tau peptide. Any of the tau isoforms can be used.

[0062] Antibodies having the binding specificity of a selected murine antibody (*e.g.* 16B5) can also be produced using a variant of the phage display method. See Winter, WO 92/20791. This method is particularly suitable for producing human antibodies. In this method, either the heavy or light chain variable region of the selected murine antibody is used as a starting material. If, for example, a light chain variable region is selected as the starting material, a phage library is constructed in which members display the same light chain variable region (*i.e.*, the murine starting material) and a different heavy chain variable region. The heavy chain variable regions can for example be obtained from a library of rearranged human heavy chain variable regions. A phage showing strong specific binding for the desired target (*e.g.*, a tau peptide) (*e.g.*, at least 10^8 and preferably at least 10^9 M^{-1}) is selected. The heavy chain variable region from this phage then serves as a starting material for constructing a further phage library. In this library, each phage displays the same heavy chain variable region (*i.e.*, the region identified from the first display library) and a different light chain variable region. The light chain variable regions can be obtained for example from a library of rearranged human variable light chain regions. Again, phage showing strong specific binding for the desired target are selected. The resulting antibodies usually have the same or similar epitope specificity as the murine starting material.

[0063] Other antibodies can be obtained by mutagenesis of cDNA encoding the heavy and light chains of an exemplary antibody, such as 16B5. Monoclonal antibodies that are at least

90%, 95% or 99% identical to 16B5 in amino acid sequence of the mature heavy and/or light chain variable regions and maintain its functional properties, and/or which differ from the respective antibody by a small number of functionally inconsequential amino acid substitutions (*e.g.*, conservative substitutions), deletions, or insertions are also included in the invention. Monoclonal antibodies having at least one and preferably all six CDR(s) as defined by Kabat that are 90%, 95%, 99% or 100% identical to corresponding CDRs of 16B5 are also included.

B. Non-human antibodies

[0064] The production of other non-human monoclonal antibodies, *e.g.*, murine, guinea pig, primate, rabbit or rat, against an immunogen can be performed by, for example, immunizing the animal with an immunogen as described above. See Harlow & Lane, *Antibodies, A Laboratory Manual* (CSHP NY, 1988) (incorporated by reference for all purposes). Such an immunogen can be obtained from a natural source, by peptide synthesis or by recombinant expression.

[0065] Optionally, the immunogen can be administered with an adjuvant. Several types of adjuvant can be used as described below. Complete Freund's adjuvant followed by incomplete adjuvant is preferred for immunization of laboratory animals. Rabbits or guinea pigs are typically used for making polyclonal antibodies. Mice are typically used for making monoclonal antibodies. Antibodies are screened for specific binding to Optionally, antibodies are further screened for binding to a specific region of tau. Such screening can be accomplished by determining binding of an antibody to a collection of deletion mutants of tau and determining which deletion mutants bind to the antibody. Binding can be assessed, for example, by Western blot, FACS™ or ELISA.

C. Humanized antibodies

[0066] A humanized antibody is a genetically engineered antibody in which the CDRs from a non-human "donor" antibody (*e.g.*, 16B5) are grafted into human "acceptor" antibody sequences (see, *e.g.*, Queen, US 5,530,101 and 5,585,089; Winter, US 5,225,539, Carter, US 6,407,213, Adair, US 5,859,205 6,881,557, Foote, US 6,881,557). The acceptor antibody sequences can be, for example, a mature human antibody sequence, a composite of such sequences, a consensus sequence of human antibody sequences, or a germline region sequence. Thus, a humanized antibody is an antibody having some or all CDRs entirely or substantially from a donor antibody and variable region framework sequences and constant regions, if present, entirely or

substantially from human antibody sequences. Similarly a humanized heavy chain has at least one, two and usually all three CDRs entirely or substantially from a donor antibody heavy chain, and a heavy chain variable region framework sequence and heavy chain constant region, if present, substantially from human heavy chain variable region framework and constant region sequences. Similarly a humanized light chain has at least one, two and usually all three CDRs entirely or substantially from a donor antibody light chain, and a light chain variable region framework sequence and light chain constant region, if present, substantially from human light chain variable region framework and constant region sequences. Other than nanobodies and dAbs, a humanized antibody comprises a humanized heavy chain and a humanized light chain. A CDR in a humanized antibody is substantially from a corresponding CDR in a non-human antibody when at least 85%, 90%, 95% or 100% of corresponding residues (as defined by Kabat) are identical between the respective CDRs. The variable region framework sequences of an antibody chain or the constant region of an antibody chain are substantially from a human variable region framework sequence or human constant region respectively when at least 85, 90, 95 or 100% of corresponding residues defined by Kabat are identical.

[0067] Although humanized antibodies often incorporate all six CDRs (preferably as defined by Kabat) from a mouse antibody, they can also be made with less than all CDRs (*e.g.*, at least 3, 4, or 5) CDRs from a mouse antibody (*e.g.*, Pascalis *et al.*, J. Immunol. 169:3076, 2002; Vajdos et al., Journal of Molecular Biology, 320: 415-428, 2002; Iwahashi et al., Mol. Immunol. 36:1079-1091, 1999; Tamura et al, Journal of Immunology, 164:1432-1441, 2000).

[0068] In some antibodies only part of the CDRs, namely the subset of CDR residues required for binding, termed the SDRs, are needed to retain binding in a humanized antibody. CDR residues not contacting antigen and not in the SDRs can be identified based on previous studies (for example residues H60-H65 in CDR H2 are often not required), from regions of Kabat CDRs lying outside Chothia hypervariable loops (Chothia, J. Mol. Biol. 196:901, 1987), by molecular modeling and/or empirically, or as described in Gonzales et al., Mol. Immunol. 41: 863, 2004. In such humanized antibodies at positions in which one or more donor CDR residues is absent or in which an entire donor CR is omitted, the amino acid occupying the position can be an amino acid occupying the corresponding position (by Kabat numbering) in the acceptor antibody sequence. The number of such substitutions of acceptor for donor amino acids in the CDRs to include reflects a balance of competing considerations. Such substitutions are potentially advantageous in decreasing the number of mouse amino acids in a humanized antibody and

consequently decreasing potential immunogenicity. However, substitutions can also cause changes of affinity, and significant reductions in affinity are preferably avoided. Positions for substitution within CDRs and amino acids to substitute can also be selected empirically.

[0069] The human acceptor antibody sequences can optionally be selected from among the many known human antibody sequences to provide a high degree of sequence identity (*e.g.*, 65-85% identity) between a human acceptor sequence variable region frameworks and corresponding variable region frameworks of a donor antibody chain.

[0070] Certain amino acids from the human variable region framework residues can be selected for substitution based on their possible influence on CDR conformation and/or binding to antigen. Investigation of such possible influences is by modeling, examination of the characteristics of the amino acids at particular locations, or empirical observation of the effects of substitution or mutagenesis of particular amino acids.

[0071] For example, when an amino acid differs between a murine variable region framework residue and a selected human variable region framework residue, the human framework amino acid can be substituted by the equivalent framework amino acid from the mouse antibody when it is reasonably expected that the amino acid:

(1) noncovalently binds antigen directly,

(2) is adjacent to a CDR region,

(3) otherwise interacts with a CDR region (*e.g.* is within about 6 Å of a CDR region), (*e.g.*, identified by modeling the light or heavy chain on the solved structure of a homologous known immunoglobulin chain); and

(4) a residue participating in the VL-VH interface.

[0072] Framework residues from classes (1)-(3) as defined by Queen, US 5,530, 101 are sometimes alternately referred to as canonical and vernier residues. Framework residues defining canonical class of the donor CDR loops determining the conformation of a CDR loop are sometimes referred to as canonical residues (Chothia and Lesk, J. Mol. Biol. 196, 901-917 (1987), Thornton & Martin *J. Mol. Biol.*, 263, 800-815, 1996). A layer of framework residues that support antigen-binding loop conformations play a role in fine-tuning the fit of an antibody to antigen are sometimes referred to as vernier residues (Foote & Winter, 1992, *J Mol Bio.* 224, 487–499). Other candidates for substitution are residues creating a potential glycosylation site. Other candidates for substitution are acceptor human framework amino acids that are unusual for

a human immunoglobulin at that position. These amino acids can be substituted with amino acids from the equivalent position of the mouse donor antibody or from the equivalent positions of more typical human immunoglobulins. Other candidates for substitution are acceptor human framework amino acids that are unusual for a human immunoglobulin at that position.

[0073] The invention provides humanized forms of the mouse 16B5 antibody. The mouse antibody comprises mature heavy and light chain variable regions having amino acid sequences comprising SEQ ID NOS. 10 and 16 respectively. The invention provides one exemplified humanized mature heavy chain variable regions (H1) and three exemplified humanized mature light chain variable region (L1, L2 and L3). The H1L2 variant has the same or better affinity as a chimeric 16B5 and seven backmutations. H1L1 and H1L3 have similar affinity to chimeric 16B5 and six backmutations

[0074] The invention provides variants of the H1L2 humanized 16B5 antibody in which the humanized mature heavy chain variable region shows at least 90%, 95%, 96%, 97%, 98% or 99% identity to SEQ ID NO:15 and the humanized mature light chain mature variable region shows at least 90%, 95%, 96%, 97% 98% or 99% sequence identity to SEQ ID NO:22. Preferably, in such antibodies some or all of the backmutations in H1L2 are retained. In other words, at least 1, 2, or 3 of positions position H13 is occupied by K, position H48 is occupied by M and position H91 is occupied by F. Preferably at least, 1, 2, 3 or all four positions position L1 is occupied by N, position L4 is occupied by L, position L36 is occupied by F and position L43 is occupied by S. The CDR regions of such humanized antibodies are preferably identical or substantially identical to the CDR regions of H1L2, which are the same as those of the mouse donor antibody. The CDR regions can be defined by any conventional definition (*e.g.*, Chothia) but are preferably as defined by Kabat.

[0075] One possibility for additional variation in 16B5 variants is additional backmutations in the variable region frameworks. Many of the framework residues not in contact with the CDRs in the humanized mAb can accommodate substitutions of amino acids from the corresponding positions of the donor mouse mAb or other mouse or human antibodies, and even many potential CDR-contact residues are also amenable to substitution or even amino acids within the CDRs may be altered, for example, with residues found at the corresponding position of the human acceptor sequence used to supply variable region frameworks. In addition, alternate human acceptor sequences can be used, for example, for the heavy and/or light chain. If different

acceptor sequences are used, one or more of the backmutations recommended above may not be performed because the corresponding donor and acceptor residues are already the same without backmutation. For example, when using a heavy chain acceptor sequence in which position H13 is already occupied by K no backmutation is necessary.

[0076] The invention also includes humanized antibodies in which the mature light and heavy chain variable regions shows at least 90, 95, 96, 97, 98 or 99% sequence identity to the mature light and heavy chain variable regions of the humanized 16B5 H1L1 antibody (SEQ ID NOs: 21 and 15, respectively) or the humanized 16B5 H1L3 antibody (SEQ ID NOs: 23 and 15, respectively).

D. Chimeric and Veneered Antibodies

[0077] The invention further provides chimeric and veneered forms of non-human antibodies, particularly 16B5 antibody.

[0078] A chimeric antibody is an antibody in which the mature variable regions of light and heavy chains of a non-human antibody (*e.g.*, a mouse) are combined with human light and heavy chain constant regions. Such antibodies substantially or entirely retain the binding specificity of the mouse antibody, and are about two-thirds human sequence.

[0079] A veneered antibody is a type of humanized antibody that retains some and usually all of the CDRs and some of the non-human variable region framework residues of a non-human antibody but replaces other variable region framework residues that may contribute to B- or T-cell epitopes, for example exposed residues (Padlan, Mol. Immunol. 28:489, 1991) with residues from the corresponding positions of a human antibody sequence. The result is an antibody in which the CDRs are entirely or substantially from a non-human antibody and the variable region frameworks of the non-human antibody are made more human-like by the substitutions. Veneered forms of either the 16B5 antibody are included in the invention.

E. Human antibodies

[0080] Human antibodies against tau are provided by a variety of techniques described below. Methods for producing human antibodies include the trioma method of Oestberg et al., *Hybridoma* 2:361-367 (1983); Oestberg, U.S. Patent No. 4,634,664; and Engleman et al., US Patent 4,634,666, use of transgenic mice including human immunoglobulin genes (see, *e.g.*, Lonberg et al., WO93/12227 (1993); US 5,877,397, US 5,874,299, US 5,814,318, US 5,789,650,

US 5,770,429, US 5,661,016, US 5,633,425, US 5,625,126, US 5,569,825, US 5,545,806, *Nature* 148, 1547-1553 (1994), *Nature Biotechnology* 14, 826 (1996), Kucherlapati, WO 91/10741 (1991) and phage display methods (see, *e.g.*, Dower et al., WO 91/17271 and McCafferty et al., WO 92/01047, US 5,877,218, US 5,871,907, US 5,858,657, US 5,837,242, US 5,733,743 and US 5,565,332.

F. Selection of Constant Region

[0081] The heavy and light chain variable regions of chimeric, humanized (including veneered), or human antibodies can be linked to at least a portion of a human constant region. The choice of constant region depends, in part, whether antibody-dependent complement and/or cellular mediated cytotoxicity is desired. For example, human isotopes IgG1 and IgG3 have complement-mediated cytotoxicity whereas human isotypes IgG2 and IgG4 have poor or no complement-mediated cytotoxicity. Light chain constant regions can be lambda or kappa. Antibodies can be expressed as tetramers containing two light and two heavy chains, as separate heavy chains, light chains, as Fab, Fab', F(ab')2, and Fv, or as single chain antibodies in which heavy and light chain variable regions are linked through a spacer.

[0082] Human constant regions show allotypic variation and isoallotypic variation between different individuals, that is, the constant regions can differ in different individuals at one or more polymorphic positions. Isoallotypes differ from allotypes in that sera recognizing an isoallotype binds to a non-polymorphic region of a one or more other isotypes. Reference to a human constant region includes a constant region with any natural allotype or any permutation of residues occupying polymorphic positions in natural allotypes or up to 3, 5 or 10 substitutions for reducing or increasing effector function as described below.

[0083] One or several amino acids at the amino or carboxy terminus of the light and/or heavy chain, such as the C-terminal lysine of the heavy chain, may be missing or derivatized in a proportion or all of the molecules. Substitutions can be made in the constant regions to reduce or increase effector function such as complement-mediated cytotoxicity or ADCC (see, *e.g.*, Winter et al., US Patent No. 5,624,821; Tso et al., US Patent No. 5,834,597; and Lazar et al., Proc. Natl. Acad. Sci. USA 103:4005, 2006), or to prolong half-life in humans (see, *e.g.*, Hinton et al., J. Biol. Chem. 279:6213, 2004). Exemplary substitutions include a Gln at position 250 and/or a Leu at position 428 (EU numbering is used in this paragraph for the constant region) for increasing the half-life of an antibody. Substitution at any or all of positions 234, 235, 236

and/or 237 reduce affinity for Fcγ receptors, particularly FcγRI receptor (*see, e.g.*, US 6,624,821). An alanine substitution at positions 234, 235 and 237 of human IgG1 is preferred for reducing effector functions. Optionally, positions 234, 236 and/or 237 in human IgG2 are substituted with alanine and position 235 with glutamine. (*See, e.g.*, US 5,624,821.)

G. Expression of Recombinant Antibodies

[0084] Chimeric, humanized (including veneered) and human antibodies are typically produced by recombinant expression. Nucleic acids encoding the antibodies can be codon-optimized for expression in the desired cell-type (*e.g.*, CHO or Sp2/0). Nucleic acids encoding the humanized 16B5 heavy and light chain variable regions disclosed herein have sequences comprising or consisting of, for example, SEQ ID NO: 25 (encoding Hu16B5 H1), SEQ ID NO: 26 (encoding Hu16B5 L1), SEQ ID NO: 27 (encoding Hu16B5 L2), or SEQ ID NO: 28 (encoding Hu16B5 L3). For variable regions including signal peptides such as SEQ ID NOS. 10 and 16, the nucleic acid can encode the variable region with or without the signal peptide. Nucleic acid segments encoding heavy and light chain can be present on the same contiguous nucleic acid molecule or on separate molecules. The heavy and light chains can be expressed from the same vector or from different vectors. Nucleic acids are typically provided in isolated form.

[0085] Nucleic acids encoding a humanized 16B5 heavy chain variable region can be linked to a nucleic acid segment encoding a human IgG1 constant region, *e.g.*, having the sequence of SEQ ID NO: 30. Such nucleic acids can also include an intron located between the segments encoding the heavy chain variable region and the IgG1 constant region, *i.e.*, 5' to the segment encoding the constant region. An exemplary nucleic acid sequence encoding a human IgG1 constant region and having a mouse intron at its 5' end is shown in SEQ ID NO: 31.

[0086] Nucleic acids encoding humanized 16B5 light chain variable regions can be linked to a nucleic acid segment encoding a human kappa constant region, *e.g.*, having the sequence of SEQ ID NO: 33. Such nucleic acids can also include an intron between the segments encoding the light chain variable region and the kappa constant region (*i.e.*, 5' to the kappa constant region). An exemplary nucleic acid sequence encoding a human kappa constant region and having a human intron at its 5' end is shown in SEQ ID NO: 34.

[0087] Recombinant polynucleotide constructs typically include an expression control sequence operably linked to the coding sequences of antibody chains, including naturally-associated or heterologous promoter regions. Preferably, the expression control sequences are eukaryotic promoter systems in vectors capable of transforming or transfecting eukaryotic host cells. Once the vector has been incorporated into the appropriate host, the host is maintained under conditions suitable for high level expression of the nucleotide sequences, and the collection and purification of the crossreacting antibodies. The vector or vectors encoding the antibody chains can also contain a selectable gene, such as dihydrofolate reductase or glutamine synthase, to allow amplification of copy number of the nucleic acids encoding the antibody chains.

[0088] E. coli is a prokaryotic host particularly useful for expressing antibodies, particularly antibody fragments. Microbes, such as yeast are also useful for expression. Saccharomyces is a preferred yeast host, with suitable vectors having expression control sequences, an origin of replication, termination sequences and the like as desired. Typical promoters include 3-phosphoglycerate kinase and other glycolytic enzymes. Inducible yeast promoters include, among others, promoters from alcohol dehydrogenase, isocytochrome C, and enzymes responsible for maltose and galactose utilizations.

[0089] Mammalian cells are a preferred host for expressing nucleotide segments encoding immunoglobulins or fragments thereof. See Winnacker, From Genes to Clones, (VCH Publishers, NY, 1987). A number of suitable host cell lines capable of secreting intact heterologous proteins have been developed in the art, and include CHO cell lines, various COS cell lines, HeLa cells, HEK293 cells, L cells, and non-antibody-producing myelomas including Sp2/0 and NS0. Preferably, the cells are nonhuman. Expression vectors for these cells can include expression control sequences, such as an origin of replication, a promoter, an enhancer (Queen et al., *Immunol. Rev.* 89:49 (1986)), and necessary processing information sites, such as ribosome binding sites, RNA splice sites, polyadenylation sites, and transcriptional terminator sequences. Preferred expression control sequences are promoters derived from endogenous genes, cytomegalovirus, SV40, adenovirus, bovine papillomavirus, and the like. See Co et al., *J. Immunol.* 148:1149 (1992).

[0090] Having introduced vector(s) encoding antibody heavy and light chains into cell culture, cell pools can be screened for growth productivity and product quality in serum-free media.

Top-producing cell pools can then be subjected ot FACS-based single-cell cloning to generate monoclonal lines. Specific productivites above 50 pg or 100 pg per cell per day, which correspond to product titers of greater than 7.5 g/L culture, are preferred. Antibodies produced by single cell clones can also be tested for turbidity, filtration properties, PAGE, IEF, UV scan, HP–SEC, carboydrate-oligosaccharide mapping, mass spectrometery, and bining assay, such as ELISA or Biacore. A selected clone can then be banked in multiple vials and stored frozen for subsequent use.

[0091] Once expressed, antibodies can be purified according to standard procedures of the art, including protein A capture, column chromatography (*e.g.*, hydrophobic interaction or ion exchange), low-pH for viral inactivation and the like (see generally, Scopes, *Protein Purification* (Springer-Verlag, NY, 1982)).

[0092] Methodology for commercial production of antibodies can be employed, including codon optimization, selection of promoters, transcription elements, and terminators, serum-free single cell cloning, cell banking, use of selection markers for amplification of copy number, CHO terminator, serum free single cell cloning, improvement of protein titers (see, *e.g.*, US 5,786,464, US 6,114,148, US 6,063,598, US 7,569,339, WO2004/050884, WO2008/012142, WO2008/012142, WO2005/019442, WO2008/107388, and WO2009/027471, and US 5,888,809).

IV. Active Immunogens

[0093] An agent used for active immunization serves to induce in a patient the same types of antibody described in connection with passive immunization above. Agents used for active immunization can be the same types of immunogens used for generating monoclonal antibodies in laboratory animals, *e.g.*, a peptide of 3-15 or 3-12 or 5-12, or 5-8 contiguous amino acids from a region of tau corresponding to residues 23-46, 25-44, 28-41 or 30-39 of SEQ ID NO. 1, such as, for example, a peptide including residues 28-30, 28-31, 28-32, 28-33, 28-34, 28-35, 28-36, 28-37, 28-38, 28-39, 28-40, 28-41, 29-31, 29-32, 29-33, 29-34, 29-35, 29-36, 29-37, 29-38, 29-39, 29-40, 29-41, 30-32, 30-33, 30-34, 30-35, 30-36, 30-37, 30-38, 30-39, 30-40, 30-41, 31-33, 31-34, 31-35, 31-36, 31-37, 31-38, 31-39, 31-40, 31-41, 32-34, 32-35, 32-36, 32-37, 32-38, 32-39, 32-40, 32-41, 33-35, 33-36, 33-37, 33-38, 33-39, 33-40, 33-41, 34-36, 34-37, 34-38, 34-39, 34-40, 34-41, 35-37, 35-38, 35-39, 35-40, 35-41, 36-38, 36-39, 36-40, 36-41 of SEQ ID NO:1. For inducing antibodies binding to the same or overlapping epitope as 16B5, the epitope

specificity of these antibodies can be mapped (*e.g.*, by testing binding to a series of overlapping peptides spanning tau). A fragment of tau consisting of or including or overlapping the epitope can then be used as an immunogen. Such fragments are typically used in unphosphorylated form.

[0094] The heterologous carrier and adjuvant, if used may be the same as used for generating monoclonal antibody, but may also be selected for better pharmaceutical suitability for use in humans. Suitable carriers include serum albumins, keyhole limpet hemocyanin, immunoglobulin molecules, thyroglobulin, ovalbumin, tetanus toxoid, or a toxoid from other pathogenic bacteria, such as diphtheria (*e.g.*, CRM197), *E. coli,* cholera, or *H. pylori*, or an attenuated toxin derivative. T cell epitopes are also suitable carrier molecules. Some conjugates can be formed by linking agents of the invention to an immunostimulatory polymer molecule (*e.g.*, tripalmitoyl-S-glycerine cysteine (Pam_3Cys), mannan (a mannose polymer), or glucan (a β 1→2 polymer)), cytokines (*e.g.*, IL-1, IL-1 alpha and β peptides, IL-2, γ-INF, IL-10, GM-CSF), and chemokines (*e.g.*, MIP1-α and β, and RANTES). Immunogens may be linked to the carriers with or without spacers amino acids (*e.g.*, gly-gly). Additional carriers include virus-like particles. Virus-like particles (VLPs), also called pseudovirions or virus-derived particles, represent subunit structures composed of multiple copies of a viral capsid and/or envelope protein capable of self-assembly into VLPs of defined spherical symmetry in vivo. (Powilleit, et al., (2007) PLoS ONE 2(5):e415.) Alternatively, peptide immunogens can be linked to at least one artificial T-cell epitope capable of binding a large proportion of MHC Class II molecules., such as the pan DR epitope ("PADRE"). PADRE is described in US 5,736,142, WO 95/07707, and Alexander J et al, Immunity, 1:751-761 (1994). Active immunogens can be presented in multimeric form in which multiple copies of an immunogen and/or its carrier are presented as a single covalent molecule.

[0095] Fragments are often administered with pharmaceutically acceptable adjuvants. The adjuvant increases the titer of induced antibodies and/or the binding affinity of induced antibodies relative to the situation if the peptide were used alone. A variety of adjuvants can be used in combination with an immunogenic fragment of tau to elicit an immune response. Preferred adjuvants augment the intrinsic response to an immunogen without causing conformational changes in the immunogen that affect the qualitative form of the response. Preferred adjuvants include aluminum salts, such aluminum hydroxide and aluminum phosphate, 3 De-O-acylated monophosphoryl lipid A (MPL™) (*see* GB 2220211 (RIBI ImmunoChem Research Inc., Hamilton, Montana, now part of Corixa). Stimulon™ QS-21 is a

triterpene glycoside or saponin isolated from the bark of the Quillaja Saponaria Molina tree found in South America (*see* Kensil *et al.*, in *Vaccine Design: The Subunit and Adjuvant Approach* (eds. Powell & Newman, Plenum Press, NY, 1995); US 5,057,540), (Aquila BioPharmaceuticals, Framingham, MA; now Antigenics, Inc., New York, NY). Other adjuvants are oil in water emulsions (such as squalene or peanut oil), optionally in combination with immune stimulants, such as monophosphoryl lipid A (*see* Stoute *et al.*, *N. Engl. J. Med.* 336, 86-91 (1997)), pluronic polymers, and killed mycobacteria. Ribi adjuvants are oil-in-water emulsions. Ribi contains a metabolizable oil (squalene) emulsified with saline containing Tween 80. Ribi also contains refined mycobacterial products which act as immunostimulants and bacterial monophosphoryl lipid A. Another adjuvant is CpG (WO 98/40100). Adjuvants can be administered as a component of a therapeutic composition with an active agent or can be administered separately, before, concurrently with, or after administration of the therapeutic agent.

[0096] Analogs of natural fragments of tau that induce antibodies against tau can also be used. For example, one or more or all L-amino acids can be substituted with D amino acids in such peptides. Also the order of amino acids can be reversed (retro peptide). Optionally a peptide includes all D-amino acids in reverse order (retro-inverso peptide). Peptides and other compounds that do not necessarily have a significant amino acid sequence similarity with tau peptides but nevertheless serve as mimetics of tau peptides and induce a similar immune response. Anti- idiotypic antibodies against monoclonal antibodies to tau as described above can also be used. Such anti-Id antibodies mimic the antigen and generate an immune response to it (see Essential Immunology, Roit ed., Blackwell Scientific Publications, Palo Alto, CA 6th ed., p. 181).

[0097] Peptides (and optionally a carrier fused to the peptide) can also be administered in the form of a nucleic acid encoding the peptide and expressed in situ in a patient. A nucleic acid segment encoding an immunogen is typically linked to regulatory elements, such as a promoter and enhancer that allow expression of the DNA segment in the intended target cells of a patient. For expression in blood cells, as is desirable for induction of an immune response, promoter and enhancer elements from light or heavy chain immunoglobulin genes or the CMV major intermediate early promoter and enhancer are suitable to direct expression. The linked regulatory elements and coding sequences are often cloned into a vector. Antibodies can also be administered in the form of nucleic acids encoding the antibody heavy and/or light chains. If

both heavy and light chains are present, the chains are preferably linked as a single chain antibody. Antibodies for passive administration can also be prepared *e.g.*, by affinity chromatography from sera of patients treated with peptide immunogens.

[0098] The DNA can be delivered in naked form (*i.e.*, without colloidal or encapsulating materials). Alternatively a number of viral vector systems can be used including retroviral systems (see, *e.g.*, Lawrie and Tumin, Cur. Opin. Genet. Develop. 3, 102-109 (1993)); adenoviral vectors {see, *e.g.*, Bett et al, J. Virol. 67, 591 1 (1993)); adeno-associated virus vectors {see, *e.g.*, Zhou et al., J. Exp. Med. 179, 1867 (1994)), viral vectors from the pox family including vaccinia virus and the avian pox viruses, viral vectors from the alpha virus genus such as those derived from Sindbis and Semliki Forest Viruses (see, *e.g.*, Dubensky et al., J. Virol. 70, 508-519 (1996)), Venezuelan equine encephalitis virus (see US 5,643,576) and rhabdoviruses, such as vesicular stomatitis virus (see WO 96/34625)and papillomaviruses (Ohe et al., Human Gene Therapy 6, 325-333 (1995); Woo et al, WO 94/12629 and Xiao & Brandsma, Nucleic Acids. Res. 24, 2630-2622 (1996)).

[0099] DNA encoding an immunogen, or a vector containing the same, can be packaged into liposomes. Suitable lipids and related analogs are described by US 5,208,036, US 5,264,618, US 5,279,833, and US 5,283,185. Vectors and DNA encoding an immunogen can also be adsorbed to or associated with particulate carriers, examples of which include polymethyl methacrylate polymers and polylactides and poly(lactide-co-glycolides), (see, *e.g.*, McGee et al., J. Micro Encap. 1996).

V. Screening methods

[0100] Antibodies can be initially screened for the intended binding specificity as described above. Active immunogens can likewise be screened for capacity to induce antibodies with such binding specificity. In this case, an active immunogen is used to immunize a laboratory animal and the resulting sera tested for the appropriate binding specificity.

[0101] Antibodies having the desired binding specificity can then be tested in cellular and animal models. The cells used for such screening are preferentially neuronal cells. A cellular model of tau pathology has been reported in which neuroblastoma cells are transfected with a four-repeat domain of tau, optionally with a mutation associated with tau pathology (*e.g.*, delta K280, see Khlistunova, Current Alzheimer Research 4, 544-546 (2007)). In another model, tau

is induced in the neuroblastoma N2a cell line by the addition of doxycyclin. The cell models enable one to study the toxicity of tau to cells in the soluble or aggregated state, the appearance of tau aggregates after switching on tau gene expression, the dissolution of tau aggregates after switching the gene expression off again, and the efficiency of antibodies in inhibiting formation of tau aggregates or disaggregating them.

[0102] Antibodies or active immunogens can also be screened in transgenic animal models of diseases associated with tau. Such transgenic animals can include a tau transgene (*e.g.*, any of the human isoforms) and optionally a human APP transgene among others, such as a kinase that phosphorylates tau, ApoE, presenilin or alpha synuclein. Such transgenic animals are disposed to develop at least one sign or symptom of a disease associated with tau.

[0103] An exemplary transgenic animal is the K3 line of mice (Itner et al., Proc. Natl. Acad. Sci. USA 105(41):15997-6002 (2008)). These mice have a human tau transgene with a K 369 I mutation (the mutation is associated with Pick's disease) and a Thy 1.2 promoter. This model shows a rapid course of neurodegeneration, motor deficit and degeneration of afferent fibers and cerebellar granule cells. Another exemplary animal is the pR5 line of mice. These mice have a human tau transgene with a P301L mutation (the mutation is associated with frontotemporal dementia) and a Thy 1.2 promoter (Taconic, Germantown, N.Y., Lewis, et al., Nat Genet. 25:402-405 (2000)). These mice have a more gradual course of neurodegeneration. The mice develop neurofibrillary tangles in several brain regions and spinal cord, which is hereby incorporated by reference in its entirety). This is an excellent model to study the consequences of tangle development and for screening therapy that may inhibit the generation of these aggregates. Another advantage of these animals is the relatively early onset of pathology. In the homozygous line, behavioral abnormalities associated with tau pathology can be observed at least as early as 3 months, but the animals remain relatively healthy at least until 8 months of age. In other words, at 8 months, the animals ambulate, feed themselves, and can perform the behavioral tasks sufficiently well to allow the treatment effect to be monitored. Active immunization of these mice for 6-13 months with - AI wI KLH-PHF-1 generated titers of about 1,000 and showed fewer neurofibrillary tangles, less pSer422, and reduced weight loss relative to untreated control ice.

[0104] The activity of antibodies or active agents can be assessed by various criteria including reduction in amount of total tau or phosphorylated tau, reduction in other pathological

characteristics, such as amyloid deposits of Aβ, and inhibition or delay or behavioral deficits. Active immunogens can also be tested for induction of antibodies in the sera. Both passive and active immunogens can be tested for passage of antibodies across the blood brain barrier into the brain of a transgenic animal. Antibodies or fragments inducing an antibody can also be tested in non-human primates that naturally or through induction develop symptoms of diseases characterized by tau. Tests on an antibody or active agent are usually performed in conjunction with a control in which a parallel experiment is conduct except that the antibody or active agent is absent (*e.g.*, replaced by vehicle). Reduction, delay or inhibition of signs or symptoms disease attributable to an antibody or active agent under test can then be assessed relative to the control.

VI. Patients amenable to treatment

[0105] The presence of neurofibrillary tangles has been found in several diseases including Alzheimer's disease, Down's syndrome, mild cognitive impairment, postencephalitic parkinsonism, posttraumatic dementia or dementia pugalistica, type C Niemann-Pick disease, supranuclear palsy, frontotemporal dementia, frontotemporal lobar degeneration, amyotrophic lateral sclerosis/parkinsonism dementia complex of Guam, and PSP progressive supranuclear palsy. The present regimes can also be used in treatment or prophylaxis of any of these diseases. Because of the widespread association between neurological diseases and conditions and tau, the present regimes can be used in treatment or prophylaxis of any subject showing elevated levels of tau or phosphorylated tau (*e.g.*, in the CSF) compared with a mean value in individuals without neurological disease. The present regimes can also be used in treatment or prophylaxis of neurological disease in individuals having a mutation in tau associated with neurological disease. The present methods are particularly suitable for treatment or prophylaxis of Alzheimer's disease, and especially in patients.

[0106] Patients amenable to treatment include individuals at risk of disease but not showing symptoms, as well as patients presently showing symptoms. Patients at risk of disease include those having a known genetic risk of disease. Such individuals include those having relatives who have experienced this disease, and those whose risk is determined by analysis of genetic or biochemical markers. Genetic markers of risk include mutations in tau, such as those discussed above, as well as mutations in other genes associated with neurological disease. For example, the ApoE4 allele in heterozygous and even more so in homozygous form is associated with risk of Alzheimer's disease. Other markers of risk of Alzheimer's disease include mutations in the

APP gene, particularly mutations at position 717 and positions 670 and 671 referred to as the Hardy and Swedish mutations respectively, mutations in the presenilin genes, PS1 and PS2, a family history of AD, hypercholesterolemia or atherosclerosis. Individuals presently suffering from Alzheimer's disease can be recognized by PET imaging, from characteristic dementia, as well as the presence of risk factors described above. In addition, a number of diagnostic tests are available for identifying individuals who have AD. These include measurement of CSF tau or phospho-tau and Aβ42 levels. Elevated tau or phospho-tau and decreased Aβ42 levels signify the presence of AD. Some mutations associated with Parkinson's disease. Ala30Pro or Ala53, or mutations in other genes associated with Parkinson's disease such as leucine-rich repeat kinase, PARK8. Individuals can also be diagnosed with any of the neurological diseases mentioned above by the criteria of the DSM IV TR.

[0107] In asymptomatic patients, treatment can begin at any age (*e.g.*, 10, 20, 30). Usually, however, it is not necessary to begin treatment until a patient reaches 40, 50, 60 or 70 years of age. Treatment typically entails multiple dosages over a period of time. Treatment can be monitored by assaying antibody levels over time. If the response falls, a booster dosage is indicated. In the case of potential Down's syndrome patients, treatment can begin antenatally by administering therapeutic agent to the mother or shortly after birth.

VII. Pharmaceutical compositions and methods of treatment

[0108] In prophylactic applications, an antibody or agent for inducing an antibody or a pharmaceutical composition the same is administered to a patient susceptible to, or otherwise at risk of a disease (*e.g.*, Alzheimer's disease) in regime (dose, frequency and route of administration) effective to reduce the risk, lessen the severity, or delay the onset of at least one sign or symptom of the disease. In particular, the regime is preferably effective to inhibit or delay tau or phospho-tau and paired filaments formed from it in the brain, and/or inhibit or delay its toxic effects and/or inhibit/or delay development of behavioral deficits. In therapeutic applications, an antibody or agent to induce an antibody is administered to a patient suspected of, or already suffering from a disease (*e.g.*, Alzheimer's disease) in a regime (dose, frequency and route of administration) effective to ameliorate or at least inhibit further deterioration of at least one sign or symptom of the disease. In particular, the regime is preferably effective to reduce or at least inhibit further increase of levels of tau, phosphor-tau, or paired filaments formed from it , associated toxicities and/or behavioral deficits.

[0109] A regime is considered therapeutically or prophylactically effective if an individual treated patient achieves an outcome more favorable than the mean outcome in a control population of comparable patients not treated by methods of the invention, or if a more favorable outcome is demonstrated in treated patients versus control patients in a controlled clinical trial (*e.g.*, a phase II, phase II/III or phase III trial) at the $p < 0.05$ or 0.01 or even 0.001 level .

[0110] Effective doses of vary depending on many different factors, such as means of administration, target site, physiological state of the patient, whether the patient is an ApoE carrier, whether the patient is human or an animal, other medications administered, and whether treatment is prophylactic or therapeutic.

[0111] An exemplary dosage range for antibodies is from about 0.01 to 5 mg/kg, and more usually 0.1 to 3 mg/kg or 0.15-2 mg/kg or 0.15-1.5 mg/kg, of patient body weight. Antibody can be administered such doses daily, on alternative days, weekly, fortnightly, monthly, quarterly, or according to any other schedule determined by empirical analysis. An exemplary treatment entails administration in multiple dosages over a prolonged period, for example, of at least six months. Additional exemplary treatment regimes entail administration once per every two weeks or once a month or once every 3 to 6 months.

[0112] The amount of an agent for active administration varies from 0.1-500 μg per patient and more usually from 1-100 or 1-10 μg per injection for human administration. The timing of injections can vary significantly from once a day, to once a year, to once a decade. A typical regimen consists of an immunization followed by booster injections at time intervals, such as 6 week intervals or two months. Another regimen consists of an immunization followed by booster injections 1, 2 and 12 months later. Another regimen entails an injection every two months for life. Alternatively, booster injections can be on an irregular basis as indicated by monitoring of immune response.

[0113] Antibodies or agents for inducing antibodies are preferably administered via a peripheral route (*i.e.*, one in which an administered or induced antibody crosses the blood brain barrier to reach an intended site in the brain. Routes of administration include topical, intravenous, oral, subcutaneous, intraarterial, intracranial, intrathecal, intraperitoneal, intranasal or intramuscular. Preferred routes for administration of antibodies are intravenous and subcutaneous. Preferred routes for active immunization are subcutaneous and intramuscular. This type of injection is most typically performed in the arm or leg muscles. In some methods,

agents are injected directly into a particular tissue where deposits have accumulated, for example intracranial injection.

[0114] Pharmaceutical compositions for parenteral administration are preferably sterile and substantially isotonic and manufactured under GMP conditions. Pharmaceutical compositions can be provided in unit dosage form (*i.e.*, the dosage for a single administration). Pharmaceutical compositions can be formulated using one or more physiologically acceptable carriers, diluents, excipients or auxiliaries. The formulation depends on the route of administration chosen. For injection, antibodies can be formulated in aqueous solutions, preferably in physiologically compatible buffers such as Hank's solution, Ringer's solution, or physiological saline or acetate buffer (to reduce discomfort at the site of injection). The solution can contain formulatory agents such as suspending, stabilizing and/or dispersing agents. Alternatively antibodies can be in lyophilized form for constitution with a suitable vehicle, *e.g.*, sterile pyrogen-free water, before use.

[0115] The present regimes can be administered in combination with another agent effective in treatment or prophylaxis of the disease being treated. For example, in the case of Alzheimer's disease, the present regimes can be combined with immunotherapy against Aβ (WO/2000/072880), cholinesterase inhibitors or memantine or in the case of Parkinson's disease immunotherapy against alpha synuclein WO/2008/103472, Levodopa, dopamine agonists, COMT inhibitors, MAO-B inhibitors, Amantadine, or anticholinergic agents.

VIII. In Vivo Imaging, Diagnostic Methods, and Optimizing Immunotherapy

[0116] The invention provides methods of in vivo imaging tau protein deposits (*e.g.*, neurofibrillary tangles and tau inclusions) in a patient. The methods work by administering a reagent, such as antibody that binds tau (*e.g.*, a mouse, humanized, chimeric or veneered16B5 antibody), to the patient and then detecting the agent after it has bound. Antibodies binding to an epitope of tau within amino acids 24 to 46 are preferred. In some methods, the antibody binds to an epitope within amino acids 25 to 44, or within amino acids 30 to 39. A clearing response to the administered antibodies can be avoided or reduced by using antibody fragments lacking a full-length constant region, such as Fabs. In some methods, the same antibody can serve as both a treatment and diagnostic reagent.

[0117] Diagnostic reagents can be administered by intravenous injection into the body of the patient, or directly into the brain by intracranial injection or by drilling a hole through the skull. The dosage of reagent should be within the same ranges as for treatment methods. Typically, the reagent is labeled, although in some methods, the primary reagent with affinity for tau is unlabeled and a secondary labeling agent is used to bind to the primary reagent. 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 SPECT.

[0118] The methods of in vivo imaging of tau protein deposits are useful to diagnose or confirm diagnosis of a tauopathy, such as Alzheimer's disease, frontotemporal lobar degeneration, progressive supranuclear palsy and Pick's disease, or susceptibility to such a disease. For example, the methods can be used on a patient presenting with symptoms of dementia. If the patient has abnormal neurofibrillary tangles, then the patient is likely suffering from Alzheimer's disease. Alternatively, if the patient has abnormal tau inclusions, then depending on the location of the inclusions, the patient may be suffering from frontotemporal lobar degeneration. The methods can also be used on asymptomatic patients. Presence of abnormal tau protein deposits indicates susceptibility to future symptomatic disease. The methods are also useful for monitoring disease progression and/or response to treatment in patients who have been previously diagnosed with a tau-related disease.

[0119] Diagnosis can be performed by comparing the number, size, and/or intensity of labeled loci, to corresponding baseline values. The base line values can represent the mean levels in a population of undiseased individuals. Baseline values can also represent previous levels determined in the same patient. For example, baseline values can be determined in a patient before beginning tau immunotherapy treatment, and measured values thereafter compared with the baseline values. A decrease in values relative to baseline signals a positive response to treatment.

[0120] In some patients, diagnosis of a tauopathy may be aided by performing a PET scan. A PET scan can be performed using, for example, a conventional PET imager and auxiliary equipment. The scan typically includes one or more regions of the brain known in general to be associated with tau protein deposits and one or more regions in which few if any deposits are generally present to serve as controls.

[0121] The signal detected in a PET scan can be represented as a multidimensional image. The multidimensional image can be in two dimensions representing a cross-section through the brain, in three dimensions, representing the three dimensional brain, or in four dimensions representing changes in the three dimensional brain over time. A color scale can be used with different colors indicating different amounts of label and, inferentially, tau protein deposit detected. The results of the scan can also be presented numerically, with numbers relating to the amount of label detected and consequently amount of tau protein deposits. The label present in a region of the brain known to be associated with deposits for a particular tauopathy (*e.g.*, Alzheimer's disease) can be compared with the label present in a region known not to be associated with deposits to provide a ratio indicative of the extent of deposits within the former region. For the same radiolabeled ligand, such ratios provide a comparable measure of tau protein deposits and changes thereof between different patients.

[0122] In some methods, a PET scan is performed concurrent with or in the same patient visit as an MRI or CAT scan. An MRI or CAT scan provides more anatomical detail of the brain than a PET scan. However, the image from a PET scan can be superimposed on an MRI or CAT scan image more precisely indicating the location of PET ligand and inferentially tau deposits relative to anatomical structures in the brain. Some machines can perform both PET scanning and MRI or CAT scanning without the patient changing positions between the scans facilitating superimposition of images.

[0123] Suitable PET ligands include radiolabeled antibodies of the invention (*e.g.*, a mouse, humanized, chimeric or veneered16B5 antibody). The radioisotope used can be, for example, C^{11}, N^{13}, O^{15}, F^{18}, or I^{123}. The interval between administering the PET ligand and performing the scan can depend on the PET ligand and particularly its rate of uptake and clearing into the brain, and the half- life of its radiolabel.

[0124] PET scans can also be performed as a prophylactic measure in asymptomatic patients or in patients who have symptoms of mild cognitive impairment but have not yet been diagnosed with a tauopathy but are at elevated risk of developing a tauopathy. For asymptomatic patients, scans are particularly useful for individuals considered at elevated risk of tauopathy because of a family history, genetic or biochemical risk factors, or mature age. Prophylactic scans can commence for example, at a patient age between 45 and 75 years. In some patients, a first scan is performed at age 50 years.

[0125] Prophylactic scans can be performed at intervals of for example, between six months and ten years, preferably between 1-5 years. In some patients, prophylactic scans are performed annually. If a PET scan performed as a prophylactic measure indicates abnormally high levels of tau protein deposits, immunotherapy can be commenced and subsequent PET scans performed as in patients diagnosed with a tauopathy. If a PET scanned performed as a prophylactic measure indicates levels of tau protein deposits within normal levels, further PET scans can performed at intervals of between six months and 10 years, and preferably 1-5 years, as before, or in response to appearance of signs and symptoms of a tauopathy or mild cognitive impairment. By combining prophylactic scans with administration of tau-directed immunotherapy if and when an above normal level of tau protein deposits is detected, levels of tau protein deposits can be reduced to, or closer to, normal levels, or at least inhibited from increasing further, and the patient can remain free of the tauopathy for a longer period than if not receiving prophylactic scans and tau-directed immunotherapy (*e.g.*, at least 5, 10, 15 or 20 years, or for the rest of the patient's life).

[0126] Normal levels of tau protein deposits can be determined by the amount of neurofibrillary tangles or tau inclusions in the brains of a representative sample of individuals in the general population who have not been diagnosed with a particular tauopathy (*e.g.*, Alzheimer's disease) and are not considered at elevated risk of developing such disease (*e.g.*, a representative sample of disease-free individuals under 50 years of age). Alternatively, a normal level can be recognized in an individual patient if the PET signal according to the present methods in a region of the brain in which tau protein deposits are known to develop is not different (within the accuracy of measurement) from the signal from a region of the brain in which it is known that such deposits do not normally develop. An elevated level in an individual can be recognized by comparison to the normal levels (*e.g.*, outside mean and variance of a standard deviation) or simply from an elevated signal beyond experimental error in a region of the brain associated with tau protein deposits compared with a region not known to be associated with deposits. For purposes of comparing the levels of tau protein deposits in an individual and population, the tau protein deposits should preferably be determined in the same region(s) of the brain, these regions including at least one region in which tau protein deposits associated with a particular tauopathy (*e.g.*, Alzheimer's disease) are known to form. A patient having an elevated level of tau protein deposits is a candidate for commencing immunotherapy.

[0127] After commencing immunotherapy, a decrease in the level of tau protein deposits can be first seen as an indication that the treatment is having the desired effect. The observed decrease can be, for example, in the range of 1-100%, 1-50%, or 1-25% of the baseline value. Such effects can be measured in one or more regions of the brain in which deposits are known to form or can be measured from an average of such regions. The total effect of treatment can be approximated by adding the percentage reduction relative to baseline to the increase in tau protein deposits that would otherwise occur in an average untreated patient.

[0128] Maintenance of tau protein deposits at an approximately constant level or even a small increase in tau protein deposits can also be an indication of response to treatment albeit a suboptimal response. Such responses can be compared with a time course of levels of tau protein deposits in patients with a particular tauopathy (*e.g.*, Alzheimer's disease) that did not receive treatment, to determine whether the immunotherapy is having an effect in inhibiting further increases of tau protein deposits.

[0129] Monitoring of changes in tau protein deposits allows adjustment of the immunotherapy or other treatment regime in response to the treatment. PET monitoring provides an indication of the nature and extent of response to treatment. Then a determination can be made whether to adjust treatment and if desired treatment can be adjusted in response to the PET monitoring. PET monitoring thus allows for tau-directed immunotherapy or other treatment regime to be adjusted before other biomarkers, MRI or cognitive measures have detectably responded. A significant change means that comparison of the value of a parameter after treatment relative to basement provides some evidence that treatment has or has not resulted in a beneficial effect. In some instances, a change of values of a parameter in a patient itself provides evidence that treatment has or has not resulted in a beneficial effect. In other instances, the change of values, if any, in a patient, is compared with the change of values, if any, in a representative control population of patients not undergoing immunotherapy. A difference in response in a particular patient from the normal response in the control patient (*e.g.*, mean plus variance of a standard deviation) can also provide evidence that an immunotherapy regime is or is not achieving a beneficial effect in a patient.

[0130] In some patients, monitoring indicates a detectable decline in tau protein deposits but that the level of tau protein deposits remains above normal. In such patients, if there are no

unacceptable side effects, the treatment regime can be continued as is or even increased in frequency of administration and/or dose if not already at the maximum recommended dose.

[0131] If the monitoring indicates levels of tau protein deposits in a patient have already been reduced to normal, or near-normal, levels of tau protein deposits, the immunotherapy regime can be adjusted from one of induction (*i.e.*, that reduces the level of tau protein deposits) to one of maintenance (*i.e.* , that maintains tau protein deposits at an approximately constant level). Such a regime can be affected by reducing the dose and or frequency of administering immunotherapy.

[0132] In other patients, monitoring can indicate that immunotherapy is having some beneficial effect but a suboptimal effect. An optimal effect can be defined as a percentage reduction in the level of tau protein deposits within the top half or quartile of the change in tau protein deposits (measured or calculated over the whole brain or representative region(s) thereof in which tau protein deposits are known to form) experienced by a representative sample of tauopathy patients undergoing immunotherapy at a given time point after commencing therapy. A patient experiencing a smaller decline or a patient whose tau protein deposits remains constant or even increases, but to a lesser extent than expected in the absence of immunotherapy (*e.g.*, as inferred from a control group of patients not administered immunotherapy) can be classified as experiencing a positive but suboptimal response. Such patients can optionally be subject to an adjustment of regime in which the dose and or frequency of administration of an agent is increased.

[0133] In some patients, tau protein deposits may increase in similar or greater fashion to tau deposits in patients not receiving immunotherapy. If such increases persist over a period of time, such as 18 months or 2 years, even after any increase in the frequency or dose of agents, immunotherapy can if desired be discontinued in favor of other treatments.

[0134] The foregoing description of diagnosing, monitoring, and adjusting treatment for tauopathies has been largely focused on using PET scans. However, any other technique for visualizing and/or measuring tau protein deposits that is amenable to the use of tau antibodies of the invention (*e.g.*, a mouse, humanized, chimeric or veneered16B5 antibody) can be used in place of PET scans to perform such methods.

EXAMPLES

EXAMPLE 1. Generation of antibody 16B5

[0135] Pan antibody 16B5, which recognizes tau whether or not it is phosphorylated, was raised to purified tau and selected based on its high affinity capture properties in an ELISA assay.

EXAMPLE 2. Cloning and sequencing of antibody 16B5

[0136] RNA were extracted from pelleted cells expressing the 16B5 antibody using Trizol LS (Invitrogen). RNA concentrations were measured using the Quant-IT kit (Invitrogen).

[0137] 5'-RACE was used to amplify the 5' end of IgG mRNA using the Smart RACE kit (Clontech). About 1 μg of RNA was used for the RT reaction and the cDNA pools were further amplified using the Universal primer provided with the Smart RACE kit and gene specific primers (GSPs) designed in ExonBIO.

[0138] Primer Sequences:

Universal Primer: CTAATACGACTCACTATAGGGC (SEQ ID NO: 7)

GSPs:

IgG1 and IgG2a: CTC AAT TTT CTT GTC CAC CTT GGT GC (SEQ ID NO: 8)

IgG2b: CTC AAG TTT TTT GTC CAC CGT GGT GC (SEQ ID NO: 9)

[0139] PCR products were gel purified and cloned into the pSUPER-blunt vector (Adexon, www.adexonbiotech.com). For the heavy chain, 15 colonies were mini-prepared and sequenced. For the light chain, colony PCR was performed to distinguish endogenous aberrant light chain, and only clones that were not amplified from the colony PCR were sequenced. Sequencing results were analyzed on NTI vector. Adaptor and GSP primer sequences were marked on the map. The regions between the adaptor and GSP sequences are IgG heavy chain sequences which include leader, signal peptide and V-region, and part of the constant region. ORFs were marked on the map.

EXAMPLE 3. Epitope mapping of antibody 16B5

[0140] Identification of epitope by peptide fragment analysis. The human tau sequence with 4 microtubule binding repeats and no N-terminal inserts, and containing a P301L mutation (rTau), was expressed in *E. coli* and purified. This form of tau has the sequence of SEQ ID NO:3, with the substitution of leucine for proline at position 243 (which corresponds to P301L using the

numbering convention based on the longest isoform of tau). Enzymatic digests of 200ug of tau were carried out with one of four different proteases: trypsin (which cleaves at the carboxyl end of arginine and lysine), chymotrypsin (which primarily cleaves at the carboxyl end of tyrosine, tryptophan, phenylalanine and leucine), LysC (which cleaves at the carboxyl end of lysine), or GluC (which cleaves after glutamate residues and rarely after aspartate residues). All proteases were obtained from Thermo Scientific, and digests were performed for 16h at 37°C. The resulting peptide fragments were incubated with 10μg of 16B5, and precipitated using Protein G magnetic beads (NEB). Precipitates were thoroughly washed in PBS containing 300mM NaCl and 0.5% NP-40, then eluted with 1M NaCl in 100 mM glycine, pH 2.8. Eluates were dried under vacuum and resuspended in 0.1% trifluoroacetic acid (TFA). Resuspended eluates were loaded onto a 4.6x50mm C18 column, then fractionated by HPLC (Agilent 1260 Infinity system) using a linear gradient of acetonitrile with 0.075% TFA. Peak fractions were collected, dried and resuspended in distilled water. Peptide masses and identities were determined by MALDI-TOF/TOF. A peak corresponding to residues 25-44 of SEQ ID NO:1 was identified in the LysC MS Spectrum. Peaks corresponding to residues 25-44 of SEQ ID NO:1 and 24-44 were identified in the Trypsin MS Spectrum. No signal was obtained from the chymotrypsin and GluC digests, suggesting that some epitopes may comprise residue 29 of SEQ ID NO:1 and/or residue 37 of SEQ ID NO:1.

[0141] Identification of epitope by mutation analysis. Using the results determined by peptide fragment analysis (described above), deletion mutagenesis of rTau was carried out by whole plasmid amplification using standard molecular biology methods. Protein was expressed in small volumes of bacterial culture, and equal volumes of clarified bacterial lysate were electrophoresed, blotted, and stained with the 16B5 antibody. To control for sample loading, Tau46, an antibody with specificity for the C-terminal region of tau (C-terminal epitope), was used to stain duplicate blots. Both antibodies were used at a concentration of 0.2 μg/mL. Images were captured using a Licor Odyssey fluorescent scanner. The following deletion mutants of tau were made and analyzed in this manner: Δ5-24, Δ23-32, Δ25-44, Δ30-39, and Δ37-46. As shown in Figures 1 and 2, the Δ25-44 and Δ30-39 deletion mutants of tau were not detected by the 16B5 antibody, providing evidence that an epitope recognized by 16B5 lies within those residues. The Δ37-46 deletion mutant of tau was only slightly detectable with 16B5, providing evidence that some of the residues within 37-46 (*e.g.*, residue 37) may play a role in the binding of 16B5 to tau. The 16B5 antibody stained the Δ23-32 deletion mutant of tau

to a lesser extent than Δ5-24 and to a greater extent than the Δ25-44 and Δ30-39 deletion mutants, providing evidence that 16B5 may also bind to a peptide comprising residues 33-36, 30-36, 33-37, 30-37 or 33-39. Taken as a whole, the data obtained from the tau deletion mutants suggests that an epitope recognized by 16B5 may comprise some or all of residues 23-32 of SEQ ID NO:1 and some or all of residues 37-46 of SEQ ID NO:1. For example, 16B5 may recognize an epitope within residues 32-38 or 28-41 of SEQ ID NO:1.

[0142] Identification of epitope by alanine scanning. Single residues within the region of tau spanning residues 30-42 were next mutated to alanine using PCR mutagenesis. The mutated proteins were expressed, and lysates were resolved by electrophoresis and blotted with either the 16B5 antibody or the Tau46 antibody, as described above. The results of this analysis are shown in Figure 3. The specific point mutants analyzed, including T30A, M31A, H32A, Q33A, D34A, Q35A, E36A, G37A, D38A, T39A, D40A, A41L, and G42A, are listed above the blots. Residues of particular interest are enclosed in boxes on each blot. Detectable binding of 16B5 was completely eliminated by the Q33A tau mutant and substantially reduced by the G37A tau mutant, providing evidence that residue 33, and to a lesser extent residue 37, may be important components of an epitope recognized by 16B5. Other residues may be important components of an epitope recognized by 16B5 in a Biacore analysis.

EXAMPLE 4. Passive immunization in the hTau.P301L transgenic mouse model of tauopathy

[0143] Immunization. 3-month-old hTau.P301L-Tg female mice in the FVB/N genetic background were used for this study. Administration of 10mg/kg of test and control antibodies was performed intraperitoneally, once a week. The treatment duration was about 5 months. Following 23 injections, the study ended with the sacrifice of the mice. Table 1 describes the test and control antibodies administered in this study.

Table 1

Dosing Scheme

	Group K	**Group M**
Antibody	16B5	6F10
Binding specificity	Within 23-46 (see Example 3)	Non-immune IgG1 isotype control
N	22	22

Table 1

Dosing Scheme

	Group K	**Group M**
Treatment	N2	N3
Dose	10 mg/kg weekly	10 mg/kg weekly
Dose volume	1.724 ml/kg	2.381 ml/kg

[0144] Premature death is a phenotype observed in transgenic murine tauopathy models. The particular model used in this study develops hyperphosphorylated Tau at the age of 6 months, although with a high variability of onset. The mice also suffer motor defects like hind limb clasping and reduced general mobility, and die prematurely at the age of 8-11 months (reMYND unpublished data, Terwel *et al.,* 2005). Mice developing end-stage disease symptoms, characterized by the presence of the clasping phenotype and weight loss, were sacrificed. An unexpectedly high number of mice died prematurely without the presence of these symptoms. The cause of death in such cases is considered to be unrelated to late-stage tauopathy or the test antibody, and instead is thought to be related to the inbred FVB/N background.

[0145] Table 2 shows an overview of the overall survival of all mice during the course of the study.

Table 2

Survival during treatment (all causes of death)

		N at study start	**N alive at sacrifice**	**% survival**
Group K	**N2**	22(23)*	11	50(47)
Group M	**N3**	22	13	59

*One mouse in Group K had to be replaced at the beginning of the study. The data can be analyzed with or without this replacement mouse.

[0146] Following sacrifice, mice were dissected and the brainstems and midbrains were homogenized using a potter-type mechanical homogenizer (VOS 14 S40, rate 750 rpm; VWR) in 10 weight-volumes of ice-cold Tris-proteinase-phosphatase-inhibitor buffer (TPPI-buffer) containing: 20 mM Tris-HCl (pH 8.1); 150 Mm NaCl; 1 mM ethylene diamine tetraacetic acid

(EDTA, Merck); 1 mM ethylene glycol tetraacetic acid (EGTA, Sigma-Aldrich); 5 mM sodium pyrophosphate (Sigma); 30 mM sodium fluoride (Sigma-Aldrich); 1 mM PMSF (Sigma); 2 mM sodium vanadate (Sigma); 10 mM 1,10-ortho- phenanthrolinemonohydrate (Sigma-Aldrich); 5 µg/ml soya bean trypsin inhibitor; 5 µg/ml pepstatin; and a cocktail of proteinase inhibitors (CPI, Roche Diagnostics GmbH, Germany). Fixed volumes of 140 µl and 100 µl of the brainstem and midbrain homogenates (TotH), respectively, (approximately half of the total volumes) were centrifuged at 136000xg, for 60 min at 4°C (TLA-55 rotor, OptimaTMTLX Ultracentrifuge, Beckman Coulter) to generate a Tris-soluble fraction (SF), with the remainder of the total homogenates being stored at -80°C. Due to a limited number of centrifuge holders (N=12), samples were randomized to equilibrate the centrifuge and divide the different treatment groups over the different centrifugation sessions.

[0147] The supernatant (S1, also referred to as "soluble fraction" or "SF") was separated from the pellet (P1), aliquoted and stored at -80°C. The P1 pellet was solubilized in 10 weight volumes of a high-salt solution (0.85 M NaCl containing TPPI-buffer) and centrifuged at 20000xg, for 30 min at 4 °C. The resulting high-salt pellet (P2) was stored at -80 °C. The supernatant (S2) was brought to 1% Sarkosyl with one tenth 10% Sarkosyl and incubated at room temperature for 60 min in a top-over-top rotary tumbler, then centrifuged at 136000xg, for 60 min at 4 °C. The Sarkosyl soluble supernatant (S3) was stored at -80 °C and the Sarkosyl insoluble pellet (P3, also referred to as "insoluble fraction" or "IF") was resuspended in 30 µl TPPI buffer and aliquoted. The total homogenate (TotH), Tris-soluble (SF), and Sarkosyl-insoluble (IF) brainstem fractions generated by the fractionation protocol described above were used in subsequent poly-acrylamide gel electrophoresis and Western blotting analyses.

[0148] Poly-acrylamide gel electrophoresis and Western blotting. For application of conventional SDS-PAGE and Western blotting, samples were denatured and reduced by incubation at 95°C for 10 min, then separated on 7.5 % Tris-HCl gels (Criterion XT Precast Gel, 26-well comb, 15 µl, 1.0 mm; Biorad). After dry electrotransfer (iBlot™ Invitrogen) to PVDF-membranes (iBlot™ Gel Transfer Stacks, PVDF, Regular, Invitrogen), the membranes were washed in 0.4% PFA for 30 min and then washed in Tris-buffered saline. Next the membranes were incubated in Tris-buffered saline (TBS, pH 7.6) containing 5% (w/v) non-fat dry milk and 0.1 % (v/v) Tween-20 for 1 hour. Blots were incubated with various anti-tau primary antibodies overnight, at the working concentrations shown in Table 3. After washing and incubation with an anti-mouse or anti-rabbit HRP-conjugated secondary antibody (goat-anti-mouse or goat-anti-

rabbit IgG, DAKO), blots were developed by the ECL detection system (SuperSignal West Femto Maximum Sensitivity Substrate, product 34096, Thermo Scientific). Images were recorded digitally (VisionWorks Acquisition, UVP) with different exposure times, and dedicated software (VisionWorks Analysis, UVP) was used for analysis of the blots. For comparison, an inter-gel reference gel was run with aliquots of four fractions being run on each gel to be compared. Anti-tau primary monoclonal antibodies used for detection included AT100 (phospho-Tau, Thermo Scientific; dilution 1:250), AT8 (phospho-Tau, Thermo Scientific; dilution 1:500), HT7 (pan Tau, Pierce; dilution 1:1000), and 1F5 (epitope unknown to the Testing Facility, Neotope, dilution 3:500). Blots were re-probed with anti-GAPDH (Abcam 9485; dilution 1:2500) as a loading control. Pan Tau antibodies are not specific for phospho-Tau.

Table 3

Summary of antibodies used for biochemistry analysis

mAb	Supplier	Specificity (human)	Stock Conc.	Work Conc.
AT100	Thermo Scientific	Phospho-PHF-tau pSer212/Thr214	200 µg/ml	0.8 µg/ml
AT 8	Thermo Scientific	Phospho-PHF-tau pSer202/Thr205	200 µg/ml	0.4 µg/ml
HT7	Pierce	between residue 159 and 163	200 µg/ml	0.2 µg/ml
1F5*	Neotope	pS^{404}	1 mg/ml	6 µg/ml
GAPDH	Abcam	Human	1mg/ml	0.4 µg/ml

*IgG2b isotype, JH131-1F5.4.1 hybridoma, lot # NB-0081

[0149] As shown in Figure 4, a statistically significant reduction in the amount of tau was observed in sarkosyl insoluble brainstem fractions from animals treated with the 16B5 antibody, as compared to animals treated with the 6F10 control antibody. Statistical significance was

assessed using the Student's t test, $p<0.05$. This reduction was observed with both phospho-tau specific antibodies (AT8, upper left-hand panel; AT100, lower left-hand panel; 1F5, upper right-hand panel) and pan-tau antibodies (HT7, lower right-hand panel). Western blots of the total homogenate also indicated a significant reduction in the ratio of phosphor-tau to total tau in the 16B5 treated animals relative to control animals treated with the 6F10 antibody, when detected with a phospho-specific antibody. *See* Figure 5, left panel (showing the signal detected with the AT8 anti-phospho-tau antibody divided by the signal detected with the HT7 pan tau antibody). In contrast, there was no significant change in the ratio of total tau to GAPDH levels in the total homogenates of the 16B5 treated animals as compared to the control animals treated with the 6F10 antibody. *See* Figure 5, right panel (showing the signal detected with the HT7 pan tau antibody divided by the signal detected with the GAPDH antibody). These data provide evidence that the level of phospho-Tau but not total tau was reduced in the homogenates.

[0150] Histological Analysis. Immuno-histochemical analysis using anti-phospho-tau antibodies was performed in the subthalamic nucleus annex zona incerta (STH/ZI) and the interposed nucleus of the cerebellum, anterior and posterior part, annex lateral cerebellar nucleus (IntA/P/LAT). Sagittal vibratome sections (40 µm) were stored in PBS with 0.1% sodium azide at 4 °C until use. Eight sections per mouse, at bregma indicated, were stained free-floating with mAbs AT8, AT100 or 1F5. Sections were selected for staining with the indicated antibodies as listed in Table 4 below. Sections of all animals selected for a particular staining were randomized for staining and blinded quantification.

[0151] Free-floating sections were incubated in Netwells™. Sections were then washed twice in PBS and incubated for 20 minutes in hydrogen peroxide 1.5% in PBS and methanol (1:1) to remove endogenous peroxidase activity. After washing the sections three times in PBS containing 0.1% Triton X100 (PBST), the sections were blocked for 30 min in 10% Fetal Calf Serum (FCS) in PBST followed by an overnight incubation with primary antibodies AT8, AT100 (Thermo scientific), using a concentrations of 0.4 µg/ml and 0.05 µg/ml, respectively, in PBST with 10% FCS. After rinsing, the sections were incubated with goat anti-mouse peroxidase labeled (GAMPO) secondary antibody (DAKO, 1/500 in PBST, 10% FCS) and the signal was developed with 3,3'-diaminobenzidine tetrahydrochloride (DAB, 1 tablet per 10 ml Tris-HCl with 3 µl H_2O_2 per 10 ml). Sections were counterstained with Mayer's hematoxylin, dehydrated in five steps (50, 70, 95 and 2x100%) in ethanol and xylene (Merck Eurolab) and mounted in Depex (Depex mounting medium, BDH Laboratory).

Table 4

Summary of antibodies used for immunohistochemical analysis

mAb	Supplier	Specificity	Host	Stock Conc.	Work Conc.
AT8	Thermo	Human	Mouse	200 µg/ml	0.4 µg/ml
AT100	Thermo	Human	Mouse	200 µg/ml	0.05

[0152] Images were acquired with an Olympus BX41 microscope equipped with a Color view II Olympus camera and analyzed with a computer using AnalySIS Five – Cell^D software. Light intensity and condenser settings for the microscope were kept constant throughout the image acquisition process. All acquired images were subjected to the same computer subroutines to minimize investigator bias. Density slice thresholding was applied uniformly throughout analysis.

[0153] The region of interest as defined below was selected for automatic quantification of the staining signal(s). Subthalamic nucleus and zona incerta were delineated by cerebral peduncle ventrally and by white mater dorsally, respectively, as well as on the basis of differences in cell density (sagittal cerebellar sections bregma 1,32-1,92). Interposed nucleus of the cerebellum, anterior and posterior part, and lateral cerebellar nucleus were delineated by white matter and changes in cell density and the third ventricle (sagittal cerebellar sections, bregma 1,92-2,64 for LAT and 0,84-1,8 for IntA/P). For each staining, 6 brain sections containing the STH/ZI and 16 sections containing the IntA/P/LAT per mouse were included in the analysis.

[0154] As shown in FIG. 6, the amount of phospho-tau detected in the cerebellar nuclei and the subthalamic region of animals treated with the 16B5 antibody was significantly reduced as compared to the amount of phospho-tau detected in the same structures in control animals treated with the 6F10 antibody. Statistical significance was assessed using the Student's t test, $p<0.05$.

EXAMPLE 5. Humanization of 16B5

[0155] Sequence analysis shows that the 16B5 antibody has a variable kappa (Vk) domain having the sequence of SEQ ID NO:16, which belongs to mouse Kabat subgroup 1, and corresponds to human Kabat subgroup 4. Kabat CDRs are underlined. The variable heavy (Vh) domain of the 16B5 antibody has the sequence of SEQ ID NO:10, which belongs to mouse Kabat subgroup 2b, and corresponds to human Kabat subgroup 1 (Kabat et al. (1991), Sequences of Proteins of Immunological Interest, Fifth Edition; NIH Publication No. 91-3242). Kabat CDRs are underlined.

[0156] The 16B5 Vk domain includes a 17 residue CDR-L1 sequence (KSSQSLLNSRTRKNYLA, SEQ ID NO: 17), a 7 residue CDR-L2 sequence (WASTRES, SEQ ID NO: 18), and an 8 residue CDR-L3 (KQSYTLRT, SEQ ID NO: 19). The CDR-L1 sequence belongs to canonical class 3, and the CDR-L2 and CDR-L3 sequences belong to class 1 (Martin & Thornton (1996), J. Mol. Biol. 263:800-15).

[0157] The 16B5 Vh domain includes a 5 residue CDR-H1 sequence (YHGMD, SEQ ID NO: 11) based on Kabat numbering or a 10 residue CDR-H1 sequence (GYPFTYHGMD, SEQ ID NO: 24) based on combined Kabat and Chothia numbering, a 17 residue CDR-H2 sequence (WINTYSGVPTYADDFKG, SEQ ID NO: 12), and an 8 residue CDR-H3 sequence (RRDFTMDF, SEQ ID NO: 13). The CDR-H1 sequence belongs to canonical class 1 and the CDR-H2 sequence belongs to class 2 (Martin & Thornton (1996), J. Mol. Biol. 263:800-15). The CDR-H3 sequence has no canonical class, but probably has a kinked base according to the rules of Shirai et al. (1999), FEBS Lett. 455:188-97.

[0158] The residues at the interface between the Vk and Vh domains are usual residues for these positions in mice.

[0159] A search was performed over the protein sequences in the PDB database (Deshpande et al. (2011), J. Virol. 85:1820-33) to find structures which would provide a rough structural model of the 16B5 antibody. The structure of the anti-Cholera toxin antibody Fab fragment Te33 (pdb code 1ZEA_H) was used for the VL with a resolution of 1.78 A. It retained the same canonical structure for the loops as 16B5. The Fab crystal structure in the Dsbb-Fab Complex (pdb code 2ZUQ_B) was used to model the VH domain of 16B5. It was solved at a resolution of 3.3 A and contained the same canonical structures for CDR-H1 and CDR-H2, and also the same length

CDR-H3 with a kinked based. The BioLuminate program was used to model a rough structure of 16B5 Fv.

[0160] A search of the non-redundant protein sequence database from NCBI with a CDR"X"ed 16B5 Fv sequence allowed selection of suitable human frameworks into which to graft the murine CDRs. For Vk, a human kappa light chain with NCBI accession code ACJ71718.pro was chosen (SEQ ID NO:20). This human kappa light chain sequence has the same canonical classes for CDR-L2 and L3. For Vh, human Ig heavy chain BAC02002.1 was chosen (SEQ ID NO:14). It shares the canonical form of 16B5 CDR-H1 and H2, and H3 is 8 residues long with a predicted kinked base.

[0161] The humanized heavy chain and light chain designs and backmutations based on these human frameworks are shown in Tables 5 and 6, respectively.

[0162] A humanized 16B5 variable heavy chain (H1) having the sequence of SEQ ID NO: 15 was designed. The design includes three backmutations: R13K; V48M; and Y98F. The K at position 13 was selected because it is more frequent than R in humans. The M at position 48 was selected because it is more frequent than V in humans. The F at position 98 was selected because it is located at an interface, making it desirable to keep the mouse residue.

[0163] Three humanized 16B5 variable light chain sequences were designed:

Version 1 (L1) has the sequence of SEQ ID NO: 21 and includes three backmutations: D1N; M4L; and Y36F. The N at position 1 was selected because it forms a potential hydrogen bond with N61 in HCDR2. The L at position 4 was selected because it contacts K96, Q97and S98 in LCDR3; it also contacts F104, an interface residue. The F at position 36 was selected because Y can hydrogen bond with D106 in HCDR3, whereas F cannot. The hydrogen bond would constitute an extra interaction which may affect HCDR3 function, and thus is preferably avoided.

[0164] Version 2 (L2) has the sequence of SEQ ID NO: 22 and includes four backmutations: D1N; M4L; Y36F; and P43S. The rationale for D1N, M4L, and Y36F are the same as for Version 1. The S at position 43 was selected because S forms a hydrogen bond with Q110 in VH, which is close to HCDR3.

[0165] Version 3 (L3) has the sequence of SEQ ID NO: 23 and includes three backmutations: M4L; Y36F; and P43S. The rationale for each of these mutations is the same as for Versions 1 and 2.

Table 5

Sequences for humanization of 16B5 heavy chain

Kabat residue #	Linear residue #	FR or CDR	Parent mouse mAb SEQ ID NO:10	Hu VH Acceptor FR B2 SEQ ID NO:14	Design v1 (R13K, V48M, Y91F) SEQ ID NO:15
1	1	Fr1	Q	Q	Q
2	2	Fr1	I	V	V
3	3	Fr1	Q	Q	Q
4	4	Fr1	L	L	L
5	5	Fr1	V	V	V
6	6	Fr1	Q	Q	Q
7	7	Fr1	S	S	S
8	8	Fr1	G	G	G
9	9	Fr1	P	S	S
10	10	Fr1	E	E	E
11	11	Fr1	L	L	L
12	12	Fr1	K	K	K
13	13	Fr1	K	R	K
14	14	Fr1	P	P	P
15	15	Fr1	G	G	G
16	16	Fr1	E	A	A
17	17	Fr1	T	S	S
18	18	Fr1	V	V	V
19	19	Fr1	K	K	K
20	20	Fr1	I	V	V
21	21	Fr1	S	S	S
22	22	Fr1	C	C	C
23	23	Fr1	K	K	K
24	24	Fr1	A	A	A
25	25	Fr1	S	S	S
26	26	Fr1	G	G	G
27	27	Fr1	Y	Y	Y
28	28	Fr1	P	S	T
29	29	Fr1	F	F	F

Table 5

Sequences for humanization of 16B5 heavy chain

Kabat residue #	Linear residue #	FR or CDR	Parent mouse mAb SEQ ID NO:10	Hu VH Acceptor FR B2 SEQ ID NO:14	Design V1 (R13K, V48M, Y91F) SEQ ID NO:15
30	30	Fr1	T	T	T
31	31	CDR-H1	Y	S	Y
32	32	CDR-H1	H	Y	H
33	33	CDR-H1	G	A	G
34	34	CDR-H1	M	V	M
35	35	CDR-H1	D	N	D
35A		CDR-H1			
35B		CDR-H1			
36	36	Fr2	W	W	W
37	37	Fr2	V	V	V
38	38	Fr2	K	R	R
39	39	Fr2	Q	Q	Q
40	40	Fr2	A	A	A
41	41	Fr2	P	P	P
42	42	Fr2	W	G	G
43	43	Fr2	G	Q	Q
44	44	Fr2	G	G	G
45	45	Fr2	L	L	L
46	46	Fr2	E	E	E
47	47	Fr2	W	W	W
48	48	Fr2	M	V	M
49	49	Fr2	G	G	G
50	50	CDR-H2	W	W	W
51	51	CDR-H2	I	I	I
52	52	CDR-H2	N	N	N
52A	53	CDR-H2	T	T	T
52B	54	CDR-H2	Y	N	Y
52C	55	CDR-H2	S	T	S
52D	56	CDR-H2	G	G	G
52E	57	CDR-H2	V	N	V
52F	58	CDR-H2	P	P	P
53	59	CDR-H2	T	T	T
54	60	CDR-H2	Y	Y	Y
55	61	CDR-H2	A	A	A

Table 5

Sequences for humanization of 16B5 heavy chain

Kabat residue #	Linear residue #	FR or CDR	Parent mouse mAb SEQ ID NO:10	Hu VH Acceptor FR B2 SEQ ID NO:14	Design V1 (R13K, V48M, Y91F) SEQ ID NO:15
56	62	CDR-H2	D	Q	D
57	63	CDR-H2	D	G	D
58	64	CDR-H2	F	F	F
59	65	CDR-H2	K	T	K
60	66	CDR-H2	G	G	G
66	67	Fr3	R	R	R
67	68	Fr3	F	F	F
68	69	Fr3	A	V	V
69	70	Fr3	F	F	F
70	71	Fr3	S	S	S
71	72	Fr3	L	L	L
72	73	Fr3	E	D	D
73	74	Fr3	T	T	T
74	75	Fr3	S	S	S
75	76	Fr3	V	V	V
76	77	Fr3	G	S	S
77	78	Fr3	T	T	T
78	79	Fr3	A	A	A
79	83	Fr3	Y	Y	Y
80	84	Fr3	L	L	L
81	85	Fr3	Q	Q	Q
82	86	Fr3	I	I	I
82A	87	Fr3	N	S	S
82B	88	Fr3	N	S	S
82C	89	Fr3	L	L	L
83	90	Fr3	K	K	K
84	91	Fr3	N	A	A
85	92	Fr3	E	A	E
86	93	Fr3	D	D	D
87	94	Fr3	T	T	T
88	95	Fr3	A	A	A
89	96	Fr3	T	V	V
90	97	Fr3	Y	Y	Y
91	98	Fr3	F	Y	F

Table 5

Sequences for humanization of 16B5 heavy chain

Kabat residue #	Linear residue #	FR or CDR	Parent mouse mAb SEQ ID NO:10	Hu VH Acceptor FR B2 SEQ ID NO:14	Design v1 (R13K, V48M, Y91F) SEQ ID NO:15
92	99	Fr3	C	C	C
93	100	Fr3	A	A	A
94	101	Fr3	R	R	R
95	102	CDR-H3	R	A	R
96	103	CDR-H3	R	R	R
97	104	CDR-H3	D	G	D
98	105	CDR-H3	F	Q	F
99	106	CDR-H3	T	N	T
100	107	CDR-H3	M	G	M
100A		CDR-H3		M	
100B					
100C					
100D					
100E					
100F					
100G					
100H					
100I					
100J					
100K					
101	108	CDR-H3	D	D	D
102	109	CDR-H3	F	V	F
103	110	Fr4	W	W	W
104	111	Fr4	G	G	G
105	112	Fr4	Q	Q	Q
106	113	Fr4	G	G	G
107	114	Fr4	T	T	T
108	115	Fr4	S	T	T
109	116	Fr4	V	V	V
110	117	Fr4	T	T	T
111	118	Fr4	V	V	V
112	119	Fr4	S	S	S
113	120	Fr4	S	S	S

Table 6

Sequences of humanized 16B5 light chain variable regions

Kabat residue #	Linear residue #	FR or CDR	Parent mouse mAb SEQ ID NO:16	Hu VL Acceptor Fr SEQ ID NO:20	Humanized Design v1 (D1N, M4L, Y36F) SEQ ID NO:21	Humanized Design v2 (D1N, M4L, Y36F, P43S) SEQ ID NO:22	Humanized Design v3 (M4L, Y36F, P43S) SEQ ID NO:23
1	1	Fr1	N	D	N	N	D
2	2	Fr1	I	I	I	I	I
3	3	Fr1	V	V	V	V	V
4	4	Fr1	L	M	L	L	L
5	5	Fr1	S	T	T	T	T
6	6	Fr1	Q	Q	Q	Q	Q
7	7	Fr1	S	S	S	S	S
8	8	Fr1	P	P	P	P	P
9	9	Fr1	S	D	D	D	D
10	10	Fr1	S	S	S	S	S
11	11	Fr1	L	L	L	L	L
12	12	Fr1	A	A	A	A	A
13	13	Fr1	V	V	V	V	V
14	14	Fr1	S	S	S	S	S
15	15	Fr1	P	L	L	L	L
16	16	Fr1	G	G	G	G	G
17	17	Fr1	E	E	E	E	E
18	18	Fr1	K	R	R	R	R
19	19	Fr1	V	A	A	A	A
20	20	Fr1	T	T	T	T	T
21	21	Fr1	M	I	I	I	I
22	22	Fr1	S	N	N	N	N
23	23	Fr1	C	C	C	C	C
24	24	CDR-L1	K	K	K	K	K
25	25	CDR-L1	S	S	S	S	S
26	26	CDR-L1	S	S	S	S	S
27	27	CDR-L1	Q	Q	Q	Q	Q
27A	28	CDR-L1	S	S	S	S	S
27B	29	CDR-L1	L	V	L	L	L
27C	30	CDR-L1	L	L	L	L	L
27D	31	CDR-L1	N	Y	N	N	N
27E	32	CDR-L1	S	S	S	S	S

Table 6

Sequences of humanized 16B5 light chain variable regions

Kabat residue #	Linear residue #	FR or CDR	Parent mouse mAb SEQ ID NO:16	Hu VL Acceptor Fr SEQ ID NO:20	Humanized Design v1 (D1N, M4L, Y36F) SEQ ID NO:21	Humanized Design v2 (D1N, M4L, Y36F, P43S) SEQ ID NO:22	Humanized Design v3 (M4L, Y36F, P43S) SEQ ID NO:23
27F	33	CDR-L1	R	S	R	R	R
28	34	CDR-L1	T	N	T	T	T
29	35	CDR-L1	R	N	R	R	R
30	36	CDR-L1	K	K	K	K	K
31	37	CDR-L1	N	N	N	N	N
32	38	CDR-L1	Y	Y	Y	Y	Y
33	39	CDR-L1	L	L	L	L	L
34	40	CDR-L1	A	A	A	A	A
35	41	Fr2	W	W	W	W	W
36	42	Fr2	F	Y	F	F	F
37	43	Fr2	Q	Q	Q	Q	Q
38	44	Fr2	Q	Q	Q	Q	Q
39	45	Fr2	K	K	K	K	K
40	46	Fr2	P	P	P	P	P
41	47	Fr2	G	G	G	G	G
42	48	Fr2	Q	Q	Q	Q	Q
43	49	Fr2	S	P	P	S	S
44	50	Fr2	P	P	P	P	P
45	51	Fr2	K	K	K	K	K
46	52	Fr2	L	L	L	L	L
47	53	Fr2	L	L	L	L	L
48	54	Fr2	I	I	I	I	I
49	55	Fr2	Y	Y	Y	Y	Y
50	56	CDR-L2	W	W	W	W	W
51	57	CDR-L2	A	A	A	A	A
52	58	CDR-L2	S	S	S	S	S
53	59	CDR-L2	T	T	T	T	T
54	60	CDR-L2	R	R	R	R	R
55	61	CDR-L2	E	E	E	E	E
56	62	CDR-L2	S	S	S	S	S
57	63	Fr3	G	G	G	G	G
58	64	Fr3	V	V	V	V	V

Table 6

Sequences of humanized 16B5 light chain variable regions

Kabat residue #	Linear residue #	FR or CDR	Parent mouse mAb SEQ ID NO:16	Hu VL Acceptor Fr SEQ ID NO:20	Humanized Design v1 (D1N, M4L, Y36F) SEQ ID NO:21	Humanized Design v2 (D1N, M4L, Y36F, P43S) SEQ ID NO:22	Humanized Design v3 (M4L, Y36F, P43S) SEQ ID NO:23
59	65	Fr3	P	P	P	P	P
60	66	Fr3	D	D	D	D	D
61	67	Fr3	R	R	R	R	R
62	68	Fr3	F	F	F	F	F
63	69	Fr3	T	S	S	S	S
64	70	Fr3	G	G	G	G	G
65	71	Fr3	S	S	S	S	S
66	72	Fr3	G	G	G	G	G
67	73	Fr3	S	S	S	S	S
68	74	Fr3	G	G	G	G	G
69	75	Fr3	T	T	T	T	T
70	76	Fr3	D	D	D	D	D
71	77	Fr3	F	F	F	F	F
72	78	Fr3	T	T	T	T	T
73	79	Fr3	L	L	L	L	L
74	80	Fr3	T	T	T	T	T
75	81	Fr3	I	I	I	I	I
76	82	Fr3	S	S	S	S	S
77	83	Fr3	S	S	S	S	S
78	84	Fr3	V	L	L	L	L
79	85	Fr3	Q	Q	Q	Q	Q
80	86	Fr3	A	A	A	A	A
81	87	Fr3	E	E	E	E	E
82	88	Fr3	D	D	D	D	D
83	89	Fr3	L	V	V	V	V
84	90	Fr3	A	A	A	A	A
85	91	Fr3	V	V	V	V	V
86	92	Fr3	Y	Y	Y	Y	Y
87	93	Fr3	Y	Y	Y	Y	Y
88	94	Fr3	C	C	C	C	C
89	95	CDR-L3	K	Q	K	K	K
90	96	CDR-L3	Q	Q	Q	Q	Q

Table 6

Sequences of humanized 16B5 light chain variable regions

Kabat residue #	Linear residue #	FR or CDR	Parent mouse mAb SEQ ID NO:16	Hu VL Acceptor Fr SEQ ID NO:20	Humanized Design v1 (D1N, M4L, Y36F) SEQ ID NO:21	Humanized Design v2 (D1N, M4L, Y36F, P43S) SEQ ID NO:22	Humanized Design v3 (M4L, Y36F, P43S) SEQ ID NO:23
91	97	CDR-L3	S	Y	S	S	S
92	98	CDR-L3	Y	Y	Y	Y	Y
93	99	CDR-L3	T	S	T	T	T
94	100	CDR-L3	L	T	L	L	L
95		CDR-L3		P			
95A		CDR-L3					
95B		CDR-L3					
95C		CDR-L3					
95D		CDR-L3					
95E		CDR-L3					
95F		CDR-L3					
96	101	CDR-L3	R	Q	R	R	R
97	102	CDR-L3	T	T	T	T	T
98	103	Fr4	F	F	F	F	F
99	104	Fr4	G	G	G	G	G
100	105	Fr4	G	G	G	G	G
101	106	Fr4	G	G	G	G	G
102	107	Fr4	T	T	T	T	T
103	108	Fr4	N	K	K	K	K
104	109	Fr4	L	V	V	V	V
105	110	Fr4	E	E	E	E	E
106	111	Fr4	I	I	I	I	I
106A	112	Fr4	K	K	K	K	K
107	113	Fr4	R	R	R	R	R

EXAMPLE 6. Tau Affinity of Humanized 16B5 Antibodies

[0166] Binding data for humanized 16B5 antibodies having an H1L1 or H1L2 design are shown in Table 7, below. For comparison, the binding data for chimeric 16B5 is also shown. The data was generated using a Biacore instrument. It was concluded that version H1L2 has the strongest

affinity – essentially the same as that of chimeric 16B5. Humanized 16B5 versions H1L1 and H1L3 also had adequate affinity.

[0167] Surface Plasmon Resonance measurements were performed using a Biacore T200 (GE Lifesciences). All experiments were performed using a mobile phase of 10mM HEPES pH 7.4, 150 mM NaCl, and 0.05% Tween-20 at 30 µl/min, over a CM5 sensor chip prepared by amine-coupling an anti-mouse or anti-human capture antibody. 16B5 (chimeric or humanized form) was bound to the immobilized capture antibody, and varied concentrations of recombinant purified hTau-P301L were applied to the antibody complex in successive iterations. Iterative steps were separated with high salt or low pH regeneration steps. The experiments were repeated with different preparations of antibody and antigen. Analysis was performed with onboard Biacore software.

Table 7

Biacore Data

	K_D (M)	K_{on}(1/Ms)	K_{on} Error	K_{off}(1/s)	K_{off} Error
Chi16B5	232 pM	1.43×10^{7}	1.5×10^{5}	3.33×10^{-3}	3.5×10^{-5}
Hu16B5H1L1	617 pM	3.5×10^{6}	1.5×10^{4}	2.15×10^{-3}	8.2×10^{-6}
Hu16B5H1L2	286 pM	1.2×10^{7}	4.6×10^{4}	3.42×10^{-3}	1.1×10^{-5}
Hu16B5H1L3	320 pM	1.25×10^{7}	6.2×10^{4}	3.98×10^{-3}	1.8×10^{-5}

EXAMPLE 7. Immunoprecipitation Detection of Tau with Humanized 16B5 Antibodies

[0168] A postmortem sample of frontal cortex from an Alzheimer disease patient with a Braak score of 6 was sequentially extracted in buffers of increasing solubilization strength, in the following order: (i) High salt buffer (20 mM Tris, 5 mM EDTA, 1 mM DTT, 10% sucrose, 7500 mM NaCl pH 7.4), (ii) Triton buffer (20 mM Tris, 5 mM EDTA, 1 mM DTT, 10% sucrose, 1% Triton X100, 500 mM NaCl pH 7.4), and (iii) Sarkosyl Buffer (10 mM Tris, 5 mM EDTA, 1 mM DTT, 10% sucrose, 500 mM NaCl, 1% Sarkosyl, pH 7.4).

[0169] For each sample, 200 micrograms of the high salt soluble, or 20 micrograms of the sarkosyl insoluble, fractions were diluted into 400 microliters of immunoprecipitation buffer (10 mM Tris, 150 mM NaCl, 0.5% Triton X100, 1 mM EGTA, 1 mM EDTA, pH 7.4). The samples were precleared with protein G magnetic beads (New England Biolabs), and 5 micrograms of antibody was added to each tube. Antibodies used included: 1) mouse non-immune IgG

antibody (mIgG), as control; 2) human non-immune IgG antibody (hIgG), as control; 3) chimeric 16B5 antibody (Chi16B5); 4) humanized 16B5, version H1L2 (h16B6-H1L2); and humanized 16B5, version H1L3 (h16B6-H1L3). Precleared lysates and antibodies were incubated for 2 hours at 4°C. Antibody/antigen complexes were precipitated by using protein G magnetic beads, and the precipitates were washed thoroughly with PBS/350 mM NaCl. After elution using Laemmli buffer, eluates were resolved by SDS-PAGE and blotted using a polyclonal tau antibody (DAKO).

[0170] As shown in Fig. 7, chimeric 16B5 and humanized 16B5 H1L2 and H1L3 recognized tau in both soluble and insoluble fractions from Alzheimer's brain.

EXAMPLE 8. Immunohistochemical Characterization of murine and humanized 16B5 tau Antibodies on Alzheimer's Disease Brain

[0171] Murine monoclonal anti-tau antibody 16B5 and its two humanized variants, h16B5-H1L2 and h16B5-N1D, were also tested immunohistochemically on fresh frozen sections of human brain cortex from Alzheimer's disease donors and non-diseased, aged controls.

Methods:

Human brain tissue

[0172] Frontal cortices were obtained from Sun Health Research Institute. Cases included six patients (mean age 86.8 ± 0.40 SEM) diagnosed with Alzheimer's disease and confirmed upon post mortem neuropathological assessment, and three non-diseased aged controls (mean age 77 ± 9.7 SEM). Demographics of the cases are listed in Table 8, below. Immunohistochemistry was performed on lightly acetone-fixed,10 um slide-mounted cryosections.

Table 8

Demographics for cases examined immunohistochemically

Case	Diagnosis	Expired Age (years)	Sex	Post-mortem interval (h)
11-21	AD	88	F	2.28
03-34	AD	88	F	3.3
08-06	AD	86	M	2.66

Table 8

Demographics for cases examined immunohistochemically

03-52	AD	86	M	2.2
01-16	AD	87	M	3
01-18	AD	86	M	3
10-63	Control	79	M	3
10-39	Control	93	M	3
10-22	Control	59	F	3.2

Immunohistochemistry

[0173] The immunoperoxidase method was the principal detection system, which consisted of either a peroxidase labeled polymer conjugated to goat anti-mouse immunoglobulins (EnVision+System HRP labeled Polymer; Dako K4001) or a Vector ABC amplification system for directly biotinylated humanized antibodies (ABC Elite Standard; PK-6100; Vector Laboratories). The staining was visualized with a DAB chromogen (Liquid DAB + Substrate Chromogen System; Dako K3468), which produced a brown deposit.

[0174] The negative control consisted of performing the entire immunohistochemical procedure on adjacent sections with an IgG isotype control antibody or an omission of the primary antibody.

Immunofluorescent labeling

[0175] Double immunofluorescent staining was conducted to determine the relationship between the murine and humanized variants of the antibody, other tau antibodies that recognize various phosphorylated epitopes, and amyloid beta. Tissue sections were stained in parallel with an antibody cocktail containing biotinylated or FITC-tagged humanized 16B5 variants (1 ug/mL) and a murine antibody (either monoclonal 16B5 (1 ug/mL), AT8 (1:1000), AT100 (1:1000), or 3D6 (1 ug/mL). The murine antibodies were detected with a goat anti-mouse secondary conjugated to a 488 or 635 fluorophore (Invitrogen). The biotinylated humanized antibodies were detected with a streptavidin 635.

Preabsorptions

[0176] To assess the specificity of the antibodies to its target antigens, 1 ug/mL of 16B5 antibodies were preabsorbed with 50 ug/mL of purified human P301L tau or wild-type synuclein (an irrelevant protein) overnight at 4°C. The antibodies were then applied to tissue and the immunohistochemistry procedure was conducted as outlined above.

Image Analysis

[0177] Slides were imaged with either an Olympus BX61 microscope, Olympus Nanozoomer 2.0HT, or a Leica SPE spectral confocal system. Images were collected as TIFF files.

Results

[0178] As shown in Table 9, below, mouse monoclonal antibody 16B5 and both humanized variants showed reactivity on Alzheimer's disease tissue, staining prominently neuropil threads, some neurofibrillary tangles (mostly globose), and some tau-positive neuritic plaques. Most of the 16B5 AD-fibrillar pathology were confined to the grey matter, but some reactivity was also detected in the white. The non-diseased control tissue, in contrast, showed diffuse background reactivity but was negative for any pathologies found in the AD tissue.

[0179] Double labeling experiments were performed with the murine monoclonal version of 16B5 and with (1) both humanized variants, (2) antibodies recognizing tau at various phosphorylated epitopes, and (3) beta amyloid to further characterize the pathologies recognized by the antibody variants.

[0180] Both h16B5-H1L2 and h16B5-N1D colocalized with monoclonal 16B5 antibody with high congruence on AD-fibrillar pathological structures. H16B5-H1L2 also detected pathologies that were shown to be immunoreactive to various phosphorylated tau epitopes, including serine202 and threonine205 (AT8), serine212 and threonine214 (AT100), and serine396 (in-house proprietary antibody, 20H1). Finally, double labeling with an amyloid beta antibody that recognizes the N-terminal amino acid sequence (3D6; aa 1-5) and 16B5 showed very little colocalization between Aβ and 16B5-immunoreactive structures on amyloid plaques.

[0181] When 16B5 immunoreactivity was compared to a well characterized commercially available monoclonal anti-tau antibody (Dako), both stained the fibrillar AD pathology which included tau-positive neuritic plaques, neuropil threads, and neurofibrillary tangles.

[0182] The specificity of the antibody was assessed by preabsorptions with purified recombinant P301L tau. A decrement in the staining was observed when 16B5 was preabsorbed with P301L tau, but staining was unaffected when the antibodies were preabsorbed with an irrelevant protein (wild-type synuclein) at the same molar concentrations.

[0183] Both the IgG-isotype control antibody and primary antibody omission sections were negative for staining across all tissues tested.

Table 9

16B5 antibodies characterized immunohistochemically

Antibody	Lot#	Stain AD Tissue	Concentration
Murine 16B5	NB-0174A	Yes	1 ug/mL
Chimeric 16B5	061512	Yes	1 ug/mL
h16B5-H1L2	NB-0248	Yes	1 ug/mL
H16B5-N1D	011113	Yes	1 ug/mL

[0184] All publications (including GenBank Accession numbers, UniProtKB/Swiss-Prot accession numbers and the like), patents and patent applications cited are herein incorporated by reference in their entirety for all purposes to the same extent as if each individual publication, patent and patent application was specifically and individually indicated to be incorporated by reference in its entirety for all purposes. In the event of any variance in sequences associated with Genbank and UniProtKB/Swiss-Prot accession numbers and the like, the application refers to the sequences associated with the cited accession numbers as of the effective filing date of the application meaning the actual filing date or earlier date of a priority application disclosing the relevant accession number. Any feature, step, element, embodiment, or aspect of the invention can be used in combination with any other unless specifically indicated otherwise. Although the present invention has been described in some detail by way of illustration and example for purposes of clarity and understanding, it will be apparent that certain changes and modifications may be practiced within the scope of the appended claims.

WHAT IS CLAIMED IS:

1. A monoclonal antibody that competes for binding to tau with antibody 16B5.

2. The monoclonal antibody of claim 1 that is a humanized, chimeric, veneered or human antibody.

3. The monoclonal antibody of claim 1 that is of human IgG isotype.

4. The monoclonal antibody of claim 1, wherein the isotype is IgG1.

5. The monoclonal antibody of claim 4, having at least one mutation in the constant region.

6. The monoclonal antibody of claim 1 that is of human IgG2 or IgG4 isotype.

7. The monoclonal antibody of claim 1 that is a humanized, chimeric or veneered form of monoclonal antibody 16B5.

8. The monoclonal antibody of claim 1 that has the three light chain CDRs as defined by Kabat and three heavy chain CDRs as defined by Kabat of monoclonal antibody 16B5.

9. A monoclonal antibody that specifically binds to an epitope within residues 24-46 of SEQ ID NO:1 (Swiss-Prot No. P10636-8).

10. The antibody of claim 9 that is human, humanized, chimeric, or veneered.

11. The antibody of claim 10 that specifically binds to an epitope within residues 25-44 of SEQ ID NO:1.

12. The antibody of claim 11 that is a humanized, chimeric or veneered antibody that binds to an epitope within residues 30-39 of SEQ ID NO:1.

13. The antibody of claim 1, that binds to tau in phosphorylated and unphosphorylated forms.

14. An antibody comprising a mature heavy chain variable region having an amino acid sequence at least 90% identical to SEQ ID NO:15 and a mature light chain variable region at least 90% identical to SEQ ID NO:22.

15. The antibody of claim 14, comprising three Kabat CDRs of SEQ ID NO:15 and three Kabat CDRs of SEQ ID NO:22.

16. The antibody of claim 14 or 15, provided at least one of positions H13, H48 and H91 is occupied by K, M and F respectively and at least one of positions L1, L4, L36 and L43 is occupied by N, L, F and S respectively.

17. The antibody of claim 16, provided positions H13, H48 and H91 are occupied by K, M and F respectively and at least two of positions L1, L4, L36 and L43 is occupied by N, L, F and S respectively.

18. The antibody of claim 17, provided positions H13, H48 and H91 are occupied by K, M and F respectively, and at least three of positions L1, L4, L36 and L43 are occupied by N, L, F and S respectively.

19. The antibody of claim 17, provided positions H13, H48 and H91 are occupied by K, M and F respectively, and positions L1, L4, L36 and L43 are occupied by N, L, F and S respectively.

20. The antibody of any of claims 14-16, comprising a mature heavy chain variable region having an amino acid sequence at least 95% identical to SEQ ID NO:15 and a mature light chain variable region at least 95% identical to SEQ ID NO:22.

21. The antibody of any of claims 14-20 wherein the mature heavy chain variable region is fused to a heavy chain constant region and the mature light chain variable region is fused to a light chain constant region.

22. The antibody of any of claims 14-21, wherein the heavy chain constant region is a mutant form of natural human constant region which has reduced binding to an Fcγ receptor relative to the natural human constant region.

23. The antibody of any of claims 21 or 22, wherein the heavy chain constant region is of IgG1 isotype.

24. The antibody of any of claims 14-23, provided any differences in CDRs of the mature heavy chain variable region and mature light variable region from SEQ ID NOS: 15 and 22 respectively reside in positions H60-H65.

25. The antibody of any of claims 14, 22, and 24, wherein the mature heavy chain variable region has an amino acid sequence designated SEQ ID NO:15 and the mature light chain variable region has an amino acid sequence designated SEQ ID NO:21, 22, or 23.

26. The antibody of any of claims 14-25, wherein the mature heavy chain variable region has an amino acid sequence designated SEQ ID NO:15 and the mature light chain variable region has an amino acid sequence designated SEQ ID NO:22.

27. The antibody of any of claims 14-26, wherein the antibody is conjugated to a cytotoxic or cytostatic agent.

28. The antibody of any of claims 1-27, wherein the antibody is a Fab fragment.

29. A polynucleotide comprising a nucleic acid encoding the heavy and/or light chains of an antibody as described in claims 14-28.

30. A method of humanizing an antibody, comprising:

determining the sequences of the heavy and light chain variable regions of a mouse antibody;

synthesizing a nucleic acid encoding a humanized heavy chain comprising CDRs of the mouse antibody heavy chain and a nucleic acid encoding a humanized light chain comprising CDRs of the mouse antibody light chain; and

expressing the nucleic acids in a host cell to produce a humanized antibody,

wherein the mouse antibody is 16B5.

31. A method of producing a humanized, chimeric or veneered antibody, comprising:

culturing cells transformed with nucleic acids encoding the heavy and light chains of the antibody, so that the cell secretes the antibody; and

purifying the antibody from cell culture media,

wherein the antibody is a humanized, chimeric or veneered form of 16B5.

32. A method of producing a cell line producing a humanized, chimeric or veneered antibody, comprising;

introducing a vector encoding heavy and light chains of an antibody and a selectable marker into cells;

propagating the cells under conditions to select for cells having increased copy number of the vector;

isolating single cells from the selected cells; and

banking cells cloned from a single cell selected based on yield of antibody; wherein the antibody is a humanized, chimeric or veneered form of 16B5.

33. A pharmaceutical composition comprising an antibody of any preceding claim and a pharmaceutically acceptable carrier.

34. An isolated fragment of tau including 3-10 contiguous residues of tau within residues 24-46 of SEQ ID NO:1 (Swiss-Prot No. P10636-8).

35. The fragment of claim 34 that includes 3-10 contiguous residues of tau within residues 30-39 of SEQ ID NO:1 (Swiss-Prot No. P10636-8).

36. The fragment of claim 34 that includes residues 33-37 of SEQ ID NO:1 (Swiss-Prot No. P10636-8).

37. The isolated fragment of claim 34, linked to a carrier molecule optionally via a spacer that helps elicit antibodies against the fragment.

38. A pharmaceutical composition comprising the fragment of claim 34 and an adjuvant acceptable for administration to humans.

39. A method of treating or effecting prophylaxis of Alzheimer's disease comprising administering an effective regime of an antibody that specifically binds to an epitope within residues 24-46 of SEQ ID NO:1 (Swiss-Prot No. P10636-8), or an agent that induces such an antibody, to a patient having or at risk of Alzheimer's disease and thereby treating or effecting prophylaxis of the disease.

40. The method of claim 39, wherein the antibody is as defined in any of claims 14-28.

41. The method of claim 39, wherein the agent that induces the antibody is a fragment of tau that includes 3-10 contiguous residues of tau within residues 24-46 of SEQ ID NO: 1.

42. The method of claim 39, wherein the patient is an ApoE4 carrier.

43. A method of treating or effecting prophylaxis of a disease associated with tau comprising administering an effective regime of an antibody that specifically binds to an epitope within residues 24-46 of SEQ ID NO:1 (Swiss-Prot No. P10636-8), or an agent that induces such an antibody, to a patient having or at risk of the disease and thereby treating or effecting prophylaxis of the disease.

44. The method of claim 43, wherein the antibody is as defined in any of claims 14-28.

45. The method of claim 43, wherein the agent that induces the antibody is a fragment of tau that includes 3-10 contiguous residues of tau within residues 24-46 of SEQ ID NO: 1.

46. The method of claim 43, wherein the disease is a neurological disease.

47. A method of reducing aberrant transmission of tau comprising administering an effective regime of an antibody that specifically binds to an epitope within residues 24-46 of SEQ ID NO:1 (Swiss-Prot No. P10636-8), or an agent that induces such an antibody, to a patient having or at risk of a disease associated with aberrant transmission of tau, and thereby treating or effecting prophylaxis of the disease.

48. The method of claim 47, wherein the antibody is as defined in any of claims 14-28.

49. The method of claim 47, wherein the agent that induces the antibody is a fragment of tau that includes 3-10 contiguous residues of tau within residues 24-46 of SEQ ID NO: 1.

50. A method of inducing phagocytosis of tau comprising administering an effective regime of an antibody that specifically binds to an epitope within residues 24-46 of SEQ ID NO:1 (Swiss-Prot No. P10636-8), or an agent that induces such an antibody, to a patient having or at risk of a disease associated with the accumulation of tau.

51. The method of claim 50, wherein the antibody is as defined in any of claims 14-28.

52. The method of claim 50, wherein the agent that induces the antibody is a fragment of tau that includes 3-10 contiguous residues of tau within residues 24-46 of SEQ ID NO: 1.

53. The method of claim 50, wherein the disease is a neurological disease.

54. A method of inhibiting tau aggregation or deposition comprising administering an effective regime of an antibody that specifically binds to an epitope within residues 24-46 of SEQ ID NO:1 (Swiss-Prot No. P10636-8), or an agent that induces such an antibody, to a patient having or at risk of a disease associated with the aggregation or deposition of tau.

55. The method of claim 54, wherein the antibody is as defined in any of claims 14-28.

56. The method of claim 54, wherein the agent that induces the antibody is a fragment of tau that includes 3-10 contiguous residues of tau within residues 24-46 of SEQ ID NO: 1.

57. The method of claim 54, wherein the disease is a neurological disease.

58. A method of inhibiting formation of tau tangles comprising administering an effective regime of an antibody that specifically binds to an epitope within residues 24-46 of SEQ ID NO:1 (Swiss-Prot No. P10636-8), or an agent that induces such an antibody, to a patient having or at risk of a disease associated with the formation of tau tangles.

59. The method of claim 58, wherein the antibody is as defined in any of claims 14-28.

60. The method of claim 58, wherein the agent that induces the antibody is a fragment of tau that includes 3-10 contiguous residues of tau within residues 24-46 of SEQ ID NO: 1.

61. The method of claim 58, wherein the disease is a neurological disease.

62. A method of screening an agent for activity against Alzheimer's disease,

comprising administering the agent to a transgenic animal expressing a tau transgene, and determining whether the agent inhibits or delays at least one sign or symptom of Alzheimer's disease, wherein the agent is an antibody that specifically binds to an epitope within residues 24-46 of SEQ ID NO:1 (Swiss-Prot No. P10636-8), or an agent that induces such an antibody.

63. A nucleic acid comprising a segment encoding a heavy chain variable region having he sequence of SEQ ID NO: 10.

64. A nucleic acid comprising a segment encoding a heavy chain variable region having the sequence of SEQ ID NO: 15.

65. The nucleic acid of claim 64, wherein the segment has a nucleotide sequence of SEQ ID NO: 25.

66. The nucleic acid of claim 64 or 65, further comprising a segment encoding an IgG1 constant region.

67. The nucleic acid of claim 66, wherein the IgG1 constant region is a human IgG1 constant region.

68. The nucleic acid of claim 67, wherein the IgG1 constant region has a sequence of SEQ ID NO: 29 provided the C-terminal lysine can be omitted.

69. The nucleic acid of claim 68, wherein the segment encoding the IgG1 constant region has a nucleotide sequence of SEQ ID NO: 30.

70. The nucleic acid of any of claims 66 to 69, further comprising an intron linking the segments encoding the heavy chain variable region and the IgG 1 constant region.

71. The nucleic acid of claim 70, wherein the segment encoding the IgG1 constant region has a nucleotide sequence of SEQ ID NO: 31.

72. A nucleic acid comprising a segment encoding a light chain variable region having the sequence of SEQ ID NO: 16.

73. A nucleic acid comprising a segment encoding a light chain variable region having a sequence of SEQ ID NO: 21, SEQ ID NO: 22 or SEQ ID NO: 23.

74. The nucleic acid of claim 73, wherein the segment encoding the light chain variable region has the sequence of SEQ ID NO: 26, SEQ ID NO: 27, or SEQ ID NO: 28.

75. The nucleic acid of claim 73 or 74, further comprising a segment encoding a kappa constant region.

76. The nucleic acid of claim 75, wherein the kappa constant region is a human kappa constant region.

77. The nucleic acid of claim 76, wherein the kappa constant region has the sequence of SEQ ID NO: 32.

78. The nucleic acid of claim 77, wherein the nucleic acid encoding the kappa constant region has the sequence of SEQ ID NO: 33.

79. The nucleic acid of any of claims 75 to 78, further comprising an intron linking the segment encoding encoding the light chain variable region to the segment encoding the kappa constant region.

80. The nucleic acid of claim 79, wherein the segment encoding the kappa constant region has the sequence of SEQ ID NO: 34.

81. The antibody of any of claims 1-28, wherein a heavy chain of the antibody comprises a human IgG1 constant domain having the sequence of SEQ ID NO: 29.

82. The antibody of any of claims 1-28, wherein a light chain of the antibody comprises a human kappa constant region having the sequence of SEQ ID NO: 32.

83. Nucleic acid(s) encoding the heavy chain variable region of SEQ ID NO:15 and/or the light chain variable region of SEQ ID NO:21, 22 or 23.

SEQUENCE LISTING

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Dolan, Philip James III
Liu, Yue
Barbour, Robin

<120> TAU IMMUNOTHERAPY

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    50                  55                  60
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65                  70                  75                  80
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Gly Tyr Ser Ser Pro Gly Ser Pro Gly Thr Pro Gly Ser Arg Ser Arg
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    210                 215                 220
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Thr Ala Pro Val Pro Met Pro Asp Leu Lys Asn Val Lys Ser Lys Ile
                245                 250                 255
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325 330 335
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340 345 350
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355 360 365
Lys Leu Thr Phe Arg Glu Asn Ala Lys Ala Lys Thr Asp His Gly Ala
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385 390 395 400
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Lys Val Ala Val Val Arg Thr Pro Pro Lys Ser Pro Ser Ser Ala Lys
195 200 205
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Lys Ser Lys Gly Ser Thr Glu Asn Leu Lys His Gln Pro Gly Gly Gly
225 230 235 240
Lys Val Gln Ile Ile Asn Lys Lys Leu Asp Leu Ser Asn Val Gln Ser
245 250 255
Lys Cys Gly Ser Lys Asp Asn Ile Lys His Val Pro Gly Gly Gly Ser
260 265 270
Val Gln Ile Val Tyr Lys Pro Val Asp Leu Ser Lys Val Thr Lys Cys
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Ser Pro Arg His Leu Ser Asn Val Ser Ser Thr Gly Ser Ile Asp Met
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        35                  40                  45
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    50                  55                  60
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65                  70                  75                  80
Lys Lys Ala Lys Gly Ala Asp Gly Lys Thr Lys Ile Ala Thr Pro Arg
                85                  90                  95
Gly Ala Ala Pro Pro Gly Gln Lys Gly Gln Ala Asn Ala Arg Ile Pro
            100                 105                 110
Ala Lys Thr Pro Pro Ala Pro Lys Thr Pro Pro Ser Ser Gly Glu Pro
        115                 120                 125
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                245                 250                 255
Thr Ser Lys Cys Gly Ser Leu Gly Asn Ile His His Lys Pro Gly Gly
            260                 265                 270
Gly Gln Val Glu Val Lys Ser Glu Lys Leu Asp Phe Lys Asp Val Gln
        275                 280                 285
Ser Lys Ile Gly Ser Leu Asp Asn Ile Thr His Val Pro Gly Gly Gly
    290                 295                 300
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65   70   75   80
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Gly Lys Thr Lys Ile Ala Thr Pro Arg Gly Ala Ala Pro Pro Gly Gln
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                85   90   95
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Trp Asn Ser Gly Ser Leu Ser Ser Gly Val His Thr Phe Pro Ala Val
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Leu Gln Ser Asp Leu Tyr Thr Leu Ser Ser Ser Val Thr Val Pro Ser
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    50   55   60
Thr Gly Arg Phe Val Phe Ser Leu Asp Thr Ser Val Ser Thr Ala Tyr
65   70   75   80
Leu Gln Ile Ser Ser Leu Lys Ala Ala Asp Thr Ala Val Tyr Tyr Cys
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    50   55   60
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        35   40   45
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    50   55   60
Lys Pro Gly Gln Ser Pro Lys Leu Leu Ile Tyr Trp Ala Ser Thr Arg
65   70   75   80
Glu Ser Gly Val Pro Asp Arg Phe Thr Gly Ser Gly Ser Gly Thr Asp
                85   90   95
Phe Thr Leu Thr Ile Ser Ser Val Gln Ala Glu Asp Leu Ala Val Tyr
            100   105   110
Tyr Cys Lys Gln Ser Tyr Thr Leu Arg Thr Phe Gly Gly Gly Thr Asn
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                165   170   175
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            180   185   190
Ser Lys Asp Ser Thr Tyr Ser Met Ser Ser Thr Leu Thr Leu Thr Lys
        195   200   205
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<400> 17
Lys Ser Ser Gln Ser Leu Leu Asn Ser Arg Thr Arg Lys Asn Tyr Leu
 1   5   10   15
Ala

<210> 18
<211> 7
<212> PRT
<213> Artificial Sequence

<220>
<223> Synthesized

<400> 18
Trp Ala Ser Thr Arg Glu Ser
 1   5

<210> 19
<211> 8
<212> PRT
<213> Artificial Sequence

<220>
<223> Synthesized

<400> 19
Lys Gln Ser Tyr Thr Leu Arg Thr
1 5

<210> 20
<211> 114
<212> PRT
<213> Artificial Sequence

<220>
<223> Synthesized

<400> 20
Asp Ile Val Met Thr Gln Ser Pro Asp Ser Leu Ala Val Ser Leu Gly
1 5 10 15
Glu Arg Ala Thr Ile Asn Cys Lys Ser Ser Gln Ser Val Leu Tyr Ser
20 25 30
Ser Asn Asn Lys Asn Tyr Leu Ala Trp Tyr Gln Gln Lys Pro Gly Gln
35 40 45
Pro Pro Lys Leu Leu Ile Tyr Trp Ala Ser Thr Arg Glu Ser Gly Val
50 55 60
Pro Asp Arg Phe Ser Gly Ser Gly Ser Gly Thr Asp Phe Thr Leu Thr
65 70 75 80
Ile Ser Ser Leu Gln Ala Glu Asp Val Ala Val Tyr Tyr Cys Gln Gln
85 90 95
Tyr Tyr Ser Thr Pro Gln Thr Phe Gly Gly Gly Thr Lys Val Glu Ile
100 105 110
Lys Arg

<210> 21
<211> 113
<212> PRT
<213> Artificial Sequence

<220>
<223> Synthesized

<400> 21
Asn Ile Val Leu Thr Gln Ser Pro Asp Ser Leu Ala Val Ser Leu Gly
1 5 10 15
Glu Arg Ala Thr Ile Asn Cys Lys Ser Ser Gln Ser Leu Leu Asn Ser
20 25 30
Arg Thr Arg Lys Asn Tyr Leu Ala Trp Phe Gln Gln Lys Pro Gly Gln
35 40 45
Pro Pro Lys Leu Leu Ile Tyr Trp Ala Ser Thr Arg Glu Ser Gly Val
50 55 60
Pro Asp Arg Phe Ser Gly Ser Gly Ser Gly Thr Asp Phe Thr Leu Thr
65 70 75 80
Ile Ser Ser Leu Gln Ala Glu Asp Val Ala Val Tyr Tyr Cys Lys Gln
85 90 95
Ser Tyr Thr Leu Arg Thr Phe Gly Gly Gly Thr Lys Val Glu Ile Lys
100 105 110
Arg

<210> 22

<211> 113
<212> PRT
<213> Artificial Sequence

<220>
<223> Synthesized

<400> 22
Asn Ile Val Leu Thr Gln Ser Pro Asp Ser Leu Ala Val Ser Leu Gly
 1   5   10   15
Glu Arg Ala Thr Ile Asn Cys Lys Ser Ser Gln Ser Leu Leu Asn Ser
            20   25   30
Arg Thr Arg Lys Asn Tyr Leu Ala Trp Phe Gln Gln Lys Pro Gly Gln
        35   40   45
Ser Pro Lys Leu Leu Ile Tyr Trp Ala Ser Thr Arg Glu Ser Gly Val
    50   55   60
Pro Asp Arg Phe Ser Gly Ser Gly Ser Gly Thr Asp Phe Thr Leu Thr
65   70   75   80
Ile Ser Ser Leu Gln Ala Glu Asp Val Ala Val Tyr Tyr Cys Lys Gln
                85   90   95
Ser Tyr Thr Leu Arg Thr Phe Gly Gly Gly Thr Lys Val Glu Ile Lys
            100   105   110
Arg

<210> 23
<211> 113
<212> PRT
<213> Artificial Sequence

<220>
<223> Synthesized

<400> 23
Asp Ile Val Leu Thr Gln Ser Pro Asp Ser Leu Ala Val Ser Leu Gly
 1   5   10   15
Glu Arg Ala Thr Ile Asn Cys Lys Ser Ser Gln Ser Leu Leu Asn Ser
            20   25   30
Arg Thr Arg Lys Asn Tyr Leu Ala Trp Phe Gln Gln Lys Pro Gly Gln
        35   40   45
Ser Pro Lys Leu Leu Ile Tyr Trp Ala Ser Thr Arg Glu Ser Gly Val
    50   55   60
Pro Asp Arg Phe Ser Gly Ser Gly Ser Gly Thr Asp Phe Thr Leu Thr
65   70   75   80
Ile Ser Ser Leu Gln Ala Glu Asp Val Ala Val Tyr Tyr Cys Lys Gln
                85   90   95
Ser Tyr Thr Leu Arg Thr Phe Gly Gly Gly Thr Lys Val Glu Ile Lys
            100   105   110
Arg

<210> 24
<211> 10
<212> PRT
<213> Artificial Sequence

<220>
<223> Synthesized

<400> 24
Gly Tyr Pro Phe Thr Tyr His Gly Met Asp
 1   5   10

<210> 25
<211> 351

<212> DNA
<213> Artificial Sequence

<220>
<223> Synthesized

<400> 25
caggtccagt tggtgcagtc tggatctgag ctgaagaagc ctggagcctc cgtcaaggtg 60
tcctgcaagg cttctgggta tcccttcaca taccatggaa tggactgggt gcgtcaggct 120
cctggtcagg gtttagagtg gatgggctgg ataaacacct actctggagt gccaacatat 180
gctgatgact tcaagggacg atttgtgttc tctttggaca cctctgtctc tactgcctat 240
ttgcagatct cttctctcaa agccgaggac acggccgtgt atttttgtgc aagacggcgt 300
gattttacaa tggacttctg gggtcaagga accaccgtga ccgtctcctc a 351

<210> 26
<211> 339
<212> DNA
<213> Artificial Sequence

<220>
<223> Synthesized

<400> 26
aacatcgtgc tgacccagag ccccgatagc ctggccgtga gcctgggcga gagagccacc 60
atcaactgca agagcagcca gagcctgctg aacagcagga ccaggaagaa ctacctggcc 120
tggttccagc agaagcccgg ccagcccccc aagctgctga tctactgggc cagcaccagg 180
gagagcggcg tgcccgatag gttcagcggc agcggcagcg gcaccgattt caccctgacc 240
atcagcagcc tgcaggccga ggatgtggcc gtgtactact gcaagcagag ctacaccctg 300
agaaccttcg gcggcggcac caaggtggaa attaaacgt 339

<210> 27
<211> 339
<212> DNA
<213> Artificial Sequence

<220>
<223> Synthesized

<400> 27
aacatcgtgc tgacccagag ccccgatagc ctggccgtga gcctgggcga gagagccacc 60
atcaactgca agagcagcca gagcctgctg aacagcagga ccaggaagaa ctacctggcc 120
tggttccagc agaagcccgg ccagagcccc aagctgctga tctactgggc cagcaccagg 180
gagagcggcg tgcccgatag gttcagcggc agcggcagcg gcaccgattt caccctgacc 240
atcagcagcc tgcaggccga ggatgtggcc gtgtactact gcaagcagag ctacaccctg 300
agaaccttcg gcggcggcac caaggtggaa attaaacgt 339

<210> 28
<211> 339
<212> DNA
<213> Artificial Sequence

<220>
<223> Synthesized

<400> 28
gacatcgtgc tgacccagag ccccgatagc ctggccgtga gcctgggcga gagagccacc 60
atcaactgca agagcagcca gagcctgctg aacagcagga ccaggaagaa ctacctggcc 120
tggttccagc agaagcccgg ccagagcccc aagctgctga tctactgggc cagcaccagg 180
gagagcggcg tgcccgatag gttcagcggc agcggcagcg gcaccgattt caccctgacc 240
atcagcagcc tgcaggccga ggatgtggcc gtgtactact gcaagcagag ctacaccctg 300
agaaccttcg gcggcggcac caaggtggaa attaaacgt 339

<210> 29
<211> 330
<212> PRT
<213> Artificial Sequence

<220>

<223> Synthesized

<400> 29

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Ala Ser Thr Lys Gly Pro Ser Val Phe Pro Leu Ala Pro Ser Ser Lys
 1               5                  10                  15
Ser Thr Ser Gly Gly Thr Ala Ala Leu Gly Cys Leu Val Lys Asp Tyr
            20                  25                  30
Phe Pro Glu Pro Val Thr Val Ser Trp Asn Ser Gly Ala Leu Thr Ser
        35                  40                  45
Gly Val His Thr Phe Pro Ala Val Leu Gln Ser Ser Gly Leu Tyr Ser
    50                  55                  60
Leu Ser Ser Val Val Thr Val Pro Ser Ser Ser Leu Gly Thr Gln Thr
65                  70                  75                  80
Tyr Ile Cys Asn Val Asn His Lys Pro Ser Asn Thr Lys Val Asp Lys
                85                  90                  95
Arg Val Glu Pro Lys Ser Cys Asp Lys Thr His Thr Cys Pro Pro Cys
            100                 105                 110
Pro Ala Pro Glu Leu Leu Gly Gly Pro Ser Val Phe Leu Phe Pro Pro
        115                 120                 125
Lys Pro Lys Asp Thr Leu Met Ile Ser Arg Thr Pro Glu Val Thr Cys
    130                 135                 140
Val Val Val Asp Val Ser His Glu Asp Pro Glu Val Lys Phe Asn Trp
145                 150                 155                 160
Tyr Val Asp Gly Val Glu Val His Asn Val Lys Thr Lys Pro Arg Glu
                165                 170                 175
Glu Gln Tyr Asn Ser Thr Tyr Arg Val Val Ser Val Leu Thr Val Leu
            180                 185                 190
His Gln Asp Trp Leu Asn Gly Lys Glu Tyr Lys Cys Lys Val Ser Asn
        195                 200                 205
Lys Ala Leu Pro Ala Pro Ile Glu Lys Thr Ile Ser Lys Ala Lys Gly
    210                 215                 220
Gln Pro Arg Glu Pro Gln Val Tyr Thr Leu Pro Pro Ser Arg Glu Glu
225                 230                 235                 240
Met Thr Lys Asn Gln Val Ser Leu Thr Cys Leu Val Lys Gly Phe Tyr
                245                 250                 255
Pro Ser Asp Ile Ala Val Glu Trp Glu Ser Asn Gly Gln Pro Glu Asn
            260                 265                 270
Asn Tyr Lys Thr Thr Pro Pro Val Leu Asp Ser Asp Gly Ser Phe Phe
        275                 280                 285
Leu Tyr Ser Lys Leu Thr Val Asp Lys Ser Arg Trp Gln Gln Gly Asn
    290                 295                 300
Val Phe Ser Cys Ser Val Met His Glu Ala Leu His Asn His Tyr Thr
305                 310                 315                 320
Gln Lys Ser Leu Ser Leu Ser Pro Gly Lys
                325                 330
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<210> 30
<211> 990
<212> DNA
<213> Artificial Sequence

<220>
<223> Synthesized

<400> 30

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gctagcacca agggcccatc ggtcttcccc ctggcaccct cctccaagag cacctctggg 60
ggcacagcgg ccctgggctg cctggtcaag gactacttcc ccgaaccggt gacggtgtcg 120
tggaactcag gcgccctgac cagcggcgtg cacaccttcc cggctgtcct acagtcctca 180
ggactctact ccctcagcag cgtggtgacc gtgccctcca gcagcttggg cacccagacc 240
tacatctgca acgtgaatca caagcccagc aacaccaagg tggacaagaa agttgagccc 300
aaatcttgtg acaaaactca cacatgccca ccgtgcccag cacctgaact cctgggggga 360
ccgtcagtct tcctcttccc cccaaaaccc aaggacaccc tcatgatctc ccggacccct 420
gaggtcacat gcgtggtggt ggacgtgagc cacgaagacc ctgaggtcaa gttcaactgg 480
tacgtggacg gcgtggaggt gcataatgcc aagacaaagc cgcgggagga gcagtacaac 540
agcacgtacc gtgtggtcag cgtcctcacc gtcctgcacc aggactggct gaatggcaag 600
gagtacaagt gcaaggtctc caacaaagcc ctcccagccc ccatcgagaa aaccatctcc 660
aaagccaaag ggcagccccg agaaccacag gtgtacaccc tgcccccatc ccgggaggag 720
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atgaccaaga accaggtcag cctgacctgc ctggtcaaag gcttctatcc cagcgacatc 780
gccgtggagt gggagagcaa tgggcagccg gagaacaact acaagaccac gcctcccgtg 840
ctggactccg acggctcctt cttcctctac agcaagctca ccgtggacaa gagcaggtgg 900
cagcagggga acgtcttctc atgctccgtg atgcatgagg ctctgcacaa ccactacacg 960
cagaagagcc tctccctgtc tccgggtaaa 990

<210> 31
<211> 1343
<212> DNA
<213> Artificial Sequence

<220>
<223> Synthesized

<400> 31
ggtgagtgga tccgcggccg ctaaactctg agggggtcgg atgacgtggc cattctttgc 60
ctaaagcatt gagtttactg caaggtcaga aaagcatgca aagccctcag aatggctgca 120
aagagctcca acaaaacaat ttagaacttt attaaggaat agggggaagc taggaagaaa 180
ctcaaaacat caagatttta aatacgcttc ttggtctcct tgctataatt atctgggata 240
agcatgctgt tttctgtctg tccctaacat gccctgtgat tatccgcaaa caacacaccc 300
aagggcagaa ctttgttact taaacaccat cctgtttgct tctttcctca gcctccacca 360
agggcccatc ggtcttcccc ctggcaccct cctccaagag cacctctggg ggcacagcgg 420
ccctgggctg cctggtcaag gactacttcc ccgaaccggt gacggtgtcg tggaactcag 480
gcgccctgac cagcggcgtg cacaccttcc cggctgtcct acagtcctca ggactctact 540
ccctcagcag cgtggtgacc gtgccctcca gcagcttggg cacccagacc tacatctgca 600
acgtgaatca caagcccagc aacaccaagg tggacaagag agttgagccc aaatcttgtg 660
acaaaactca cacatgccca ccgtgcccag cacctgaact cctgggggga ccgtcagtct 720
tcctcttccc cccaaaaccc aaggacaccc tcatgatctc ccggacccct gaggtcacat 780
gcgtggtggt ggacgtgagc cacgaagacc ctgaggtcaa gttcaactgg tacgtggacg 840
gcgtggaggt gcataatgcc aagacaaagc cgcgggagga gcagtacaac agcacgtacc 900
gtgtggtcag cgtcctcacc gtcctgcacc aggactggct gaatggcaag gagtacaagt 960
gcaaggtctc caacaaagcc ctcccagccc ccatcgagaa aaccatctcc aaagccaaag 1020
ggcagccccg agaaccacag gtgtacacgc tgccccatc ccgggaggag atgaccaaga 1080
accaggtcag cctgacctgc ctggtcaaag gcttctatcc cagcgacatc gccgtggagt 1140
gggagagcaa tgggcagccg gagaacaact acaagaccac gcctcccgtg ctggactccg 1200
acggctcctt cttcctctat agcaagctca ccgtggacaa gagcaggtgg cagcagggga 1260
acgtcttctc atgctccgtg atgcatgagg ctctgcacaa ccactacacg cagaagagcc 1320
tctccctgtc cccgggtaaa tga 1343

<210> 32
<211> 106
<212> PRT
<213> Artificial Sequence

<220>
<223> Synthesized

<400> 32
Thr Val Ala Ala Pro Ser Val Phe Ile Phe Pro Pro Ser Asp Glu Gln
 1   5   10   15
Leu Lys Ser Gly Thr Ala Ser Val Val Cys Leu Leu Asn Asn Phe Tyr
            20   25   30
Pro Arg Glu Ala Lys Val Gln Trp Lys Val Asp Asn Ala Leu Gln Ser
        35   40   45
Gly Asn Ser Gln Glu Ser Val Thr Glu Gln Asp Ser Lys Asp Ser Thr
    50   55   60
Tyr Ser Leu Ser Ser Thr Leu Thr Leu Ser Lys Ala Asp Tyr Glu Lys
65   70   75   80
His Lys Val Tyr Ala Cys Glu Val Thr His Gln Gly Leu Ser Ser Pro
                85   90   95
Val Thr Lys Ser Phe Asn Arg Gly Glu Cys
            100   105

<210> 33
<211> 318
<212> DNA
<213> Artificial Sequence

<220>
<223> Synthesized

<400> 33
acggtggctg caccatctgt cttcatcttc ccgccatctg atgagcagtt gaaatctgga 60
actgcctctg ttgtgtgcct gctgaataac ttctatccca gagaggccaa agtacagtgg 120
aaggtggata acgccctcca atcgggtaac tcccaggaga gtgtcacaga gcaggacagc 180
aaggacagca cctacagcct cagcagcacc ctgacgctga gcaaagcaga ctacgagaaa 240
cacaaagtct acgcctgcga agtcacccat cagggcctga gctcgcccgt cacaaagagc 300
ttcaacaggg gagagtgt 318

<210> 34
<211> 671
<212> DNA
<213> Artificial Sequence

<220>
<223> Synthesized

<400> 34
cgtgagtgga tccgcggccg ctaaactctg agggggtcgg atgacgtggc cattctttgc 60
ctaaagcatt gagtttactg caaggtcaga aaagcatgca aagccctcag aatggctgca 120
aagagctcca acaaaacaat ttagaacttt attaaggaat agggggaagc taggaagaaa 180
ctcaaaacat caagatttta aatacgcttc ttggtctcct tgctataatt atctgggata 240
agcatgctgt tttctgtctg tccctaacat gccctgtgat tatccgcaaa caacacaccc 300
aagggcagaa ctttgttact taaacaccat cctgtttgct tctttcctca ggaactgtgg 360
ctgcaccatc tgtcttcatc ttcccgccat ctgatgagca gttgaaatct ggaactgcct 420
ctgttgtgtg cctgctgaat aacttctatc ccagagaggc caaagtacag tggaaggtgg 480
ataacgccct ccaatcgggt aactcccagg agagtgtcac agagcaggac agcaaggaca 540
gcacctacag cctcagcagc accctgacgc tgagcaaagc agactacgag aaacacaaag 600
tctacgcctg cgaagtcacc catcagggcc tgagctcgcc cgtcacaaag agcttcaaca 660
ggggagagtg t 671