HUMANIZED ANTIBODY FOR TREATING OR PREVENTING COGNITIVE DISORDERS, PROCESS FOR PRODUCING THE SAME, AND AGENT FOR TREATING OR PREVENTING COGNITIVE DISORDERS USING THE SAME

I. CROSS REFERENCE TO RELATED APPLICATIONS

[0001] This application claims the benefit of Japanese Application No. 2017-035594, filed on February 27, 2017, the content of which is expressly incorporated herein by reference in its entirety.

II. SEQUENCE LISTING

[0002] The instant application contains a Sequence Listing which has been submitted electronically in ASCII format and is hereby incorporated by reference in its entirety. Said ASCII copy, created on February 27, 2018, is named 063785-06-5002-WO-SeqLstg-_ST25.txt and is 323,584 bytes in size.

III. TECHNICAL FIELD

[0003] The present disclosure relates to a humanized antibody for use in the therapeutic or prophylactic treatment of cognitive disorders, a process of producing the humanized antibody, a therapeutic or prophylactic agent for cognitive disorders using the antibody, and methods for treatment or prevention of cognitive disorders using the therapeutic agents disclosed herein. More specifically, the present disclosure relates to a novel humanized anti-phosphorylated tau antibody having excellent effect of improving cognitive function, a process of producing the humanized antibody, a therapeutic or prophylactic agent for cognitive disorders containing the humanized antibody, and methods for treatment or prevention of cognitive disorders using the humanized anti-phosphorylated tau antibody.

IV. BACKGROUND OF THE INVENTION

[0004] A cognitive disorder, or dementia, is a condition where developed intelligence deteriorates due to some acquired cause, resulting in a hindrance to social adaptation. Cognitive disorders are classified as neurodegenerative diseases, vascular cognitive diseases, prion diseases, infectious diseases, metabolic/endocrine diseases, trauma and cerebral surgical diseases, and toxic diseases (see Toshifumi Kishimoto & Shigeki Takahashi (Edit.), "STEP Series Seishinka" (Japanese document), 2th Edition, Kaibashobo, 2008, pp.103-104). There were about 2.1 million dementia patients in Japan as of 2010, with a morbidity prevalence rate of about 8 to 10%, or even more than 10%, among the elderly over age 65, and this has been recognized as a serious problem in the worldwide aging society (Takashi Asada, "Igaku no

Ayumi" (Japanese document), supplementary volume, "Cognitive disorders", Ishiyaku Publishing, 2011, pp.5-10). Data on underlying diseases of cognitive disorders indicate that the majority are neurodegenerative diseases such as Alzheimer's disease (AD) and frontotemporal lobar degeneration (FTLD), with about 35% being AD, about 15% being a combination of AD and cerebrovascular disease, and 5% being FTLD (Id.) Cognitive disorders associated with neurodegenerative diseases are characterized by insidious onset of memory impairment and/or personality changes which progresses over a period of at least six months or more. Neurodegenerative processes correlate highly with the degree of impairment of cognitive function, and a consistent characteristics involved therein is the presence of neurofibrillary tangles (NFT) (Alistair Burns et al. (Edit.), Dementia, 3rd Edition, 2005, CRC Press, pp.408-464).

[0005] The tau protein is a protein encoded by the MAPT gene, which is located on chromosome 17 (17q21) in the human genome. Tau protein is one of the microtubule-binding proteins abundantly expressed in the central nervous system. The tau has been found to be a major constituent protein in the paired helical filaments and straight filaments forming NFT in AD, one of the most prominent neurodegenerative diseases, and its intracellular accumulation has been demonstrated in a variety of neuropathological conditions.

[0006] Tau was first implicated in neurodegenerative diseases based on the relationship between mutations in the MAPT gene and accumulation of tau in chromosome 17-linked frontotemporal dementia with Parkinsonism (FTDP-17). More than 40 gene mutations in the MAPT gene have been reported in relation to FTDP-17 (Tetsuaki Arai, "Shinkei Naika" (Japanese document), Vol.72, special number, (Suppl.6), 2010, pp.46-51). These gene mutations are suggested as altering the proportions of tau isoforms or altering the structure and changing the interaction between mutant tau and microtubules, thus contributing to development of pathology. However, unlike familial neurodegenerative diseases, mutations in MAPT are rarely observed in sporadic neurodegenerative diseases such as AD. In addition, tau accumulated in neurodegenerative diseases is characterized by being highly modified via phosphorylation. Moreover, in patients exhibiting mild cognitive impairment (MCI), a correlation is observed between the level of phosphorylated tau in the spinal fluid and the degree of pituitary atrophy, suggesting phosphorylated tau as a highly reliable biomarker for neurodegeneration in patients with tauopathy (Wendy Noble et al., Expert Opinion on Drug Discovery, 2011, Vol.6, No.8, pp.797-810). Based on these findings, attempts have been made to develop a treatment using inhibitors against kinases, which are enzymes involved in phosphorylation, and particularly

against GSK-3 beta, for inhibiting excessive phosphorylation of tau (Id). However, there is a concern about possible side effects, since kinases such as GSK-3 beta are implicated not only in pathological conditions, but also in function controls in normal physiological processes. In fact, some of the sites where tau is phosphorylated by GSK-3 beta coincide with sites of tau phosphorylation seen in fetal and normal human brains (Burnes, et. al., supra) suggesting the possibility that this strategy may affect normal tau function.

[0007] Although it was believed that extracellular tau originates from leakage from degenerated nerve cells as a consequence of cell death, recent studies have suggested that following excessive intracellular phosphorylation, tau is processed and then actively secreted out of the cell. Phosphorylated tau secreted from the cell is thought to be dephosphorylated at certain phosphorylation sites and subsequently to act on muscarine receptors M1 and M3 of surrounding cells, thus resulting in various effects such as promoting intracellular tau phosphorylation and contributing to cell death (Miguel Diaz-Hernandes et al., Journal of Biological Chemistry, 2010, Vol.285, No.42, pp.32539-32548 and Venessa Plouffe et al., PLoS ONE, 2012, Vol.7, p.36873). Experiments relating to immunotherapy for tauopathies using tau protein as the target have been reported, as attempts aimed at executing specific action against tau (see Noble, supra, Kishimoto, supra, Asada, supra and Burns, supra).

[0008] The major symptoms in human cognitive disorders are memory impairment and cognitive function impairmen, which is especially important given the role of cognitive function in memory-based judgment, communication and performance. Motor function, on the other hand, while being a symptom found in chromosome 17-linked frontotemporal dementia with Parkinsonism (FTDP-17) and terminal-stage Alzheimer's disease, is not necessarily a major symptom exhibited in cognitive disorders. Therefore, the main issue to be considered for treating cognitive disorders is improvement of cognitive functions. However, there is currently suitable animal models for testing tauopathy-associated cognitive function impairment that would allow identification of a therapeutic or prophylactic agent for treating cognitive disorders. In addition, no such agent exists that exhibits specific and superior effects against cognitive disorders.

[0009] In view of the therapeutic and prophylactic applications against cognitive disorders in human subjects, there is a demand for a humanized anti-phosphorylated tau antibody which not only has high binding affinity to phosphorylated tau, but also exhibits reduced antigenicity to the human body.

V. Summary of the Invention

[0010] Against this background, among a large number of phosphorylated sites in the tau protein which can undergo abnormal phosphorylation in the conditions of Alzheimer's disease, the present inventors focused on the phosphorylated site of the serine residue at position 413 (Ser413), and succeeded in producing a polyclonal rabbit antibody and a monoclonal mouse antibody which are specific to the Ser413-phosphorylated tau. The present inventors also administered the monoclonal mouse antibodies to model mice of cognitive disorders which showed abnormal expression of phosphorylated tau proteins, and confirmed that improvement in cognitive functions was observed (WO2013/180238A).

[0011] In some embodiments, the present disclosure provides proteins, such as antibodies, that include an antigen binding portion that specifically binds to Tau protein that is phosphorylated at serine residue 413 (pS413-Tau). In some embodiments, the present disclosure provides nucleic acids encoding the proteins. In some embodiments, the present disclosure provides cells that include such nucleic acids encoding the proteins. In some embodiments, the present disclosure provides methods for treating a tauopathy (e.g., Alzheimer's disease) by administering the proteins, nucleic acids, or cells disclosed herein to a patient in need thereof.

[0012] Accordingly, in some aspects the invention provides a humanized antibody or a fragment or derivative thereof, which causes an antigen-antibody reaction with a tau protein or a tau peptide phosphorylated at least on an amino acid residue corresponding to Ser413 of SEQ ID NO: 1. In some cases, the humanized antibody or fragment or derivative thereof has an equilibrium dissociation constant of 1.86×10^{-8} M or less for the tau protein or tau peptide.

[0013] In further aspects, the humanized antibody or fragment or derivative thereof has an improved selective affinity with the phosphorylated tau protein (or tau peptide, e.g. SEQ ID NO:8) compared to a non-phosphorylated tau protein (or tau peptide, e.g. SEQ ID NO:69).

[0014] In an additional aspect, the humanized antibody or fragment or derivative thereof described herein has an improved ability to enter the brain when administered into the blood.

[0015] In a further aspect, the humanized antibody or fragment or derivative thereof comprises CDRs as follows:a CDR-H1 sequence, an amino acid sequence having 80% or more homology with sequence 1M; as a CDR-H2 sequence, an amino acid sequence having 84% or more homology with sequence 2M or 2H1; as a CDR-H3 sequence, an amino acid sequence having 80% or more homology with sequence 3M; as a CDR-L1 sequence, an amino acid sequence having 87% or more homology with sequence 4L1 or 4M; as a CDR-L2 sequence, an

amino acid sequence having 85% or more homology with sequence 5M; and as a CDR-L3 sequence, an amino acid sequence having 77% or more homology with sequence 6M.

[0016] In an additional aspect, the humanized antibody or fragment or derivative thereof comprises CDRs as follows: as a CDR-H1 sequence, the amino acid sequence of sequence 1M; as a CDR-H2 sequence, the amino acid sequence of sequence 2H1 or an amino acid sequence differs from sequence 2H1 in that Ala at position 15 is substituted with Asp; as a CDR-H3 sequence, the amino acid sequence of sequence 3M; as a CDR-L1 sequence, the amino acid sequence of sequence 4L1 or an amino acid sequence that differs from sequence 4L1 in that Ser at position 5 is substituted with Asn; as a CDR-L2 sequence, the amino acid sequence of sequence 5M; andas a CDR-L3 sequence, the amino acid sequence of sequence 6M.

[0017] In a further aspect, the humanized antibody or fragment or derivative thereof has CDRs as follows: (1) as a CDR-H1 sequence, the amino acid sequence of 1M; as a CDR-H2 sequence, the amino acid sequence of 2H1; as a CDR-H3 sequence, the amino acid sequence of 3M; as a CDR-L1 sequence, the amino acid sequence of 4L1; as a CDR-L2 sequence, the amino acid sequence of 5M; and as a CDR-L3 sequence, the amino acid sequence of 6M, or (2) as a CDR-H1 sequence, the amino acid sequence of 1M; as a CDR-H2 sequence, the amino acid sequence of 2H1; as a CDR-H3 sequence, the amino acid sequence of 3M; as a CDR-L1 sequence, the amino acid sequence of 4M; as a CDR-L2 sequence, the amino acid sequence of 5M; and as a CDR-L3 sequence, the amino acid sequence of 6M, or (3) as a CDR-H1 sequence, the amino acid sequence of 1M; as a CDR-H2 sequence, the amino acid sequence of 2M; as a CDR-H3 sequence, the amino acid sequence of 3M; as a CDR-L1 sequence, the amino acid sequence of 4M; as a CDR-L2 sequence, the amino acid sequence of 5M; and as a CDR-L3 sequence, the amino acid sequence of 6M.

[0018] In additional aspects the humanized antibody or fragment or derivative thereof comprises: as a heavy chain variable region, an amino acid sequence having 90% or more homology with a sequence selected from sequences VH11, VH12, VH47, VH61, VH62, VH64, and VH65; and as a light chain variable region, an amino acid sequence having 90% or more homology with a sequence selected from sequences VL15, VL36, VL46, VL47, VL48, and VL50.

[0019] In a further aspect, the humanized antibody or fragment or derivative thereof comprises: as a heavy chain variable region, the amino acid sequence of sequence VH65 or an amino acid sequence which differs from sequence VH65 in comprising one or more

substitutions selected from the group consisting of a substitution of Ala with Thr at position 28 (Kabat numbering: H28), a substitution of Asn with Ser at position 30 (Kabat numbering: H30), a substitution of Val with Gly at position 49 (Kabat numbering: H49), a substitution of Ala with Asp at position 64 (Kabat numbering: H61), and a substitution of Gln with Lys at position 78 (Kabat numbering: H75); and as a light chain variable region, the amino acid sequence of sequence VL47 or an amino acid sequence which differs from sequence VL47 in comprising one or more substitutions selected from the group consisting of a substitution of Asp with Glu at position 17 (Kabat numbering: L17), a substitution of Ser with Asn at position 28 (Kabat numbering: L27A), a substitution of Gln with Leu at position 42 (Kabat numbering: L37),a substitution of Gln with Arg at position 50 (Kabat numbering: L45), anda substitution of Arg with Leu at position 51 (Kabat numbering: L46).

[0020] In a further aspect, the humanized antibody or fragment or derivative thereof comprises any of the following sequence combinations: (a) sequence VH11 as a heavy chain variable region and sequence VL15 as a light chain variable region; (b) sequence VH11 as a heavy chain variable region and sequence VL36 as a light chain variable region; (c) sequence VH11 as a heavy chain variable region and sequence VL46 as a light chain variable region; (d) sequence VH11 as a heavy chain variable region and sequence VL47 as a light chain variable region; (e) sequence VH11 as a heavy chain variable region and sequence VL48 as a light chain variable region; (f) sequence VH11 as a heavy chain variable region and sequence VL50 as a light chain variable region; (g) sequence VH12 as a heavy chain variable region and sequence VL48 as a light chain variable region; (h) sequence VH47 as a heavy chain variable region and sequence VL48 as a light chain variable region; (i) sequence VH61 as a heavy chain variable region and sequence VL48 as a light chain variable region; (j) sequence VH62 as a heavy chain variable region and sequence VL48 as a light chain variable region; (k) sequence VH64 as a heavy chain variable region and sequence VL47 as a light chain variable region; (l) sequence VH64 as a heavy chain variable region and sequence VL48, and (m) sequence VH65 as a heavy chain variable region and sequence VL47.

[0021] In an additional aspect, the invention provides an agent for treating or preventing dementia, comprising the humanized antibody or fragment or derivative thereof as described herein.

[0022] In a further aspect, the invention provides methods and agents for treating or preventing dementia or a tauopathy, including Alzheimer's disease, corticobasal degeneration,

progressive supranuclear palsy, Pick's disease, argyrophilic grain dementia (argyrophilic grain disease), multiple system tauopathy with presenile dementia (MSTD), frontotemporal dementia and parkinsonism linked to chromosome 17 (FTDP-17), dementia with neurofibrillary tangles, diffuse neurofibrillary tangle with calcification (DNTC), white matter tauopathy with globular glial inclusions (WMT-GGI) or frontotemporal lobar degeneration with tau pathology (FTLD-tau).

[0023] In an additional aspect, the invention provides pharmaceutical compositions comprising the humanized antibody or fragment or derivative thereof as described herein and a pharmaceutically acceptable carrier.

[0024] In a further aspect, the invention provides anti-pSer413 tau antibodies comprising: a) a heavy variable domain comprising a vhCDR1 comprising SEQ ID NO:86, a vhCDR2 comprising SEQ ID NO:115 and a vhCDR3 comprising SEQ ID NO:88; and b) a light variable domain comprising a set of vlCDRs selected from the group consisting of: i) a vlCDR1 comprising SEQ ID NO:102, a vlCDR2 comprising SEQ ID NO:82 and a vlCDR3 comprising SEQ ID NO:83; ii) a vlCDR1 comprising SEQ ID NO:91, a vlCDR2 comprising SEQ ID NO82: and a vlCDR3 comprising SEQ ID NO:83; iii) a vlCDR1 comprising SEQ ID NO:92, a vlCDR2 comprising SEQ ID NO:82 and a vlCDR3 comprising SEQ ID NO:83; iv) a vlCDR1 comprising SEQ ID NO:93, a vlCDR2 comprising SEQ ID NO82: and a vlCDR3 comprising SEQ ID NO:83; v) a vlCDR1 comprising SEQ ID NO:94, a vlCDR2 comprising SEQ ID NO:82 and a vlCDR3 comprising SEQ ID NO:83; vi) a vlCDR1 comprising SEQ ID NO:95, a vlCDR2 comprising SEQ ID NO:82 and a vlCDR3 comprising SEQ ID NO:83; vii) a vlCDR1 comprising SEQ ID NO:96, a vlCDR2 comprising SEQ ID NO:82 and a vlCDR3 comprising SEQ ID NO:83; viii) a vlCDR1 comprising SEQ ID NO:97, a vlCDR2 comprising SEQ ID NO:82 and a vlCDR3 comprising SEQ ID NO:83; ix) a vlCDR1 comprising SEQ ID NO:98, a vlCDR2 comprising SEQ ID NO:82 and a vlCDR3 comprising SEQ ID NO:83; x) a vlCDR1 comprising SEQ ID NO:99, a vlCDR2 comprising SEQ ID NO:82 and a vlCDR3 comprising SEQ ID NO:83; xi) a vlCDR1 comprising SEQ ID NO:100, a vlCDR2 comprising SEQ ID NO:82 and a vlCDR3 comprising SEQ ID NO:83; and xii) a vlCDR1 comprising SEQ ID NO:101, a vlCDR2 comprising SEQ ID NO:82 and a vlCDR3 comprising SEQ ID NO:83. In some cases, the antibody has a ratio of antibody binding to the phosphorylated peptide of SEQ ID NO:8 to antibody binding to the non-phosphorylated peptide of SEQ ID NO:69 of at least about 40.

[0025] In additional aspects, the invention provides anti-pSer413 tau antibodies comprising: a) a heavy variable domain selected from SEQ ID NO:116 and SEQ ID NO:117; and b) a light variable domain selected from SEQ ID NO:103, SEQ ID NO:104, SEQ ID NO:105, SEQ ID NO:106, SEQ ID NO:107, SEQ ID NO:108, SEQ ID NO:109, SEQ ID NO:110, SEQ ID NO:111, SEQ ID NO:112, SEQ ID NO:113 and SEQ ID NO:114. In some cases, the antibody has a ratio of antibody binding to the phosphorylated peptide of SEQ ID NO:8 to antibody binding to the non-phosphorylated peptide of SEQ ID NO:69 of at least about 40.

[0026] In an additional aspect, the antibodies of the invention comprises a heavy constant domain selected from SEQ ID NO:135, SEQ ID NO:136, SEQ ID NO:137, SEQ ID NO:138, SEQ ID NO:139, SEQ ID NO:140, SEQ ID NO:141, SEQ ID NO:142 and SEQ ID NO:144.

[0027] In a further aspect, the antibodies of the invention comprise a light constant domain selected from SEQ ID NO:79 and SEQ ID NO:80.

[0028] In an additional aspect, the antibodies of the invention comprise a variable heavy domain selected from SEQ ID NO:116 and SEQ ID NO:117.

[0029] In a further aspect, the antibodies of invention comprise a variable light domain selected from SEQ ID NO:103, SEQ ID NO:104, SEQ ID NO:105, SEQ ID NO:106, SEQ ID NO:107, SEQ ID NO:108, SEQ ID NO:109, SEQ ID NO:110, SEQ ID NO:111, SEQ ID NO:112, SEQ ID NO:113 and SEQ ID NO:114.

[0030] In an additional aspect, the antibodies comprise heavy and light chains selected from the pairs LC SEQ ID NO:184 and HC SEQ ID NO:144; LC SEQ ID NO:184 and HC SEQ ID NO:145; LC SEQ ID NO:184 and HC SEQ ID NO:146; LC SEQ ID NO:184 and HC SEQ ID NO:147; LC SEQ ID NO:184 and HC SEQ ID NO:148; LC SEQ ID NO:184 and HC SEQ ID NO:149; LC SEQ ID NO:184 and HC SEQ ID NO:150; LC SEQ ID NO:184 and HC SEQ ID NO:151; LC SEQ ID NO:184 and HC SEQ ID NO:152; LC SEQ ID NO:184 and HC SEQ ID NO:153; LC SEQ ID NO:184 and HC SEQ ID NO:154; LC SEQ ID NO:184 and HC SEQ ID NO:155; LC SEQ ID NO:184 and HC SEQ ID NO:156; LC SEQ ID NO:184 and HC SEQ ID NO:157; LC SEQ ID NO:184 and HC SEQ ID NO:158; LC SEQ ID NO:184 and HC SEQ ID NO:159; LC SEQ ID NO:184 and HC SEQ ID NO:160; LC SEQ ID NO:184 and HC SEQ ID NO:161; LC SEQ ID NO:185 and HC SEQ ID NO:144; LC SEQ ID NO:185 and HC SEQ ID NO:145; LC SEQ ID NO:185 and HC SEQ ID NO:146; LC SEQ ID NO:185 and HC SEQ ID NO:147; LC SEQ ID NO:185 and HC SEQ ID NO:148; LC SEQ ID NO:185 and HC SEQ

ID NO:149; LC SEQ ID NO:185 and HC SEQ ID NO:150; LC SEQ ID NO:185 and HC SEQ ID NO:151; LC SEQ ID NO:185 and HC SEQ ID NO:152; LC SEQ ID NO:185 and HC SEQ ID NO:153; LC SEQ ID NO:185 and HC SEQ ID NO:154; LC SEQ ID NO:185 and HC SEQ ID NO:155; LC SEQ ID NO:185 and HC SEQ ID NO:156; LC SEQ ID NO:185 and HC SEQ ID NO:157; LC SEQ ID NO:185 and HC SEQ ID NO:158; LC SEQ ID NO:185 and HC SEQ ID NO:159; LC SEQ ID NO:185 and HC SEQ ID NO:160 and LC SEQ ID NO:185 and HC SEQ ID NO:161.

[0031] In a further aspect, the antibody of the invention has a light chain comprising or consisting of SEQ ID NO:184 and a heavy chain comprising or consisting of SEQ ID NO:144.

[0032] In an additional aspect, the antibody of the invention has a light chain comprising or consisting of SEQ ID NO:184 and a heavy chain comprising or consisting of SEQ ID NO:152.

[0033] In a further aspect, the invention provides nucleic acid compositions comprising: a) a first nucleic acid encoding a light chain selected from SEQ ID NO:162, SEQ ID NO:163, SEQ ID NO:164, SEQ ID NO:165, SEQ ID NO:166, SEQ ID NO:167, SEQ ID NO:168, SEQ ID NO:169, SEQ ID NO:170, SEQ ID NO:171, SEQ ID NO:172, SEQ ID NO:173, SEQ ID NO:174, SEQ ID NO:175, SEQ ID NO:176, SEQ ID NO:177, SEQ ID NO:178, SEQ ID NO:179, SEQ ID NO:180, SEQ ID NO:181, SEQ ID NO:182, SEQ ID NO:183, SEQ ID NO:184 and SEQ ID NO:185; and b) a second nucleic acid encoding a heavy chain selected from SEQ ID NO:144; SEQ ID NO:145; SEQ ID NO:146; SEQ ID NO:147; SEQ ID NO:148; SEQ ID NO:149; SEQ ID NO:150; SEQ ID NO:151; SEQ ID NO:152; SEQ ID NO:153; SEQ ID NO:154; SEQ ID NO:155; SEQ ID NO:156; SEQ ID NO:157; SEQ ID NO:158; SEQ ID NO:159; SEQ ID NO:160 and SEQ ID NO:161.

[0034] In an additional aspect, the invention provides expression vector compositions comprising a first and a second nucleic acid, wherein the first nucleic acid is contained in a first expression vector and the second nucleic acid is contained in a second expression vector.

[0035] In a further aspect, the invention provides an expression vector composition wherein the first nucleic acid and the second nucleic acid is contained in a single expression vector.

[0036] In an additional aspect, the invention provides host cells comprising the expression vector composition, and methods of making an anti-pSer413 tau antibody comprising

culturing the host cell under conditions wherein said antibody is expressed and recovering said antibody.

[0037] In a further aspect, the invention provides methods of treating a tauopathy in a subject comprising administering the antibodies of the invention.

VI. BRIEF DESCRIPTION OF THE DRAWINGS

[0038] The invention may be best understood from the following detailed description when read in conjunction with the accompanying drawings. Included in the drawings are the following figures:

[0039] **FIG. 1A and FIG. 1B** collectively show an alignment of various human isoforms of Tau protein, prepared using ClustalW.

[0040] **FIG. 2A** shows an alignment of light chain variable region sequences, highlighting CDR-L1, CDR-L2, and CDR-L3 sequences, according to the Kabat numbering system, of several anti-pS413-Tau binding proteins, in accordance with some embodiments of the disclosure.

[0041] **FIG. 2B** shows an alignment of heavy chain variable region sequences, highlighting CDR-H1, CDR-H2, and CDR-H3 sequences, according to the Kabat numbering system, of several anti-pS413-Tau binding proteins, in accordance with some embodiments of the disclosure.

[0042] **FIG. 3** illustrates the selective binding affinity of several anti-pS413-Tau antibodies for phosphorylated peptide PD17P, relative to non-phosphorylated peptide PD17, in accordance with some embodiments of the disclosure.

[0043] **FIG. 4** illustrates the binding affinity of several anti-pS413-Tau antibodies for phosphorylated Tau in brain homogenate from an Alzheimer's patient, in accordance with some embodiments of the disclosure.

[0044] **FIG. 5A and FIG. 5B** show pharmacokinetics of humanized antibody variants in mouse plasma over time. **FIG. 5A** demonstrates that five initial humanized antibody variants 2, 5, 6, 9, and 10 exhibited rapid elimination from the mouse plasma. **FIG. 5B** demonstrates that three later developed humanized antibody variants L15H11, L46H11, and L47H65 exhibited pharmacokinetics profiles comparable to the chimeric antibody.

[0045] **FIG. 6A and FIG. 6B** show different humanized antibody variants have different intracerebral migration. **FIG. 6A** shows concentration of various humanized antibodies in brain homogenate. **FIG. 6B** shows the ratio of the level of representative humanized antibodies in the brain to the level of the corresponding antibodies in the plasma.

[0046] **FIG. 7A and FIG. 7B** show alignment of amino acid sequences of the light chain variable region (**FIG. 7A**) and the heave chain variable region (**FIG. 7B**) of representative humanized antibody variants with the parent mouse antibody.

[0047] **FIG. 8A – FIG. 8D** demonstrate that the parent mouse antibody (**FIG. 8A and 8B**) and the chimeric antibody (**FIG. 8C and 8D**) bind to a phosphorylated peptide (**FIG. 8A and 8C**) but not to the non-phosphorylated counterpart (**FIG. 8B and 8D**).

[0048] **FIG. 9A – FIG. 9C** show the binding of representative humanized antibody variants to pS413 peptide.

[0049] **FIG. 10A – FIG. 10E** show comparable binding characteristics of the parent mouse antibody (**FIG. 10A**), the chimeric antibody (**FIG. 10B**), and selected humanized antibody variants (**FIG. 10C-10E**) to the S413-phosphorylated Tau protein in brain homogenates from Alzheimer's disease patients.

[0050] **FIG. 11A – FIG. 11E** show the variable heavy and variable light regions of some of the anti-pSer413 tau protein humanized antibodies of the invention.

[0051] **FIG. 12** shows a number of different human IgG constant domains that find use in combinations of the variable heavy and variable light chains of anti-pSer413 tau antibodies. "Human_IgG1 _ " is the constant domain (CH1-hinge-CH2-CH3) of human wild type IgG1. "Human_IgG1_L234A_L235A" is the constant domain (CH1-hinge-CH2-CH3) of human wild type IgG1 with two amino acid substitutions, L234A and L235A (sometimes referred to as "LALA" mutations) that reduce/ablate effector function. "Human_IgG1_ _L234A_L235A_D265S" is the constant domain (CH1-hinge-CH2-CH3) of human wild type IgG1 with three amino acid substitutions, L234A, L235A and D265S that reduce/ablate effector function. "Human_IgG1_YTE_ " or "Human IgG1_YTE (M252Y_S254T_T256E)" is the constant domain (CH1-hinge-CH2-CH3) of human wild type IgG1 with three amino acid substitutions, M252Y, S254T, T256E that increase half life of the antibody in serum. "Human_IgG1_N297A_ " is the constant domain (CH1-hinge-CH2-CH3) of human wild type IgG1 with the amino acid substitution N297A, which eliminates a glycosylation site and reduces/ablates effector function. "Human_IgG1_N297Q_ " is the constant domain (CH1-

hinge-CH2-CH3) of human wild type IgG1 with the amino acid substitution N297Q, which eliminates a glycosylation site and reduces/ablates effector function. "_Human_IgG2" is the constant domain (CH1-hinge-CH2-CH3) of human wild type IgG2. "_Human_IgG4" is the constant domain (CH1-hinge-CH2-CH3) of human wild type IgG4. "Human_IgG4_S228P_" is the constant domain (CH1-hinge-CH2-CH3) of human wild type IgG4 with an amino acid substitution S228P to prevent arm switching

[0052] **FIG. 13** shows full length heavy chains for the H11 and H65 variable regions in conjunction with the backbones of **FIG.12.** A "slash" ("/") indicates the junction of the variable and constant domains, and the CDRs are underlined.

[0053] **FIG. 14** shows the full length light chains for 12 different variable light domains with either the human kappa or lambda light constant domains. A "slash" ("/") indicates the junction of the variable and constant domains, and the CDRs are underlined.

[0054] **FIG. 15** depicts a matrix of possible combinations of heavy and light chains of the invention. An "A" in the box indicates that the heavy constant domain "Human_IgG1 _ " is used. A "B" in the box indicates that the heavy constant domain "Human_IgG1_L234A_L235A" is used. A "C" in the box indicates that the heavy constant domain "Human_IgG1_ _L234A_L235A_D265S" is used. A "D" in the box means that the heavy constant domain "Human_IgG1_YTE_ " is used. An "E" in the box means that the heavy constant domain "Human_IgG1_N297A_ " is used. An "F" in the box means that the heavy constant domain "Human_IgG1_N297Q_ " is used. A "G" in the box means that the heavy constant domain "_Human_IgG2" is used. An "H" in the box means that the heavy constant domain "_Human_IgG4" is used. An "I" in the box means that the heavy constant domain "Human_IgG4_S228P_" is used. A "J" in the box means that a human IgG1 constant domain with from 1, 2, 3, 4, 5, 6, 7, 8, 9 or 10 amino acid substitutions is used.

VII. Detailed Description

A. Overview

[0055] The present disclosure provides antibodies that include an antigen binding portion that specifically binds to Tau protein that is phosphorylated at serine residue 413 (pSer413-Tau). Also provided are nucleic acids encoding the proteins, and cells that include such nucleic acids. In some embodiments, the methods and compositions provided are used in the treatment of tauopathies (e.g., Alzheimer's disease).

[0056] The MAPT gene encoding tau has been identified as consisting of 13 exons disposed on the genome, which can be expressed as multiple different protein isoforms via alternative splicing (see Arai, supra). The tau protein comprises an N-terminal acidic domain containing 0-2 repetitive sequences (N) of 29 amino acids depending on alternative splicing of exon 2 and exon 3 (N0-N2), an intermediate domain rich in proline, and a C-terminal microtubule-binding domain (encoded by exons 9 to 12) containing 3 (3R) or 4 (4R) repetitive sequences (R) that contribute to microtubule binding (Burns, supra and Arai, supra). Therefore, human tau has six representative isoforms: 3R0N (352 amino acids), 3R1N (381 amino acids), 3R2N (410 amino acids), 4R0N (383 amino acids), 4R1N (412 amino acids), and 4R2N (441 amino acids), depending on the number of 29 amino acid repetitive sequences (N) and microtubule-binding repetitive sequences (R) that it contains. Of these isoforms, only 3R0N is present in the embryonic brain, whereas all six isoforms are present in the adult human brain, with 4R being most abundant (Burns, supra). The difference between the 3R and 4R isoforms results from whether exon 10 is removed via alternative splicing (3R) or present (4R). In order to unambiguously identify the position of an amino acid residue in any of these tau isoforms, the amino acid numbers (1 to 441) of the longest isoform, i.e., 4R2N (defined in SEQ ID NO:1), are used as a reference. For example, "Ser413" refers to the serine residue at the 413th amino acid position in 4R2N (defined in SEQ ID NO:1), which corresponds to the serine at the 384th in 4R1N (defined in SEQ ID NO:2), the 355th in 4R0N (defined in SEQ ID NO:3), the 382nd in 3R2N (defined in SEQ ID NO:4), the 353rd in 3R1N (defined in SEQ ID NO:5), and the 324th in 3R0N (defined in SEQ ID NO:6).

[0057] Tau has a phosphorylated amino acid residue at the position corresponding to the serine residue at position 413 (Ser413, when phosphorylated it is referred to herein as "pSer413") of the amino acid sequence defined in SEQ ID NO:1 (and thus the tau protein phosphorylated at Ser413 is "pSer413 tau" or "pSer413-tau") which is a site specifically phosphorylated in AD. As previously shown in WO 2013/180238, administration of antibodies that participate in specific antigen-antibody reactions with PSer413-tau to transgenic mice which develop cognitive function impairment while maturing, resulted in restoration of cognitive functions to almost the same level as that of the control group. Interestingly, administration of similar concentration of a monoclonal antibody against a tau protein having a phosphorylated amino acid residue at the position corresponding to Ser396 of the amino acid sequence defined in SEQ ID NO:1, which has a stronger affinity to an equivalent antigen than the above antibody, did not result in sufficient improvement in cognitive functions. Since there was no specific

information about a region including the amino acid residue at the position corresponding to Ser413 of the amino acid sequence defined in SEQ ID NO:1 in relation to the structure and functions of tau, it was a totally unexpected result that the antibody that binds with this region had such a strong effect of improving cognitive function.

[0058] Accordingly, the present invention is directed to humanized and optimized anti-pSer413 tau antibodies useful as a therapeutic or prophylactic agent for treating cognitive disorders such as tauopathy in a human subject.

[0059] The humanized anti-phosphorylated tau antibody according to the present invention exhibits high binding affinity to phosphorylated tau while having significantly reduced antigenicity to the human body, and can effectively be used as a therapeutic or prophylactic agent for cognitive disorders such as tauopathy in a human subject. Additionally, amino acid modifications within the CDRs as compared to the murine CDRs reduce deamidation leading to increased stability; see Examples 8, 9 and 10.

B. Definitions

[0060] As used herein, each of the following terms has the meaning associated with it in this section.

[0061] The articles "a" and "an" are used herein to refer to one or to more than one (*i.e.*, to at least one) of the grammatical object of the article. By way of example, "an element" means one element or more than one element.

[0062] "About" as used herein when referring to a measurable value such as an amount, a temporal duration, and the like, is meant to encompass variations of ±20% or ±10%, more preferably ±5%, even more preferably ±1%, and still more preferably ±0.1% from the specified value, as such variations are appropriate to perform the disclosed methods.

[0063] By "ablation" herein is meant a decrease or removal of activity. Thus for example, "ablating FcγR binding" means the Fc region amino acid variant has less than 50% starting binding as compared to an Fc region not containing the specific variant, with less than 70-80-90-95-98% loss of activity being preferred, and in general, with the activity being below the level of detectable binding in a Biacore assay.

[0064] By "ADCC" or "antibody dependent cell-mediated cytotoxicity" as used herein is meant the cell-mediated reaction wherein nonspecific cytotoxic cells that express FcγRs recognize bound antibody on a target cell and subsequently cause lysis of the target cell. ADCC

is correlated with binding to FcγRIIIa; increased binding to FcγRIIIa leads to an increase in ADCC activity. As is discussed herein, many embodiments of the invention ablate ADCC activity entirely.

[0065] By "ADCP" or antibody dependent cell-mediated phagocytosis as used herein is meant the cell-mediated reaction wherein nonspecific cytotoxic cells that express FcγRs recognize bound antibody on a target cell and subsequently cause phagocytosis of the target cell.

[0066] By "antigen binding domain" or "ABD" herein is meant a set of six **C**omplementary **D**etermining **R**egions (CDRs) that, when present as part of a polypeptide sequence, specifically binds a target antigen as discussed herein. Thus, an "antigen binding domain" binds a target antigen as outlined herein. As is known in the art, these CDRs are generally present as a first set of variable heavy CDRs (vhCDRs or V_HCDRs or CDR-HC) and a second set of variable light CDRs (vlCDRs or V_LCDRs or CDR-LC), each comprising three CDRs: vhCDR1, vhCDR2, vhCDR3 for the heavy chain and vlCDR1, vlCDR2 and vlCDR3 for the light chain. The CDRs are present in the variable heavy and variable light domains, respectively, and together form an Fv region. Thus, in some cases, the six CDRs of the antigen binding domain are contributed by a variable heavy and variable light chain. In a "Fab" format, the set of 6 CDRs are contributed by two different polypeptide sequences, the variable heavy domain (vh or V_H; containing the vhCDR1, vhCDR2 and vhCDR3) and the variable light domain (vl or V_L; containing the vlCDR1, vlCDR2 and vlCDR3), with the C-terminus of the vh domain being attached to the N-terminus of the CH1 domain of the heavy chain and the C-terminus of the vl domain being attached to the N-terminus of the constant light domain (and thus forming the light chain). In a scFv format, the vh and vl domains are covalently attached, generally through the use of a linker as outlined herein, into a single polypeptide sequence, which can be either (starting from the N-terminus) vh-linker-vl or vl-linker-vh, with the former being generally preferred (including optional domain linkers on each side, depending on the format used. As is understood in the art, the CDRs are separated by framework regions in each of the variable heavy and variable light domains: for the light variable domain, these are FR1-vlCDR1-FR2-vlCDR2-FR3-vlCDR3-FR4, and for the heavy variable domain, these are FR1-vhCDR1-FR2-vhCDR2-FR3-vhCDR3-FR4, with the framework regions showing high identity to human germline sequences. Antigen binding domains of the invention include, Fab, Fv and scFv.

[0067] By "modification" herein is meant an amino acid substitution, insertion, and/or deletion in a polypeptide sequence or an alteration to a moiety chemically linked to a protein. For example, a modification may be an altered carbohydrate or PEG structure attached to a protein. By "amino acid modification" herein is meant an amino acid substitution, insertion, and/or deletion in a polypeptide sequence. For clarity, unless otherwise noted, the amino acid modification is always to an amino acid coded for by DNA, e.g. the 20 amino acids that have codons in DNA and RNA.

[0068] By "amino acid substitution" or "substitution" herein is meant the replacement of an amino acid at a particular position in a parent polypeptide sequence with a different amino acid. In particular, in some embodiments, the substitution is to an amino acid that is not naturally occurring at the particular position, either not naturally occurring within the organism or in any organism. For example, the substitution M252Y refers to a variant polypeptide, in this case an Fc variant, in which the methionine at position 252 is replaced with tyrosine. For clarity, a protein which has been engineered to change the nucleic acid coding sequence but not change the starting amino acid (for example exchanging CGG (encoding arginine) to CGA (still encoding arginine) to increase host organism expression levels) is not an "amino acid substitution"; that is, despite the creation of a new gene encoding the same protein, if the protein has the same amino acid at the particular position that it started with, it is not an amino acid substitution.

[0069] By "variant protein" or "protein variant", or "variant" as used herein is meant a protein that differs from that of a parent protein by virtue of at least one amino acid modification. Protein variant may refer to the protein itself, a composition comprising the protein, or the amino sequence that encodes it. Preferably, the protein variant has at least one amino acid modification compared to the parent protein, *e.g.*. from about one to about seventy amino acid modifications, and preferably from about one to about five amino acid modifications compared to the parent. As described below, in some embodiments the parent polypeptide, for example an Fc parent polypeptide, is a human wild type sequence, such as the Fc region from IgG1, IgG2, IgG3 or IgG4. The protein variant sequence herein will preferably possess at least about 80% identity with a parent protein sequence, and most preferably at least about 90% identity, more preferably at least about 95%-98%-99% identity. Variant protein can refer to the variant protein itself, compositions comprising the protein variant, or the DNA sequence that encodes it.

[0070] Accordingly, by "antibody variant" or "variant antibody" as used herein is meant an antibody that differs from a parent antibody by virtue of at least one amino acid modification, "IgG variant" or "variant IgG" as used herein is meant an antibody that differs from a parent IgG (again, in many cases, from a human IgG sequence) by virtue of at least one amino acid modification, and "immunoglobulin variant" or "variant immunoglobulin" as used herein is meant an immunoglobulin sequence that differs from that of a parent immunoglobulin sequence by virtue of at least one amino acid modification. "Fc variant" or "variant Fc" as used herein is meant a protein comprising an amino acid modification in an Fc domain. The Fc variants of the present invention are defined according to the amino acid modifications that compose them. Thus, for example M252Y or 252Y is an Fc variant with the substitution tyrosine at position 252 relative to the parent Fc polypeptide, wherein the numbering is according to the EU index. Likewise, M252Y/S254T/T256E defines an Fc variant with the substitutions M252Y, S254T and T256E relative to the parent Fc polypeptide. The identity of the WT amino acid may be unspecified, in which case the aforementioned variant is referred to as 252Y/254T/256E. It is noted that the order in which substitutions are provided is arbitrary, that is to say that, for example, 252Y/254T/256E is the same Fc variant as 254T/252Y/256E, and so on. For all positions discussed in the present invention that relate to antibodies, unless otherwise noted, amino acid position numbering is according to Kabat for the variable domain numbering and is according to the EU index for the constant domains, including the Fc domain. The EU index or EU index as in Kabat or EU numbering scheme refers to the numbering of the EU antibody (Edelman et al., 1969, Proc Natl Acad Sci USA 63:78-85, hereby entirely incorporated by reference.) The modification can be an addition, deletion, or substitution. Substitutions can include naturally occurring amino acids and, in some cases, synthetic amino acids.

[0071] As used herein, "protein" herein is meant at least two covalently attached amino acids, which includes proteins, polypeptides, oligopeptides and peptides. The peptidyl group may comprise naturally occurring amino acids and peptide bonds.

[0072] By "Fab" or "Fab region" as used herein is meant the polypeptide that comprises the VH, CH1, VL, and CL immunoglobulin domains. Fab may refer to this region in isolation, or this region in the context of a full length antibody, antibody fragment or Fab fusion protein.

[0073] By "Fv" or "Fv fragment" or "Fv region" as used herein is meant a polypeptide that comprises the VL and VH domains of a single antigen binding domain (ABD). As will be

appreciated by those in the art, these generally are made up of two chains, or can be combined (generally with a linker as discussed herein) to form a scFv.

[0074] By "amino acid" and "amino acid identity" as used herein is meant one of the 20 naturally occurring amino acids that are coded for by DNA and RNA.

[0075] By "effector function" as used herein is meant a biochemical event that results from the interaction of an antibody Fc region with an Fc receptor or ligand. Effector functions include but are not limited to ADCC, ADCP, and CDC.

[0076] By "Fc gamma receptor", "FcγR" or "FcgammaR" as used herein is meant any member of the family of proteins that bind the IgG antibody Fc region and is encoded by an FcγR gene. In humans this family includes but is not limited to FcγRI (CD64), including isoforms FcγRIa, FcγRIb, and FcγRIc; FcγRII (CD32), including isoforms FcγRIIa (including allotypes H131 and R131), FcγRIIb (including FcγRIIb-1 and FcγRIIb-2), and FcγRIIc; and FcγRIII (CD16), including isoforms FcγRIIIa (including allotypes V158 and F158) and FcγRIIIb (including allotypes FcγRIIb-NA1 and FcγRIIb-NA2) (Jefferis et al., 2002, Immunol Lett 82:57-65, entirely incorporated by reference), as well as any undiscovered human FcγRs or FcγR isoforms or allotypes. In some cases, as outlined herein, binding to one or more of the FcγR receptors is reduced or ablated. For example, reducing binding to FcγRIIIa reduces ADCC, and in some cases, reducing binding to FcγRIIIa and FcγRIIb is desired.

[0077] By "FcRn" or "neonatal Fc Receptor" as used herein is meant a protein that binds the IgG antibody Fc region and is encoded at least in part by an FcRn gene. The FcRn may be from any organism, including but not limited to humans, mice, rats, rabbits, and monkeys. As is known in the art, the functional FcRn protein comprises two polypeptides, often referred to as the heavy chain and light chain. The light chain is beta-2-microglobulin and the heavy chain is encoded by the FcRn gene. Unless otherwise noted herein, FcRn or an FcRn protein refers to the complex of FcRn heavy chain with beta-2-microglobulin.

[0078] By "parent polypeptide" as used herein is meant a starting polypeptide that is subsequently modified to generate a variant. The parent polypeptide may be a naturally occurring polypeptide, or a variant or engineered version of a naturally occurring polypeptide. Parent polypeptide may refer to the polypeptide itself, compositions that comprise the parent polypeptide, or the amino acid sequence that encodes it. Accordingly, by "parent immunoglobulin" as used herein is meant an unmodified immunoglobulin polypeptide that is modified to generate a variant, and by "parent antibody" as used herein is meant an unmodified

antibody that is modified to generate a variant antibody. It should be noted that "parent antibody" includes known commercial, recombinantly produced antibodies as outlined below.

[0079] By “heavy constant region” herein is meant the CH1-hinge-CH2-CH3 portion of an antibody, generally from human IgG1, IgG2 or IgG4.

[0080] By "target antigen" as used herein is meant the molecule that is bound specifically by the variable region of a given antibody. In the present case, the target antigen is a tau protein that is phosphorylated at position Ser413 (“pSer413-tau”).

[0081] By "target cell" as used herein is meant a cell that expresses a target antigen.

[0082] By "variable region" as used herein is meant the region of an immunoglobulin that comprises one or more Ig domains substantially encoded by any of the V.kappa., V.lamda., and/or VH genes that make up the kappa, lambda, and heavy chain immunoglobulin genetic loci respectively.

[0083] By "wild type or WT" herein is meant an amino acid sequence or a nucleotide sequence that is found in nature, including allelic variations. A WT protein has an amino acid sequence or a nucleotide sequence that has not been intentionally modified.

[0084] The term “tau protein” used herein means any one of six isoforms of human tau protein having the amino acid sequences define in SEQ ID NOS:1 to 6, i.e., 4R2N (defined in SEQ ID NO:1), 4R1N (defined in SEQ ID NO:2), 4R0N (defined in SEQ ID NO:3), 3R2N (defined in SEQ ID NO:4), 3R1N (defined in SEQ ID NO:5), and 3R0N (defined in SEQ ID NO:6), as well as gene variants thereof. As explained in the BACKGROUND OF THE INVENTION section above, more than 40 mutations have been implicated with FTDP-17, which is a familial neurodegenerative disease relating to cognitive disorders. However, the sites of mutations in a tau protein should not be limited to those implicated with FTDP-17. The number of amino acid mutations introduced into the amino acid sequences of SEQ ID NOS:1 to 6 should not be limited, but may be 1 to 50, particularly 1 to 30, more particularly 1 to 10. However, the amino acid residue corresponding to the amino acid sequence defined in the serine residue at position 413 of SEQ ID NO:1 (Ser413) should preferably be conserved. The tau protein according to the present invention also encompasses proteins having similarities or identities of 80% or more to the amino acid sequence of human tau protein defined in SEQ ID NO:1 in accordance with the BLAST method (with default conditions of PBLAST provided by NCBI) and their isoforms. Such tau proteins include tau derived from non-human species such as chimpanzees, macaques, horses, pigs, dogs, mice, rabbits, and rats. It is possible to produce a

therapeutic or prophylactic agent targeted to tau derived from such a non-human animal for the purpose of improving the cognitive function of the target animal.

[0085] The term “tau peptide” used herein means a peptide including a part of the amino acid sequence of a tau protein. The position and length of the tau peptide’s amino acid sequence derived from a tau protein should not be limited, but should preferably contain, e.g., a series of at least three consecutive amino acids, particularly at least five consecutive amino acids, more particularly at least eight consecutive amino acids, derived from the amino acid residues corresponding to amino acid numbers 410 to 421 of SEQ ID NO:1, at least including the amino acid corresponding to Ser413. The length of the tau peptide should also not be limited, but should preferably have an amino acid length of four or more, particularly six or more, more particularly eight or more.

[0086] The term “tau” used herein means a tau protein or a tau peptide collectively.

[0087] The term “anti-pSer413 tau protein” means an antibody as described herein that preferentially binds to a tau protein that is phosphorylated at the serine at position 413 as compared to the binding of a tau protein is not phosphorylated at the serine at position 413. The tau protein phosphorylated at Ser413 can be human and/or murine.

[0088] By "position" as used herein is meant a location in the sequence of a protein. Positions may be numbered sequentially, or according to an established format, for example the EU index for antibody numbering.

[0089] By "residue" as used herein is meant a position in a protein and its associated amino acid identity. For example, Asparagine 297 (also referred to as Asn297 or N297) is a residue at position 297 in the human antibody IgG1.

[0090] In the context of the present invention, the position of an amino acid residue in a tau protein or a tau peptide is indicated by an amino acid number, which is identified based on the amino acid sequence defined in SEQ ID NO:1 for clarification. For example, the amino acid residue corresponding to Ser413 of SEQ ID NO:1 means the serine residue at position 413 of SEQ ID NO:1 (4R2N), position 384 of SEQ ID NO:2 (4R1N), position 355 of SEQ ID NO:3 (4R0N), position 382 of SEQ ID NO:4 (3R2N), position 353 of SEQ ID NO:5 (3R1N), or position 324 of SEQ ID NO:6 (3R0N). Correspondence of the positions of amino acid residues between tau isoforms is shown in Table 1 below.

Table 1: Isoforms of human tau protein

Isoform	4R2N	4R1N	4R0N	3R2N	3R1N	3R0N
Sequence	SEQ ID NO:1	SEQ ID NO:2	SEQ ID NO:3	SEQ ID NO:4	SEQ ID NO:5	SEQ ID NO:6
Amino acid residue	Amino acid number					
Asn	410	381	352	379	350	321
Val	411	382	353	380	351	322
Ser	412	383	354	381	352	323
Ser	413	384	355	382	353	324
Thr	414	385	356	383	354	325
Gly	415	386	357	384	355	326
Ser	416	387	358	385	356	327
Ile	417	388	359	386	357	328
Asp	418	389	360	387	358	329
Met	419	390	361	388	359	330
Val	420	391	362	389	360	331
Asp	421	392	363	390	361	332

[0091] While Table 1 shows the positions of amino acid residues of these isoforms corresponding to positions 410 to 421 of the amino acid sequence defined in SEQ ID NO:1, correspondence of the positions of amino acid residues in other regions can easily be recognized based on, *e.g.*, FIGS. 1A and 1B. A person skilled in the art would be able to determine corresponding positions of amino acids in isoforms or homologues using pairwise sequence alignment such as Needleman-Wunsch method or Smith-Waterman method, or multiple sequence alignment such as ClustalW method or PRRP method. As an example of analysis of corresponding positions, FIGS. 1A and 1B show an alignment of the amino acid sequences of the six human isoforms (indicated with one letter code) based on ClustalW. These figures indicate that the structure surrounding the amino acid residue corresponding to Ser413 of the amino acid sequence defined in SEQ ID NO:1 is conserved among the six isoforms.

[0092] The antibodies of the present invention are generally isolated or recombinant. "Isolated," when used to describe the various polypeptides disclosed herein, means a polypeptide that has been identified and separated and/or recovered from a cell or cell culture from which it was expressed. Ordinarily, an isolated polypeptide will be prepared by at least one purification step. An "isolated antibody," refers to an antibody which is substantially free of other antibodies having different antigenic specificities. "Recombinant" means the antibodies are generated using recombinant nucleic acid techniques in exogeneous host cells.

[0093] "Percent (%) amino acid sequence identity" with respect to a protein sequence is defined as the percentage of amino acid residues in a candidate sequence that are identical with

the amino acid residues in the specific (parental) sequence, after aligning the sequences and introducing gaps, if necessary, to achieve the maximum percent sequence identity, and not considering any conservative substitutions as part of the sequence identity. Alignment for purposes of determining percent amino acid sequence identity can be achieved in various ways that are within the skill in the art, for instance, using publicly available computer software such as BLAST, BLAST-2, ALIGN or Megalign (DNASTAR) software. Those skilled in the art can determine appropriate parameters for measuring alignment, including any algorithms needed to achieve maximal alignment over the full length of the sequences being compared. One particular program is the ALIGN-2 program outlined at paragraphs [0279] to [0280] of US Pub. No. 20160244525, hereby incorporated by reference. Another approximate alignment for nucleic acid sequences is provided by the local homology algorithm of Smith and Waterman, *Advances in Applied Mathematics*, 2:482-489 (1981). This algorithm can be applied to amino acid sequences by using the scoring matrix developed by Dayhoff, *Atlas of Protein Sequences and Structure*, M.O. Dayhoff ed., 5 suppl. 3:353-358, National Biomedical Research Foundation, Washington, D.C., USA, and normalized by Gribskov, *Nucl. Acids Res.* 14(6):6745-6763 (1986).

[0094] An example of an implementation of this algorithm to determine percent identity of a sequence is provided by the Genetics Computer Group (Madison, WI) in the "BestFit" utility application. The default parameters for this method are described in the Wisconsin Sequence Analysis Package Program Manual, Version 8 (1995) (available from Genetics Computer Group, Madison, WI). Another method of establishing percent identity in the context of the present invention is to use the MPSRCH package of programs copyrighted by the University of Edinburgh, developed by John F. Collins and Shane S. Sturrok, and distributed by IntelliGenetics, Inc. (Mountain View, CA). From this suite of packages, the Smith-Waterman algorithm can be employed where default parameters are used for the scoring table (for example, gap open penalty of 12, gap extension penalty of one, and a gap of six). From the data generated the "Match" value reflects "sequence identity." Other suitable programs for calculating the percent identity or similarity between sequences are generally known in the art, for example, another alignment program is BLAST, used with default parameters. For example, BLASTN and BLASTP can be used using the following default parameters: genetic code = standard; filter = none; strand = both; cutoff = 60; expect = 10; Matrix = BLOSUM62; Descriptions = 50 sequences; sort by = HIGH SCORE; Databases = non-redundant, GenBank + EMBL + DDBJ + PDB + GenBank CDS translations + Swiss protein + Spupdate + PIR. Details of these programs

can be found at the internet address located by placing http:// in front of blast.ncbi.nlm.nih.gov/Blast.cgi.

[0095] The degree of identity between an amino acid sequence of the present invention ("invention sequence") and the parental amino acid sequence is calculated as the number of exact matches in an alignment of the two sequences, divided by the length of the "invention sequence," or the length of the parental sequence, whichever is the shortest. The result is expressed in percent identity.

[0096] In some embodiments, two or more amino acid sequences are at least 50%, 60%, 70%, 80%, or 90% identical. In some embodiments, two or more amino acid sequences are at least 95%, 97%, 98%, 99%, or even 100% identical.

[0097] "Specific binding" or "specifically binds to" or is "specific for" a particular antigen or an epitope means binding that is measurably different from a non-specific interaction. Specific binding can be measured, for example, by determining binding of a molecule compared to binding of a control molecule, which generally is a molecule of similar structure that does not have binding activity. For example, specific binding can be determined by competition with a control molecule that is similar to the target.

[0098] The term "K_{assoc}" or "K_a", as used herein, is intended to refer to the association rate of a particular antibody-antigen interaction, whereas the term "K_{dis}" or "K_d," as used herein, is intended to refer to the dissociation rate of a particular antibody-antigen interaction. The term "K_D", as used herein, is intended to refer to the dissociation constant, which is obtained from the ratio of K_d to K_a (*i.e.*, K_d/K_a) and is expressed as a molar concentration (M). K_D values for antibodies can be determined using methods well established in the art. In some embodiments, the method for determining the K_D of an antibody is by using surface plasmon resonance, for example, by using a biosensor system such as a BIACORE® system. In certain embodiments, the K_D value is measured with a phosphorylated Tau protein. In some embodiments, the K_D value is measured with a phosphorylated peptide. In some embodiments, the K_D value is measured with the antigen (*e.g.*, the phosphorylated Tau protein or the phosphorylated peptide) immobilized. In other embodiments, the K_D value is measured with the antibody (*e.g.*, parent mouse antibody, chimeric antibody, or humanized antibody variants) immobilized. In yet other embodiments, the K_D value is measured with a phosphorylated Tau protein as the analyte. In still other embodiments, the KD value is measured with a phosphorylated peptide as the analyte.

In certain embodiments, the KD value is measured in a bivalent binding mode. In other embodiments, the KD value is measured in a monovalent binding mode.

[0099] A "disease" includes a state of health of an animal, including a human, wherein the animal cannot maintain homeostasis, and wherein if the disease is not ameliorated then the animal's health continues to deteriorate.

[00100] In contrast, a "disorder" in an animal, including a human, includes a state of health in which the animal is able to maintain homeostasis, but in which the animal's state of health is less favorable than it would be in the absence of the disorder. Left untreated, a disorder does not necessarily cause a further decrease in the animal's state of health.

[00101] The terms "treatment", "treating", "treat", and the like, refer to obtaining a desired pharmacologic and/or physiologic effect. The effect may be prophylactic in terms of completely or partially preventing a disease or symptom thereof or reducing the likelihood of a disease or symptom thereof and/or may be therapeutic in terms of a partial or complete cure for a disease and/or adverse effect attributable to the disease. "Treatment", as used herein, covers any treatment of a disease in a mammal, particularly in a human, and includes: (a) preventing the disease from occurring in a subject which may be predisposed to the disease but has not yet been diagnosed as having it; (b) inhibiting the disease, *i.e.*, arresting its development or progression; and (c) relieving the disease, *i.e.*, causing regression of the disease and/or relieving one or more disease symptoms. "Treatment" is also meant to encompass delivery of an agent in order to provide for a pharmacologic effect, even in the absence of a disease or condition. For example, "treatment" encompasses delivery of a composition that can elicit an immune response or confer immunity in the absence of a disease condition, *e.g.*, in the case of a vaccine.

[00102] As used herein, the term "mammal" refers to any mammal, including, but not limited to, mammals of the order *Rodentia*, such as mice and hamsters, and mammals of the order *Logomorpha*, such as rabbits. In some embodiments, the mammals are from the order *Carnivora*, including felines (cats) and canines (dogs). In some embodiments, the mammals are from the order *Artiodactyla*, including bovines (cows) and swines (pigs) or of the order *Perssodactyla*, including Equines (horses). It is most preferred that the mammals are of the order *Primates*, *Ceboids*, or *Simoids* (monkeys) or of the order *Anthropoids* (humans and apes). In some embodiments, the mammal is a human.

[00103] The term "regression," as well as words stemming therefrom, as used herein, does not necessarily imply 100% or complete regression. Rather, there are varying degrees of

regression of which one of ordinary skill in the art recognizes as having a potential benefit or therapeutic effect. In this respect, the disclosed methods can provide any amount of any level of regression of a tauopathy in a mammal. Furthermore, the regression provided by the inventive method can include regression of one or more conditions or symptoms of the disease, *e.g.*, a tauopathy. Also, for purposes herein, "regression" can encompass delaying the onset of the disease, delaying the onset of a symptom, and/or delaying the onset of a condition thereof. With respect to progressive diseases and disorders, "regression" can encompass slowing the progression of the disease or disorder, slowing the progression of a symptom of the disease or disorder, and/or slowing the progression of a condition thereof.

[00104] An "effective amount" or "therapeutically effective amount" of a composition includes that amount of the composition which is sufficient to provide a beneficial effect to the subject to which the composition is administered. An "effective amount" of a delivery vehicle includes that amount sufficient to effectively bind or deliver a composition.

[00105] By "individual" or "host" or "subject" or "patient" is meant any mammalian subject for whom diagnosis, treatment, or therapy is desired, particularly humans. Other subjects may include cattle, dogs, cats, guinea pigs, rabbits, rats, mice, horses, and so on.

[00106] The term "in combination with" as used herein refers to uses where, for example, a first therapy is administered during the entire course of administration of a second therapy; where the first therapy is administered for a period of time that is overlapping with the administration of the second therapy, *e.g.* where administration of the first therapy begins before the administration of the second therapy and the administration of the first therapy ends before the administration of the second therapy ends; where the administration of the second therapy begins before the administration of the first therapy and the administration of the second therapy ends before the administration of the first therapy ends; where the administration of the first therapy begins before administration of the second therapy begins and the administration of the second therapy ends before the administration of the first therapy ends; where the administration of the second therapy begins before administration of the first therapy begins and the administration of the first therapy ends before the administration of the second therapy ends. As such, "in combination" can also refer to regimen involving administration of two or more therapies. "In combination with" as used herein also refers to administration of two or more therapies which may be administered in the same or different formulations, by the same or different routes, and in the same or different dosage form type.

[00107] "Encoding" includes the inherent property of specific sequences of nucleotides in a polynucleotide, such as a gene, a cDNA, or an mRNA, to serve as templates for synthesis of other polymers and macromolecules in biological processes having either a defined sequence of nucleotides (*i.e.*, rRNA, tRNA and mRNA) or a defined sequence of amino acids and the biological properties resulting therefrom. Thus, a gene encodes a protein if, for example, transcription and translation of mRNA corresponding to that gene produces the protein in a cell or other biological system. Both the coding strand, the nucleotide sequence of which is identical to the mRNA sequence and is usually provided in sequence listings, and the non-coding strand, used as the template for transcription of a gene or cDNA, can be referred to as encoding the protein or other product of that gene or cDNA.

[00108] The term "nucleic acid" includes RNA or DNA molecules having more than one nucleotide in any form including single-stranded, double-stranded, oligonucleotide or polynucleotide. The term "nucleotide sequence" includes the ordering of nucleotides in an oligonucleotide or polynucleotide in a single-stranded form of nucleic acid.

[00109] By "nucleic acid construct" it is meant a nucleic acid sequence that has been constructed to comprise one or more functional units not found together in nature. Examples include circular, linear, double-stranded, extrachromosomal DNA molecules (plasmids), cosmids (plasmids containing COS sequences from lambda phage), viral genomes including non-native nucleic acid sequences, and the like.

[00110] The term "operably linked" as used herein includes a polynucleotide in functional relationship with a second polynucleotide, *e.g.* a single-stranded or double-stranded nucleic acid moiety comprising the two polynucleotides arranged within the nucleic acid moiety in such a manner that at least one of the two polynucleotides is able to exert a physiological effect by which it is characterized, upon the other. By way of example, a promoter operably linked to the coding region of a gene is able to promote transcription of the coding region. The order specified when indicating operably linkage is not important. For example, the phrases: "the promoter is operably linked to the nucleotide sequence" and "the nucleotide sequence is operably linked to the promoter" are used interchangeably herein and are considered equivalent. In some cases, when the nucleic acid encoding the desired protein further comprises a promoter/regulatory sequence, the promoter/regulatory sequence is positioned at the 5' end of the desired protein coding sequence such that it drives expression of the desired protein in a cell.

[00111] The terms "oligonucleotide," "polynucleotide," and "nucleic acid molecule", used interchangeably herein, refer to a polymeric forms of nucleotides of any length, either ribonucleotides or deoxyribonucleotides. Thus, this term includes, but is not limited to, single-, double-, or multi-stranded DNA or RNA, genomic DNA, cDNA, DNA-RNA hybrids, or a polymer comprising purine and pyrimidine bases or other natural, chemically or biochemically modified, non-natural, or derivatized nucleotide bases. The backbone of the polynucleotide can comprise sugars and phosphate groups (as may typically be found in RNA or DNA), or modified or substituted sugar or phosphate groups.

[00112] The term "recombinant," as applied to a polynucleotide means the polynucleotide is the product of various combinations of cloning, restriction or ligation steps, and other procedures resulting in a construct distinct and/or different from a polynucleotide found in nature. The terms respectively include replicates of the original polynucleotide construct and progeny of the original virus construct.

[00113] The term "promoter" as used herein includes a DNA sequence operably linked to a nucleic acid sequence to be transcribed such as a nucleic acid sequence encoding a desired molecule. A promoter is generally positioned upstream of a nucleic acid sequence to be transcribed and provides a site for specific binding by RNA polymerase and other transcription factors.

[00114] A "vector" is capable of transferring gene sequences to target-cells. Typically, "vector construct," "expression vector," and "gene transfer vector," mean any nucleic acid construct capable of directing the expression of a gene of interest and which can transfer gene sequences to target-cells, which can be accomplished by genomic integration of all or a portion of the vector, or transient or inheritable maintenance of the vector as an extrachromosomal element. Thus, the term includes cloning, and expression vehicles, as well as integrating vectors.

[00115] The term "regulatory element" as used herein includes a nucleotide sequence which controls some aspect of the expression of nucleic acid sequences. Examples of regulatory elements illustratively include an enhancer, an internal ribosome entry site (IRES), an intron, an origin of replication, a polyadenylation signal (pA), a promoter, an enhancer, a transcription termination sequence, and an upstream regulatory domain, which contribute to the replication, transcription, and/or post-transcriptional processing of a nucleic acid sequence. In cases, regulatory elements can also include *cis*-regulatory DNA elements as well as transposable elements (TEs). Those of ordinary skill in the art are capable of selecting and using these and

other regulatory elements in an expression construct with no more than routine experimentation. Expression constructs can be generated using a genetic recombinant approach or synthetically using well-known methodology.

[00116] A "control element" or "control sequence" is a nucleotide sequence involved in an interaction of molecules contributing to the functional regulation of a polynucleotide, including replication, duplication, transcription, splicing, translation, or degradation of the polynucleotide. The regulation may affect the frequency, speed, or specificity of the process, and may be enhancing or inhibitory in nature. Control elements known in the art include, for example, transcriptional regulatory sequences such as promoters and enhancers. A promoter is a DNA region capable under certain conditions of binding RNA polymerase and initiating transcription of a coding region usually located downstream (in the 3' direction) from the promoter.

[00117] The statement that an amino acid residue is "phosphorylated" used herein means that a phosphate group is ester-linked to the side chain of the amino acid residue. Typical amino acid residues that may be phosphorylated include serine (Ser), threonine (Thr), and tyrosine (Tyr).

VIII. Antibodies

[00118] The present invention is directed to antibodies and antigen binding fragments that bind human tau protein that has been phosphorylated at position 413.

[00119] Traditional antibody structural units typically comprise a tetramer. Each tetramer is typically composed of two identical pairs of polypeptide chains, each pair having one "light" (typically having a molecular weight of about 25 kDa) and one "heavy" chain (typically having a molecular weight of about 50-70 kDa). Human light chains are classified as kappa and lambda light chains. The present invention is directed to antibodies that generally are based on the IgG class, which has several subclasses, including, but not limited to IgG1, IgG2, IgG3, and IgG4. In general, IgG1, IgG2 and IgG4 are used more frequently than IgG3. It should be noted that IgG1 has different allotypes with polymorphisms at 356 (D or E) and 358 (L or M). The sequences depicted herein use the 356D/358L allotype, however the other allotype is included herein. That is, any sequence inclusive of an IgG1 Fc domain included herein can have 356E/358M replacing the 356D/358L allotype.

[00120] As will be appreciated by those in the art, the exact numbering and placement of the CDRs can be different among different numbering systems. However, it should be understood that the disclosure of a variable heavy and/or variable light sequence includes the disclosure of the associated (inherent) CDRs. Accordingly, the disclosure of each variable heavy region is a disclosure of the vhCDRs (e.g. vhCDR1, vhCDR2 and vhCDR3) and the disclosure of each variable light region is a disclosure of the vlCDRs (e.g. vlCDR1, vlCDR2 and vlCDR3).

[00121] Methods for identifying the sequence of each of CDR-H1, CDR-H2, CDR-H3, CDR-L1, CDR-L2, and CDR-L3 of an antibody include: Kabat method (Kabat et al., The Journal of Immunology, 1991, Vol.147, No.5, pp.1709-1719) and Chothia method (Al-Lazikani et al., Journal of Molecular Biology, 1997, Vol.273, No.4, pp.927-948). These methods are within the technical common knowledge to persons skilled in the art, the summaries thereof being available, e.g., on the website of Dr. Andrew C.R. Martin's Group (http://www.bioinf.org.uk/abs/).

[00122] A useful comparison of CDR numbering is as below, see Lafranc et al., Dev. Comp. Immunol. 27(1):55-77 (2003):

TABLE 1

	Kabat+ Chothia	IMGT	Kabat	AbM	Chothia	Contact
vhCDR1	26-35	27-38	31-35	26-35	26-32	30-35
vhCDR2	50-65	56-65	50-65	50-58	52-56	47-58
vhCDR3	95-102	105-117	95-102	95-102	95-102	93-101
vlCDR1	24-34	27-38	24-34	24-34	24-34	30-36
vlCDR2	50-56	56-65	50-56	50-56	50-56	46-55
vlCDR3	89-97	105-117	89-97	89-97	89-97	89-96

[00123] Throughout the present specification, the Kabat numbering system is generally used when referring to a residue in the variable domain (approximately, residues 1-107 of the

light chain variable region and residues 1-113 of the heavy chain variable region) and the EU numbering system for constant regions such as Fc regions (e.g, Kabat et al., supra (1991)).

[00124] The present invention provides a number of different CDR sets. In this case, a "full CDR set" comprises the three variable light and three variable heavy CDRs, e.g. a vlCDR1, vlCDR2, vlCDR3, vhCDR1, vhCDR2 and vhCDR3. These can be part of a larger variable light or variable heavy domain, respectfully. In addition, as more fully outlined herein, the variable heavy and variable light domains can be on separate polypeptide chains, when a heavy and light chain is used (for example when Fabs are used), or on a single polypeptide chain in the case of scFv sequences.

[00125] The CDRs contribute to the formation of the antigen-binding, or more specifically, epitope binding site of antibodies. "Epitope" refers to a determinant that interacts with a specific antigen binding site in the variable region of an antibody molecule known as a paratope. Epitopes are groupings of molecules such as amino acids or sugar side chains and usually have specific structural characteristics, as well as specific charge characteristics. A single antigen may have more than one epitope.

[00126] In the present case, the epitope comprises not only the amino acid residues directly involved in the binding of the antibodies of the invention but also the phosphorylation at Ser413.

[00127] The carboxy-terminal portion of each chain defines a constant region primarily responsible for effector function. Kabat et al. collected numerous primary sequences of the variable regions of heavy chains and light chains. Based on the degree of conservation of the sequences, they classified individual primary sequences into the CDR and the framework and made a list thereof (see SEQUENCES OF IMMUNOLOGICAL INTEREST, 5th edition, NIH publication, No. 91-3242, E.A. Kabat et al., entirely incorporated by reference).

[00128] In the IgG subclass of immunoglobulins, there are several immunoglobulin domains in the heavy chain. By "immunoglobulin (Ig) domain" herein is meant a region of an immunoglobulin having a distinct tertiary structure. Of interest in the present invention are the heavy chain domains, including, the constant heavy (CH) domains and the hinge domains. In the context of IgG antibodies, the IgG isotypes each have three CH regions. Accordingly, "CH" domains in the context of IgG are as follows: "CH1" refers to positions 118-220 according to the EU index as in Kabat. "CH2" refers to positions 237-340 according to the EU index as in Kabat, and "CH3" refers to positions 341-447 according to the EU index as in Kabat.

[00129] Another type of Ig domain of the heavy chain is the hinge region. By "hinge" or "hinge region" or "antibody hinge region" or "immunoglobulin hinge region" herein is meant the flexible polypeptide comprising the amino acids between the first and second constant domains of an antibody. Structurally, the IgG CH1 domain ends at EU position 220, and the IgG CH2 domain begins at residue EU position 237. Thus for IgG the antibody hinge is herein defined to include positions 221 (D221 in IgG1) to 236 (G236 in IgG1), wherein the numbering is according to the EU index as in Kabat. In some embodiments, for example in the context of an Fc region, the lower hinge is included, with the "lower hinge" generally referring to positions 226 or 230. As noted herein, pI variants can be made in the hinge region as well.

[00130] The light chain generally comprises two domains, the variable light domain (containing the light chain CDRs and together with the variable heavy domains forming the Fv region), and a constant light chain region (often referred to as CL or Cκ).

[00131] Another region of interest for additional substitutions, outlined below, is the Fc region.

[00132] Thus, the present invention provides different antibody domains. As described herein and known in the art, the antibodies of the invention comprise different domains within the heavy and light chains, which can be overlapping as well. These domains include, but are not limited to, the Fc domain, the CH1 domain, the CH2 domain, the CH3 domain, the hinge domain, the heavy constant domain (CH1-hinge-Fc domain or CH1-hinge-CH2-CH3), the variable heavy domain, the variable light domain, the light constant domain, Fab domains and scFv domains.

[00133] Thus, the "Fc domain" includes the -CH2-CH3 domain, and optionally a hinge domain. The heavy chain comprises a variable heavy domain and a constant domain, which includes a CH1-hinge-Fc domain comprising a CH2-CH3. The light chain comprises a variable light chain and the light constant domain. A scFv comprises a variable heavy chain, an scFv linker, and a variable light domain. Some embodiments of the invention comprise at least one scFv domain, which, while not naturally occurring, generally includes a variable heavy domain and a variable light domain, linked together by a scFv linker. As outlined herein, while the scFv domain is generally from N- to C-terminus oriented as vh-scFv linker-vl, this can be reversed for any of the scFv domains (or those constructed using vh and vl sequences from Fabs), to vl-scFv linker-vh.

[00134] As shown herein, there are a number of suitable scFv linkers that can be used, including traditional peptide bonds, generated by recombinant techniques. The linker peptide may predominantly include the following amino acid residues: Gly, Ser, Ala, or Thr. The linker peptide should have a length that is adequate to link two molecules in such a way that they assume the correct conformation relative to one another so that they retain the desired activity. In one embodiment, the linker is from about 1 to 50 amino acids in length, preferably about 1 to 30 amino acids in length. In one embodiment, linkers of 1 to 20 amino acids in length may be used, with from about 5 to about 10 amino acids finding use in some embodiments. Useful linkers include glycine-serine polymers, including for example (GS)n, (GSGGS)n, (GGGGS)n, and (GGGS)n, where n is an integer of at least one (and generally from 3 to 4), glycine-alanine polymers, alanine-serine polymers, and other flexible linkers. Alternatively, a variety of nonproteinaceous polymers, including but not limited to polyethylene glycol (PEG), polypropylene glycol, polyoxyalkylenes, or copolymers of polyethylene glycol and polypropylene glycol, may find use as linkers, that is may find use as linkers.

[00135] Other linker sequences may include any sequence of any length of CL/CH1 domain but not all residues of CL/CH1 domain; for example the first 5-12 amino acid residues of the CL/CH1 domains. Linkers can be derived from immunoglobulin light chain, for example Cκ or Cλ. Linkers can be derived from immunoglobulin heavy chains of any isotype, including for example Cγ1, Cγ2, Cγ3, Cγ4, Cα1, Cα2, Cδ, Cε, and Cμ. Linker sequences may also be derived from other proteins such as Ig-like proteins (e.g. TCR, FcR, KIR), hinge region-derived sequences, and other natural sequences from other proteins.

[00136] In some embodiments, the linker is a "domain linker", used to link any two domains as outlined herein together. While any suitable linker can be used, many embodiments utilize a glycine-serine polymer, including for example (GS)n, (GSGGS)n, (GGGGS)n, and (GGGS)n, where n is an integer of at least one (and generally from 3 to 4 to 5) as well as any peptide sequence that allows for recombinant attachment of the two domains with sufficient length and flexibility to allow each domain to retain its biological function.

A. Chimeric and Humanized Antibodies

[00137] In some embodiments, the antibodies herein can be derived from a mixture from different species, e.g. a chimeric antibody and/or a humanized antibody. In general, both "chimeric antibodies" and "humanized antibodies" refer to antibodies that combine regions from more than one species. For example, "chimeric antibodies" traditionally comprise variable

region(s) from a mouse (or rat, in some cases) and the constant region(s) from a human. "Humanized antibodies" generally refer to non-human antibodies that have had the variable-domain framework regions swapped for sequences found in human antibodies. Generally, in a humanized antibody, the entire antibody, except the CDRs, is encoded by a polynucleotide of human origin or is identical to such an antibody except within its CDRs. The CDRs, some or all of which are encoded by nucleic acids originating in a non-human organism, are grafted into the beta-sheet framework of a human antibody variable region to create an antibody, the specificity of which is determined by the engrafted CDRs. The creation of such antibodies is described in, e.g., WO 92/11018, Jones, 1986, Nature 321:522-525, Verhoeyen et al., 1988, Science 239:1534-1536, all entirely incorporated by reference. "Backmutation" of selected acceptor framework residues to the corresponding donor residues is often required to regain affinity that is lost in the initial grafted construct (US 5530101; US 5585089; US 5693761; US 5693762; US 6180370; US 5859205; US 5821337; US 6054297; US 6407213, all entirely incorporated by reference). The humanized antibody optimally also will comprise at least a portion of an immunoglobulin constant region, typically that of a human immunoglobulin, and thus will typically comprise a human Fc region. Humanized antibodies can also be generated using mice with a genetically engineered immune system. Roque et al., 2004, Biotechnol. Prog. 20:639-654, entirely incorporated by reference. A variety of techniques and methods for humanizing and reshaping non-human antibodies are well known in the art (See Tsurushita & Vasquez, 2004, Humanization of Monoclonal Antibodies, Molecular Biology of B Cells, 533-545, Elsevier Science (USA), and references cited therein, all entirely incorporated by reference). Humanization methods include but are not limited to methods described in Jones et al., 1986, Nature 321:522-525; Riechmann et al.,1988; Nature 332:323-329; Verhoeyen et al., 1988, Science, 239:1534-1536; Queen et al., 1989, Proc Natl Acad Sci, USA 86:10029-33; He et al., 1998, J. Immunol. 160: 1029-1035; Carter et al., 1992, Proc Natl Acad Sci USA 89:4285-9, Presta et al., 1997, Cancer Res. 57(20):4593-9; Gorman et al., 1991, Proc. Natl. Acad. Sci. USA 88:4181-4185; O'Connor et al., 1998, Protein Eng 11:321-8, all entirely incorporated by reference. Humanization or other methods of reducing the immunogenicity of nonhuman antibody variable regions may include resurfacing methods, as described for example in Roguska et al., 1994, Proc. Natl. Acad. Sci. USA 91:969-973, entirely incorporated by reference.

[00138] In certain embodiments, the antibodies of the invention comprise a heavy chain variable region from a particular germline heavy chain immunoglobulin gene and/or a light

chain variable region from a particular germline light chain immunoglobulin gene. For example, such antibodies may comprise or consist of a human antibody comprising heavy or light chain variable regions that are "the product of" or "derived from" a particular germline sequence. A human antibody that is "the product of" or "derived from" a human germline immunoglobulin sequence can be identified as such by comparing the amino acid sequence of the human antibody to the amino acid sequences of human germline immunoglobulins and selecting the human germline immunoglobulin sequence that is closest in sequence (i.e., greatest % identity) to the sequence of the human antibody. A human antibody that is "the product of" or "derived from" a particular human germline immunoglobulin sequence may contain amino acid differences as compared to the germline sequence, due to, for example, naturally-occurring somatic mutations or intentional introduction of site-directed mutation. However, a humanized antibody typically is at least 80% identical in amino acids sequence to an amino acid sequence encoded by a human germline immunoglobulin gene and contains amino acid residues that identify the antibody as being derived from human sequences when compared to the germline immunoglobulin amino acid sequences of other species (e.g., murine germline sequences). In certain cases, a humanized antibody may be at least 95, 96, 97, 98 or 99%, or even at least 96%, 97%, 98%, or 99% identical in amino acid sequence to the amino acid sequence encoded by the germline immunoglobulin gene. Typically, a humanized antibody derived from a particular human germline sequence will display no more than 10-20 amino acid differences from the amino acid sequence encoded by the human germline immunoglobulin gene. In certain cases, the humanized antibody may display no more than 5, or even no more than 4, 3, 2, or 1 amino acid difference from the amino acid sequence encoded by the germline immunoglobulin gene.

[00139] In one embodiment, the parent antibody has been affinity matured, as is known in the art. Structure-based methods may be employed for humanization and affinity maturation, for example as described in US Patent No 7,657,380. Selection based methods may be employed to humanize and/or affinity mature antibody variable regions, including but not limited to methods described in Wu et al., 1999, J. Mol. Biol. 294:151-162; Baca et al., 1997, J. Biol. Chem. 272(16):10678-10684; Rosok et al., 1996, J. Biol. Chem. 271(37): 22611-22618; Rader et al., 1998, Proc. Natl. Acad. Sci. USA 95: 8910-8915; Krauss et al., 2003, Protein Engineering 16(10):753-759, all entirely incorporated by reference. Other humanization methods may involve the grafting of only parts of the CDRs, including but not limited to methods described in Tan et al., 2002, J. Immunol. 169:1119-1125; De Pascalis et al., 2002, J. Immunol. 169:3076-3084, all entirely incorporated by reference.

[00140] The term "antibody" is used in the broadest sense and includes, for example, an intact immunoglobulin or an antigen binding portion. Antigen binding portions may be produced by recombinant DNA techniques or by enzymatic or chemical cleavage of intact antibodies. Thus the term antibody includes traditional tetrameric antibodies of two heavy chains and two light chains, as well as antigen binding fragments such as Fv, Fab and scFvs. In some cases, the invention provides bispecific antibodies that include at least one antigen binding domain as outlined herein.

[00141] The humanized anti-phosphorylated tau antibody according to the present invention should preferably exhibit improved plasma kinetics. The improvement in plasma kinetics of a humanized antibody herein means that a parameter representing the kinetics of the humanized antibody in plasma is altered in comparison to a non-humanized antibody corresponding to the humanized antibody. Examples of parameters representing plasma kinetics include: plasma half-life, average retention time in plasma, and plasma clearance. Such a parameter being "altered" means that the parameter is increased or decreased. The non-humanized antibody "corresponding" to the humanized antibody means a non-humanized antibody used as a basis for producing the humanized antibody, e.g., a mouse antibody or a chimeric antibody.

[00142] The term "chimeric antibody" refers to an antibody that contains one or more regions from one antibody and one or more regions from one or more other antibodies. A "CDR grafted antibody" is an antibody comprising one or more CDRs derived from an antibody of a particular species or isotype and the framework of another antibody of the same or different species or isotype.

IX. Compositions

[00143] The present invention provides a number of different antigen binding domains (generally incorporated into antibodies) that bind to human tau protein that is phosphorylated at Ser413 (pSer413), in an "antigen-antibody reaction".

[00144] . The "antigen-antibody reaction" is measured by the affinity of an antibody to a phosphorylated Tau protein or a phosphorylated Tau peptide, *i.e.*, equilibrium dissociation constant (K_D). In certain embodiments, the K_D value is measured with a phosphorylated Tau protein. In some embodiments, the K_D value is measured with a phosphorylated Tau peptide. In one embodiment, the phosphorylated Tau peptide is phosphorylated at only one residue (*e.g.*,

SEQ ID NO:75). In another embodiment, the phosphorylated peptide is phosphorylated at multiple residues (*e.g.*, SEQ ID NO:76 or SEQ ID NO:78). In some embodiments, the K_D value is measured with the antigen (*e.g.*, the phosphorylated Tau protein or the phosphorylated Tau peptide) immobilized, in which cases the affinity measurement includes an avidity component, *i.e.*, in a bivalent binding mode. In other embodiments, the K_D value is measured with the antibody (*e.g.*, the mouse parent antibody, the chimeric antibody, or the humanized antibody variants) immobilized, in which case the affinity measurement does not include an avidity component, *i.e.*, in a monovalent binding mode. In yet other embodiments, the K_D value is measured with the antibody immobilized and with the phosphorylated Tau protein as the analyte. In still other embodiments, the K_D value is measured with the antibody immobilized and with the phosphorylated peptide as the analyte. In certain embodiments, the K_D value is measured in a bivalent binding mode. In other embodiments, the K_D value is measured in a monovalent binding mode.

[00145] Thus, in certain embodiments, the K_D value is 5×10^{-8} M or less; in some embodiments, the K_D value is 5×10^{-9} M or less; in other embodiments, the K_D value is 5×10^{-10} M or less; in yet other embodiments, the K_D value is 1.86×10^{-8} M or less; in still other embodiments, the K_D value is 2×10^{-9} M or less; in yet still other embodiments, the K_D value is 2×10^{-10} M or less. In a specific embodiment, the antibody disclosed herein binds to a phosphorylated Tau protein or a phosphorylated Tau peptide with a K_D of 5×10^{-8} M or less, measured with the antibody immobilized and the phosphorylated tau protein or phosphorylated tau peptide as an analyte. In another specific embodiment, the antibody disclosed herein binds to a phosphorylated Tau protein or a phosphorylated Tau peptide with a K_D of 5×10^{-9} M or less, measured with the phosphorylated Tau protein or the phosphorylated Tau peptide immobilized and the antibody as an analyte. In yet another specific embodiment, the antibody disclosed herein binds to a phosphorylated Tau protein or a phosphorylated Tau peptide with a K_D of 2×10^{-9} M or less, measured with the phosphorylated Tau protein or the phosphorylated Tau peptide immobilized and the antibody as an analyte. In one another specific embodiment, the antibody disclosed herein binds to a phosphorylated Tau protein or a phosphorylated Tau peptide with a K_D of 5×10^{-10} M or less, measured with the phosphorylated Tau protein or the phosphorylated Tau peptide immobilized and the antibody as an analyte. In another specific embodiment, the antibody disclosed herein binds to a phosphorylated Tau protein or a phosphorylated Tau peptide with a K_D of 2×10^{-10} M or less, measured with the phosphorylated Tau protein or the phosphorylated Tau peptide immobilized and the antibody as an analyte.

[00146] Additionally, as outlined herein, the antibodies of the invention preferentially bind to tau peptides or proteins that are phosphorylated at position S413 over proteins or peptides that do not contain a phosphate at position 413.

[00147] The humanized anti-phosphorylated tau antibody according to the present invention is an antibody which can cause an antigen-antibody reaction with the phosphorylated tau protein or phosphorylated tau peptide according to the present invention.

[00148] Phosphorylated tau proteins and tau peptides with which the humanized anti-phosphorylated tau antibody according to the present invention can cause antigen-antibody reaction are those that phosphorylated at an amino acid residue which is phosphorylated specifically in neurodegenerative diseases such as AD. Specifically, such tau proteins and tau peptides are phosphorylated at least at the serine residue at position 413 of the amino acid sequence defined in SEQ ID NO:1 (Ser413), and also may be phosphorylated at one or more of the amino acid residues corresponding to amino acid numbers 410 to 421, i.e., one or more of the amino acid residues corresponding to the serine residue at position 412 (Ser412), the threonine residue at position 414 (Thr414), and the serine residue at position 416 (Ser416) of the amino acid sequence defined in SEQ ID NO:1. Typical such phosphorylated peptides include, although not limited to: pSer412/pSer413(Cys-), which has the amino acid sequence defined in SEQ ID NO:7; pSer413(Cys-), which has the amino acid sequence defined in SEQ ID NO:8, and pSer409/pSer412/pSer413(Cys-), which has the amino acid sequence defined in SEQ ID NO:9.

[00149] The phosphorylated tau peptide according to the present invention can be used in the production of the humanized anti-phosphorylated tau antibody according to the present invention, or as an antigen for studying reactivity.

[00150] The phosphorylated tau peptide according to the present invention can be produced by a person skilled in the art by using appropriate synthesis methods or genetic engineering methods. Examples of methods for producing the phosphorylated peptide via synthesis include Boc method (Wakamiya T., Chemistry Letters, Vol.22, p.1401, 1993), Fmoc method (Perich, J. W., International Journal of Peptide and Protein Research, Vol.40, pp.134-140, 1992), and JP3587390B and WO2013/180238, although a person skilled in the art can employ any other methods as appropriate. Examples of methods for producing the phosphorylated peptide via genetic engineering includes a method in which a genetic material (DNA or RNA) having a nucleic acid sequence encoding a peptide to be produced or a precursor thereof is prepared and, after optional steps such as introduction into an expression vector and

addition of a promoter sequence, introduced into a suitable host for expression or subjected to a cell-free protein synthesis system. In this case, the peptide can be phosphorylated at a desired site by causing phosphorylation reaction in the host by means of enzymes such as kinases, which may be either inherent to the host or caused to express via genetic engineering, or by recovering the target peptide from the host and then causing phosphorylation reaction using enzymes such as kinases. In the latter case, phosphorylation reaction can be caused in vitro by subjecting the target peptide to an enzyme which is known to play a role in phosphorylation reaction of tau, such as glycogen synthase kinase 3 (GSK3) or cyclin-dependent protein kinase 5 (CDK5). From among the peptides phosphorylated in the host or in vitro in accordance with the above methods, peptides phosphorylated at a desired amino acid residue can be recovered by, e.g., selecting ones which specifically bound to the anti-phosphorylated tau antibody mentioned above via antibody-antigen reaction.

[00151] The phosphorylated tau peptide according to the present invention can be modified at its N-terminal sequence and/or C-terminal sequence with a substance having other functions suitable for desired purposes. For example, the phosphorylated tau peptide according to the present invention may be added at its N-terminal and/or C-terminal with, e.g., methionine residue, acetyl group or pyroglutamic acid, or modified at its N-terminal and/or C-terminal with, e.g., fluorescent material. Alternatively, the N-terminal and/or C-terminal of the phosphorylated tau peptide according to the present invention may be modified with, e.g., a peptide or a protein. Examples of peptides usable for such a modification include suitable tag sequences (typically histidine tag or FLAG tag), peptides having amino acid sequences which can be recognized by a T-cell receptor (TCR) or a major histocompatibility complex (MHC), proteins derived from viruses or bacteria or peptides having sequences derived from such proteins. In addition, the phosphorylated tau peptide according to the present invention includes ones having at least one which amino acid residue other than the N-terminal and C-terminal residues modified with any other compound or peptide. A person skilled in the art would be able to carry out such a modification to a phosphorylated tau peptide using any known methods, such as the ones described in Hermanson et al., “Bioconjugate Techniques”, (US), Academic Press, 1996.

[00152] A person skilled in the art would be able to carry out measurement of an antigen-antibody reaction by selecting an appropriate binding assay in a system of a solid phase or liquid phase. Examples of such assays include, although not limited to: enzyme-linked immunosorbent assay (ELISA), enzyme immunoassay (EIA), surface plasmon resonance (SPR), fluorescence resonance energy transfer (FRET), and luminescence resonance energy transfer (LRET).

Measurement of antigen-antibody binding affinity can be carried out by, e.g., labelling an antibody and/or an antigen with, e.g., an enzyme, a fluorescent material, a luminescent material, or radioisotope, and detecting the antigen-antibody reaction using a method suitable for mesuring the physical and/or chemical properties characteristic to the label used.

[00153] The humanized anti-phosphorylated tau antibody according to the present invention should preferably have an improved selective binding affinity to a phosphorylated tau protein or tau peptide over its binding affinity to a non-phosphorylated tau protein or tau peptide. The improvement in the selective binding affinity of an antibody to a phosphorylated tau protein or tau peptide in this context means that the ratio of the antibody's binding affinity to the phosphorylated tau protein or tau peptide to its binding affinity to the corresponding non-phosphorylated tau protein or tau peptide is increased. Such selective binding affinity can be analyzed by, e.g., using a plate on which the phosphorylated tau protein or tau peptide has been immobilized and a plate on which the corresponding non-phosphorylated tau protein or tau peptide has been immobilized, measuring the antibody's binding affinity to each of these proteins or peptides by means of, e.g., ELISA (e.g., as an absorbance OD value of emission wavelength), and dividing the value of the binding affinity (e.g., absorbance OD value) obtained for phosphorylated tau protein or tau peptide by the value obtained for the corresponding non-phosphorylated tau protein or tau peptide. Specific conditions for ELISA may be those described in the Examples section below.

[00154] The humanized anti-phosphorylated tau antibody according to the present invention should preferably have a ratio of the selective binding affinity to the phosphorylated tau protein or tau peptide to the binding affinity for the non-phosphorylated tau protein or tau peptide of at least about 40 or more, particularly at least about 50 or more, more particularly at least about 60 or more, provided that the selective binding affinity is obtained in accordance with the method described in Example 2, below. In one embodiment, the ratio is calculated by comparing the binding affinity of the anti-pSer413 tau antibodies to a phosphorylated peptide such as SEQ ID NO:8 to the binding affinity to the non-phosphorylated peptide of SEQ ID NO:69.

[00155] The humanized anti-phosphorylated tau antibody according to the present invention should preferably exhibit an improved ability to enter the brain when administered into the blood. The improvement in the ability of an antibody to enter the brain when administered into the blood in this context means that when the antibody is administered into the

blood of a human or any other mammal (e.g., a mouse or a rat), the ratio of the antibody concentration in the brain to the antibody concentration in the plasma is increased. An antibody's ability to enter the brain can be analyzed, e.g., by administering the antibody to an animal (e.g., a mouse or a rat) via blood and, after a predetermined period (e.g., one week), collecting the blood and preparing brain homogenate, measuring the antibody concentration in each of the obtained plasma sample and the obtained brain homogenate sample using a known method (e.g., Sandwich ELISA using an anti-human polyclonal antibody), and dividing the antibody concentration in the brain by the antibody concentration in the plasma. Specific conditions for ELISA may be those described in the Examples section below. It is known that the concentration of an antibody entering the brain is generally about 0.1 to 0.3% of that in the plasma.

[00156] The humanized anti-phosphorylated tau antibody according to the present invention should preferably have a ratio of the antibody concentration in the brain to the antibody concentration in the plasma of 0.30% or more, particularly 0.35% or more, more particularly 0.40% or more, even more particularly 0.45% or more, provided that the ratio is obtained in accordance with the method described in the "[7] Example 7: Analysis of intracerebral migration" section of the Examples below.

A. Antigen Binding Domains of the Invention

[00157] The invention provides antigen binding domains that bind to human pSer413 tau protein preferentially over binding to tau proteins that are not phosphorylated at Ser413 as described herein.

[00158] In one embodiment, the invention provides antigen binding domains that comprise a vlCDR1 having SEQ ID NO:91, a vlCDR2 having SEQ ID NO:82; a vlCDR3 having SEQ ID NO:83; a vhCDR1 having SEQ ID NO:86; a vhCDR2 having SEQ ID NO:115; and a vhCDR3 having SEQ ID NO:88.

[00159] In one embodiment, the invention provides antigen binding domains that comprise a vlCDR1 having SEQ ID NO:92, a vlCDR2 having SEQ ID NO:82; a vlCDR3 having SEQ ID NO:83; a vhCDR1 having SEQ ID NO:86; a vhCDR2 having SEQ ID NO:115; and a vhCDR3 having SEQ ID NO:88.

[00160] In one embodiment, the invention provides antigen binding domains that comprise a vlCDR1 having SEQ ID NO:93, a vlCDR2 having SEQ ID NO:82; a vlCDR3 having

SEQ ID NO:83; a vhCDR1 having SEQ ID NO:86; a vhCDR2 having SEQ ID NO:115; and a vhCDR3 having SEQ ID NO:88.

[00161] In one embodiment, the invention provides antigen binding domains that comprise a vlCDR1 having SEQ ID NO:94, a vlCDR2 having SEQ ID NO:82; a vlCDR3 having SEQ ID NO:83; a vhCDR1 having SEQ ID NO:86; a vhCDR2 having SEQ ID NO:115; and a vhCDR3 having SEQ ID NO:88.

[00162] In one embodiment, the invention provides antigen binding domains that comprise a vlCDR1 having SEQ ID NO:95, a vlCDR2 having SEQ ID NO:82; a vlCDR3 having SEQ ID NO:83; a vhCDR1 having SEQ ID NO:86; a vhCDR2 having SEQ ID NO:115; and a vhCDR3 having SEQ ID NO:88.

[00163] In one embodiment, the invention provides antigen binding domains that comprise a vlCDR1 having SEQ ID NO:96, a vlCDR2 having SEQ ID NO:82; a vlCDR3 having SEQ ID NO:83; a vhCDR1 having SEQ ID NO:86; a vhCDR2 having SEQ ID NO:115; and a vhCDR3 having SEQ ID NO:88.

[00164] In one embodiment, the invention provides antigen binding domains that comprise a vlCDR1 having SEQ ID NO:97, a vlCDR2 having SEQ ID NO:82; a vlCDR3 having SEQ ID NO:83; a vhCDR1 having SEQ ID NO:86; a vhCDR2 having SEQ ID NO:115; and a vhCDR3 having SEQ ID NO:88.

[00165] In one embodiment, the invention provides antigen binding domains that comprise a vlCDR1 having SEQ ID NO:98, a vlCDR2 having SEQ ID NO:82; a vlCDR3 having SEQ ID NO:83; a vhCDR1 having SEQ ID NO:86; a vhCDR2 having SEQ ID NO:115; and a vhCDR3 having SEQ ID NO:88.

[00166] In one embodiment, the invention provides antigen binding domains that comprise a vlCDR1 having SEQ ID NO:99, a vlCDR2 having SEQ ID NO:82; a vlCDR3 having SEQ ID NO:83; a vhCDR1 having SEQ ID NO:86; a vhCDR2 having SEQ ID NO:115; and a vhCDR3 having SEQ ID NO:88.

[00167] In one embodiment, the invention provides antigen binding domains that comprise a vlCDR1 having SEQ ID NO:100, a vlCDR2 having SEQ ID NO:82; a vlCDR3 having SEQ ID NO:83; a vhCDR1 having SEQ ID NO:86; a vhCDR2 having SEQ ID NO:115; and a vhCDR3 having SEQ ID NO:88.

[00168] In one embodiment, the invention provides antigen binding domains that comprise a vlCDR1 having SEQ ID NO:101, a vlCDR2 having SEQ ID NO:82; a vlCDR3 having SEQ ID NO:83; a vhCDR1 having SEQ ID NO:86; a vhCDR2 having SEQ ID NO:115; and a vhCDR3 having SEQ ID NO:88.

[00169] In one embodiment, the invention provides antigen binding domains that comprise a vlCDR1 having SEQ ID NO:102, a vlCDR2 having SEQ ID NO:82; a vlCDR3 having SEQ ID NO:83; a vhCDR1 having SEQ ID NO:86; a vhCDR2 having SEQ ID NO:115; and a vhCDR3 having SEQ ID NO:88.

[00170] The invention provides antigen binding domains to human pSer413 tau protein. As shown in the examples, a number of humanized variable heavy and variable light domains are provided, which can be combined in any combination, and can be added to human IgG constant domains in any combination.

[00171] In one embodiment, the variable light domain Ta1505-VL46 (SEQ ID NO:84) is combined with the variable heavy domain Ta1505-VH11 (SEQ ID NO:116) to form an Fv domain that binds pSer413 tau. In this embodiment, the human heavy constant domain is selected from SEQ ID NO:s118, 119, 120, 121, 122, 123, 124, 125 and 126 and the human light kappa constant domain, SEQ ID NO:52.

[00172] In one embodiment, the variable light domain Ta1505-VL46 (SEQ ID NO:84) is combined with the variable heavy domain Ta1505-VH65 (SEQ ID NO:117). In this embodiment, the human heavy constant domain is selected from SEQ ID NO:s118, 119, 120, 121, 122, 123, 124, 125 and 126 and the human light kappa constant domain, SEQ ID NO:52.

[00173] In one embodiment, the variable light domain Ta1505-VL47 (SEQ ID NO:104) is combined with the variable heavy domain Ta1505-VH11 (SEQ ID NO:116). In this embodiment, the human heavy constant domain is selected from SEQ ID NO:s118, 119, 120, 121, 122, 123, 124, 125 and 126 and the human light kappa constant domain, SEQ ID NO:52.

[00174] In one embodiment, the variable light domain Ta1505-VL47 (SEQ ID NO:104) is combined with the variable heavy domain Ta1505-VH65 (SEQ ID NO:117). In this embodiment, the human heavy constant domain is selected from SEQ ID NO:s118, 119, 120, 121, 122, 123, 124, 125 and 126 and the human light kappa constant domain, SEQ ID NO:52.

[00175] In one embodiment, the variable light domain Ta1505-VL46_G34A (SEQ ID NO:105) is combined with the variable heavy domain Ta1505-VH11 (SEQ ID NO:116). In this

embodiment, the human heavy constant domain is selected from SEQ ID NO:s118, 119, 120, 121, 122, 123, 124, 125 and 126 and the human light kappa constant domain, SEQ ID NO:52.

[00176] In one embodiment, the variable light domain Ta1505-VL46_G34A (SEQ ID NO:105) is combined with the variable heavy domain Ta1505-VH65 (SEQ ID NO:117). In this embodiment, the human heavy constant domain is selected from SEQ ID NO:s118, 119, 120, 121, 122, 123, 124, 125 and 126 and the human light kappa constant domain, SEQ ID NO:52.

[00177] In one embodiment, the variable light domain Ta1505-VL46_G34S (SEQ ID NO:106) is combined with the variable heavy domain Ta1505-VH11 (SEQ ID NO116). In this embodiment, the human heavy constant domain is selected from SEQ ID NO:s118, 119, 120, 121, 122, 123, 124, 125 and 126 and the human light kappa constant domain, SEQ ID NO:52.

[00178] In one embodiment, the variable light domain Ta1505-VL46_G34S (SEQ ID NO:106) is combined with the variable heavy domain Ta1505-VH65 (SEQ ID NO:117). In this embodiment, the human heavy constant domain is selected from SEQ ID NO:s118, 119, 120, 121, 122, 123, 124, 125 and 126 and the human light kappa constant domain, SEQ ID NO:52.

[00179] In one embodiment, the variable light domain Ta1505-VL46_G34T (SEQ ID NO:107) is combined with the variable heavy domain Ta1505-VH11 (SEQ ID NO:116). In this embodiment, the human heavy constant domain is selected from SEQ ID NO:s118, 119, 120, 121, 122, 123, 124, 125 and 126 and the human light kappa constant domain, SEQ ID NO:52.

[00180] In one embodiment, the variable light domain Ta1505-VL46_G34T (SEQ ID NO:107) is combined with the variable heavy domain Ta1505-VH65 (SEQ ID NO:117). In this embodiment, the human heavy constant domain is selected from SEQ ID NO:s118, 119, 120, 121, 122, 123, 124, 125 and 126 and the human light kappa constant domain, SEQ ID NO:52.

[00181] In one embodiment, the variable light domain Ta1505-VL46_N33Q (SEQ ID NO:108) is combined with the variable heavy domain Ta1505-VH11 (SEQ ID NO:116). In this embodiment, the human heavy constant domain is selected from SEQ ID NO:s118, 119, 120, 121, 122, 123, 124, 125 and 126 and the human light kappa constant domain, SEQ ID NO:52.

[00182] In one embodiment, the variable light domain Ta1505-VL46_N33Q (SEQ ID NO:108) is combined with the variable heavy domain Ta1505-VH65 (SEQ ID NO:117). In this embodiment, the human heavy constant domain is selected from SEQ ID NO:s118, 119, 120, 121, 122, 123, 124, 125 and 126 and the human light kappa constant domain, SEQ ID NO:52.

[00183] In one embodiment, the variable light domain Ta1505-VL46_N33Q_G34A (SEQ ID NO:109) is combined with the variable heavy domain Ta1505-VH11 (SEQ ID NO:116). In this embodiment, the human heavy constant domain is selected from SEQ ID NO:s118, 119, 120, 121, 122, 123, 124, 125 and 126 and the human light kappa constant domain, SEQ ID NO:52.

[00184] In one embodiment, the variable light domain Ta1505-VL46_N33Q_G34A (SEQ ID NO:109) is combined with the variable heavy domain Ta1505-VH65 (SEQ ID NO:117). In this embodiment, the human heavy constant domain is selected from SEQ ID NO:s118, 119, 120, 121, 122, 123, 124, 125 and 126 and the human light kappa constant domain, SEQ ID NO:52.

[00185] In one embodiment, the variable light domain Ta1505-VL46_N33D (SEQ ID NO:110) is combined with the variable heavy domain Ta1505-VH11 (SEQ ID NO:116). In this embodiment, the human heavy constant domain is selected from SEQ ID NO:s118, 119, 120, 121, 122, 123, 124, 125 and 126 and the human light kappa constant domain, SEQ ID NO:52.

[00186] In one embodiment, the variable light domain Ta1505-VL46_N33D (SEQ ID NO:110) is combined with the variable heavy domain Ta1505-VH65 (SEQ ID NO:117). In this embodiment, the human heavy constant domain is selected from SEQ ID NO:s118, 119, 120, 121, 122, 123, 124, 125 and 126 and the human light kappa constant domain, SEQ ID NO:52.

[00187] In one embodiment, the variable light domain Ta1505-VL46_N33S (SEQ ID NO:111) is combined with the variable heavy domain Ta1505-VH11 (SEQ ID NO:116). In this embodiment, the human heavy constant domain is selected from SEQ ID NO:s118, 119, 120, 121, 122, 123, 124, 125 and 126 and the human light kappa constant domain, SEQ ID NO:52.

[00188] In one embodiment, the variable light domain Ta1505-VL46_N33S (SEQ ID NO:111) is combined with the variable heavy domain Ta1505-VH65 (SEQ ID NO:117). In this embodiment, the human heavy constant domain is selected from SEQ ID NO:s118, 119, 120, 121, 122, 123, 124, 125 and 126 and the human light kappa constant domain, SEQ ID NO:52.

[00189] In one embodiment, the variable light domain Ta1505-VL46_N33T (SEQ ID NO:112) is combined with the variable heavy domain Ta1505-VH11 (SEQ ID NO:116). In this embodiment, the human heavy constant domain is selected from SEQ ID NO:s118, 119, 120, 121, 122, 123, 124, 125 and 126 and the human light kappa constant domain, SEQ ID NO:52.

[00190] In one embodiment, the variable light domain Ta1505-VL46_N33T (SEQ ID NO:112) is combined with the variable heavy domain Ta1505-VH65 (SEQ ID NO:117). In this embodiment, the human heavy constant domain is selected from SEQ ID NO:s118, 119, 120, 121, 122, 123, 124, 125 and 126 and the human light kappa constant domain, SEQ ID NO:52.

[00191] In one embodiment, the variable light domain Ta1505-VL46_S28N (SEQ ID NO:113) is combined with the variable heavy domain Ta1505-VH11 (SEQ ID NO:116). In this embodiment, the human heavy constant domain is selected from SEQ ID NO:s118, 119, 120, 121, 122, 123, 124, 125 and 126 and the human light kappa constant domain, SEQ ID NO:52.

[00192] In one embodiment, the variable light domain Ta1505-VL46_S28N (SEQ ID NO:113) is combined with the variable heavy domain Ta1505-VH65 (SEQ ID NO:117). In this embodiment, the human heavy constant domain is selected from SEQ ID NO:s118, 119, 120, 121, 122, 123, 124, 125 and 126 and the human light kappa constant domain, SEQ ID NO:52.

[00193] In one embodiment, the variable light domain Ta1505-VL46_G34A_S28N (SEQ ID NO:114) is combined with the variable heavy domain Ta1505-VH11 (SEQ ID NO:116). In this embodiment, the human heavy constant domain is selected from SEQ ID NO:s118, 119, 120, 121, 122, 123, 124, 125 and 126 and the human light kappa constant domain, SEQ ID NO:52.

[00194] In one embodiment, the variable light domain Ta1505-VL46_G34A_S28N (SEQ ID NO:114) is combined with the variable heavy domain Ta1505-VH65 (SEQ ID NO:117). In this embodiment, the human heavy constant domain is selected from SEQ ID NO:s118, 119, 120, 121, 122, 123, 124, 125 and 126 and the human light kappa constant domain, SEQ ID NO:52.

[00195] The humanized anti-phosphorylated tau antibody according to the present invention should also preferably have a heavy chain variable region and a light chain variable region having specific amino acid sequences, specifically as mentioned below.

[00196] As a heavy chain variable region, the antibody of the present invention should preferably include an amino acid sequence having 80% or more, particularly 85% or more, more particularly 90% or more, even more particularly 95% or more, still even more particularly 100%, identity with a sequence selected from sequence VH11 (defined in SEQ ID NO:18), sequence VH12 (defined in SEQ ID NO:20), sequence VH47 (defined in SEQ ID NO:22), sequence VH61 (defined in SEQ ID NO:24), sequence VH62 (defined in SEQ ID NO:26), sequence VH64 (defined in SEQ ID NO:28), and sequence VH65 (defined in SEQ ID NO:30).

In one embodiment, an antibody of the present invention could include, as a heavy chain variable region, the amino acid sequence of sequence VH65, or an amino acid sequence derived from sequence VH65 by one or more substitutions selected from the group consisting of: substitution of Ala at position 28 (Kabat No.: H28) with Thr; substitution of Asn at position 30 (Kabat No.: H30) with Ser; substitution of Val at position 49 (Kabat No.: H49) with Gly; substitution of Ala at position 64 (Kabat No.: H61) with Asp; and substitution of Gln at position 78 (Kabat No.: H75) with Lys.

[00197] As a light chain variable region, the antibody of the present invention should preferably include an amino acid sequence having 80% or more, particularly 85% or more, more particularly 90% or more, even more particularly 95% or more, still even more particularly 100%, identity with a sequence selected from sequence VL15 (defined in SEQ ID NO:32), sequence VL36 (defined in SEQ ID NO:34), sequence VL46 (defined in SEQ ID NO:36), sequence VL47 (defined in SEQ ID NO:38), sequence VL48 (defined in SEQ ID NO:40), and sequence VL50 (defined in SEQ ID NO:42). In one embodiment, an antibody of the present invention could include, as a light chain variable region, the amino acid sequence of sequence VL47, or an amino acid sequence derived from sequence VL47 by one or more substitutions selected from the group consisting of: substitution of Asp at position 17 (Kabat No.: L17) with Glu; substitution of Ser at position 28 (Kabat No.: L27A) with Asn; substitution of Gln at position 42 (Kabat No.: L37) with Leu; substitution of Gln at position 50 (Kabat No.: L45) with Arg; and substitution of Arg at position 51 (Kabat No.: L46) with Leu.

[00198] In another embodiment, the humanized anti-phosphorylated tau antibody according to the present invention could include any combination selected from:

[00199] (1) sequence VH11 (SEQ ID NO:116) as a heavy chain variable region and sequence VL15 (SEQ ID NO:32) as a light chain variable region;

[00200] (2) sequence VH11 (SEQ ID NO:116) as a heavy chain variable region and sequence VL36 (SEQ ID NO:34) as a light chain variable region;

[00201] (3) sequence VH11 (SEQ ID NO:116) as a heavy chain variable region and sequence VL46 (SEQ ID NO:36) as a light chain variable region;

[00202] (4) sequence VH11 (SEQ ID NO:116) as a heavy chain variable region and sequence VL47 (SEQ ID NO:38) as a light chain variable region;

[00203] (5) sequence VH11 (SEQ ID NO:116) as a heavy chain variable region and sequence VL48 (SEQ ID NO:40) as a light chain variable region;

[00204] (6) sequence VH11 (SEQ ID NO:116) as a heavy chain variable region and sequence VL50 (SEQ ID NO:42) as a light chain variable region;

[00205] (7) sequence VH12 (SEQ ID NO:20) as a heavy chain variable region and sequence VL48 (SEQ ID NO:40) as a light chain variable region;

[00206] (8) sequence VH47 (SEQ ID NO:22) as a heavy chain variable region and sequence VL48 (SEQ ID NO:40_) as a light chain variable region;

[00207] (9) sequence VH61 (SEQ ID NO:24) as a heavy chain variable region and sequence VL48 (SEQ ID NO:40) as a light chain variable region;

[00208] (10) sequence VH62 (SEQ ID NO:26) as a heavy chain variable region and sequence VL48 (SEQ ID NO:40) as a light chain variable region;

[00209] (11) sequence VH64 (SEQ ID NO:28) as a heavy chain variable region and sequence VL47 (SEQ ID NO:38) as a light chain variable region;

[00210] (12) sequence VH64 (SEQ ID NO:28) as a heavy chain variable region and sequence VL48 (SEQ ID NO:40) as a light chain variable region;

[00211] (13) sequence VH65 (SEQ ID NO:117) as a heavy chain variable region and sequence VL47 (SEQ ID NO:37) as a light chain variable region;

[00212] By selecting amino acid sequences of CDRs and/or variable regions of a heavy chain and a light chain from those mentioned above and combining them with amino acid sequences of framework regions and/or constant regions of a heavy chain and a light chain of a human antibody as appropriate, a person skilled in the art will be able to design a humanized anti-phosphorylated tau antibody according to the present invention. Amino acid sequences of framework regions and/or constant regions of a heavy chain and a light chain of a human antibody can be selected from, e.g., those of human IgG, IgA, IgM, IgE, and IgD subtypes or variants thereof.

B. Specific anti-pSer413 tau Antibodies

[00213] The invention provides a number of specific antibodies comprising a pair of sequences, a heavy chain and a light chain as follows:

[00214] mAb-aPT1: LC SEQ ID NO:184 and HC SEQ ID NO:144

[00215] mAb-aPT2: LC SEQ ID NO:184 and HC SEQ ID NO:145

[00216] mAb-aPT3: LC SEQ ID NO:184 and HC SEQ ID NO:146

[00217] mAb-aPT4: LC SEQ ID NO:184 and HC SEQ ID NO:147

[00218] mAb-aPT5: LC SEQ ID NO:184 and HC SEQ ID NO:148

[00219] mAb-aPT6: LC SEQ ID NO:184 and HC SEQ ID NO:149

[00220] mAb-aPT7: LC SEQ ID NO:184 and HC SEQ ID NO:150

[00221] mAb-aPT8: LC SEQ ID NO:184 and HC SEQ ID NO:151

[00222] mAb-aPT9: LC SEQ ID NO:184 and HC SEQ ID NO:152

[00223] mAb-aPT10: LC SEQ ID NO:184 and HC SEQ ID NO:153

[00224] mAb-aPT11: LC SEQ ID NO:184 and HC SEQ ID NO:154

[00225] mAb-aPT12: LC SEQ ID NO:184 and HC SEQ ID NO:155

[00226] mAb-aPT13: LC SEQ ID NO:184 and HC SEQ ID NO:156

[00227] mAb-aPT14: LC SEQ ID NO:184 and HC SEQ ID NO:157

[00228] mAb-aPT15: LC SEQ ID NO:184 and HC SEQ ID NO:158

[00229] mAb-aPT16: LC SEQ ID NO:184 and HC SEQ ID NO:159

[00230] mAb-aPT17: LC SEQ ID NO:184 and HC SEQ ID NO:160

[00231] mAb-aPT18: LC SEQ ID NO:184 and HC SEQ ID NO:161

[00232] mAb-aPT19: LC SEQ ID NO:185 and HC SEQ ID NO:144

[00233] mAb-aPT20: LC SEQ ID NO:185 and HC SEQ ID NO:145

[00234] mAb-aPT21: LC SEQ ID NO:185 and HC SEQ ID NO:146

[00235] mAb-aPT22: LC SEQ ID NO:185 and HC SEQ ID NO:147

[00236] mAb-aPT23: LC SEQ ID NO:185 and HC SEQ ID NO:148

[00237] mAb-aPT24: LC SEQ ID NO:185 and HC SEQ ID NO:149

[00238] mAb-aPT25: LC SEQ ID NO:185 and HC SEQ ID NO:150

[00239] mAb-aPT26: LC SEQ ID NO:185 and HC SEQ ID NO:151

[00240] mAb-aPT27: LC SEQ ID NO:185 and HC SEQ ID NO:152

[00241] mAb-aPT28: LC SEQ ID NO:185 and HC SEQ ID NO:153

[00242] mAb-aPT29: LC SEQ ID NO:185 and HC SEQ ID NO:154

[00243] mAb-aPT30: LC SEQ ID NO:185 and HC SEQ ID NO:155

[00244] mAb-aPT31: LC SEQ ID NO:185 and HC SEQ ID NO:156

[00245] mAb-aPT32: LC SEQ ID NO:185 and HC SEQ ID NO:157

[00246] mAb-aPT33: LC SEQ ID NO:185 and HC SEQ ID NO:158

[00247] mAb-aPT34: LC SEQ ID NO:185 and HC SEQ ID NO:159

[00248] mAb-aPT35: LC SEQ ID NO:185 and HC SEQ ID NO:160

[00249] mAb-aPT36: LC SEQ ID NO:185 and HC SEQ ID NO:161

C. Antibodies that Compete for Binding

[00250] In addition to the antigen binding domains and antibodies containing them that bind to human tau protein that is phosphorylated at position 413 (pSer413), the invention provides antibodies that compete for binding with the recited antibodies herein.

[00251] A humanized antibody which causes competitive binding to pSer413 tau with the humanized anti-phosphorylated tau antibody according to the present invention is also included in the scope of the present invention. The term "competitive binding" used herein means that when there are two or more monoclonal antibodies together with an antigen, the binding of one of the antibodies to the antigen is inhibited by the binding of the other antibody to the antigen. The competitive binding can usually be measured by, e.g., adding, to a constant amount (concentration) of a monoclonal antibody, another monoclonal antibody with varying the amount (concentration) thereof, and determining the amount (concentration) of the latter monoclonal antibody at which the binding amount of the former monoclonal antibody, existing in the constant amount, is decreased. The degree of inhibition thereof can be expressed in the unit of IC50 or Ki. The humanized antibody which causes competitive binding with the humanized anti-phosphorylated tau antibody according to the present invention means an antibody having a IC50 of 1μM or less, particularly 100nM or less, more particularly 10nM or less when measuring an antigen-antibody binding using the humanized anti-phosphorylated tau antibody according to the present invention at 10nM.

[00252] Competitive binding studies can be done as is known in the art, generally using SPR/Biacore® or Octet® binding assays, as well as ELISA and cell based assays.

[00253] In some embodiments, the variable heavy and variable light domains of the competitive binding antibodies have at least 85%, 90%, 95%, 98% or 99% identity to at least one of the variable regions outlined herein, and in the constant domains, have at least 99 or 100% identity to a human IgG sequence outlined in Figure 13.

[00254]

D. Amino Acid Substitutions

[00255] In addition to the sequences outlined herein, the invention provides antigen binding domains and antibodies containing them that can have one or more amino acid substitutions as compared to the parent, starting sequence.

[00256] Thus for example, for all of the variable heavy and light domains listed herein, further variants can be made. As outlined herein, in some embodiments the set of 6 CDRs can have from 0, 1, 2, 3, 4 or 5 amino acid modifications (with amino acid substitutions finding particular use). In these embodiments, generally a single CDR has no more than 1 or 2 amino acid substitutions, and the mutated sequences retain binding to pSer413 tau protein. However, when amino acid variants are made to the CDRs of the sequences outlined herein, the resulting CDRs are not to the murine CDRs outlined in PCT/JP13/65090 and shown in SEQ ID NOs:81, 82 and 83 (variable light CDRs) and SEQ ID NOs:86, 87 and 88 (variable heavy CDRs).

[00257] In some cases, changes in the framework region(s) of the variable heavy and/or light domains can be made. In this embodiment, preferred variants in the framework regions (e.g. excluding the CDRs) retain at least about 80, 85, 90 or 95% identity to a human germline sequence. Thus, for example, the identical CDRs as described herein can be combined with different framework sequences from human germline sequences, as long as the framework regions retain at least 80, 85, 90 or 95% identity to a human germline sequence.

[00258] In some embodiments, for the humanized light chains outlined herein, the closest human light chain germline is IGKV2-30 that has an 81% identity to the sequences herein. Thus additional variants can be made in light framework regions as long as the identity is at least 80%, 85%, 90% or 95% identity to IGKV2-30.

[00259] For the humanized heavy chains herein, the closest human germline for heavy chain is IGHV3-73 that has an 85% identity to the sequences herein. Thus additional variants

can be made in light framework regions as long as the identity is at least 80%, 85%, 90% or 95% identity to IGHV3-73.

[00260] Alternatively, the CDRs can have amino acid modifications (e.g. from 1, 2, 3, 4 or 5 amino acid modifications in the set of CDRs (that is, the CDRs can be modified as long as the total number of changes in the set of 6 CDRs is less than 6 amino acid modifications, with any combination of CDRs being changed; e.g. there may be one change in vlCDR1, two in vhCDR2, none in vhCDR3, etc.)), as well as having framework region changes, as long as the framework regions retain at least 80, 85 or 90% identity to a human germline sequence, and the resulting CDRs are not to the murine CDRs outlined in PCT/JP13/65090 and shown in SEQ ID NOs:81, 82 and 83 (variable light CDRs) and SEQ ID NOs:86, 87 and 88 (variable heavy CDRs).

[00261] In general, the percentage identity for comparison between anti-pSer413 tau antibodies is at least 75%, at least 80%, at least 90%, with at least about 95, 96, 97, 98 or 99% percent identity being preferred. The percentage identity may be along the whole amino acid sequence, for example the entire heavy or light chain or along a portion of the chains. For example, included within the definition of the anti-pSer413 tau antibodies of the invention are those that share identity along the entire variable region (for example, where the identity is 95 or 98% identical along the variable regions), or along the entire constant region.

X. Nucleic Acids of the Invention

[00262] Nucleic acid compositions encoding the anti-pSer413 tau antibodies of the invention are also provided, as well as expression vectors containing the nucleic acids and host cells transformed with the nucleic acid and/or expression vector compositions. As will be appreciated by those in the art, the protein sequences depicted herein can be encoded by any number of possible nucleic acid sequences, due to the degeneracy of the genetic code.

[00263] Thus, the invention further provides nucleic acid compositions encoding the antigen binding domains and antibodies containing them. As will be appreciated by those in the art, in the case of antigen binding domains, the nucleic acid compositions generally include a first nucleic acid encoding the variable heavy domain and a second nucleic acid encoding the variable light domain. In the case of scFvs, a single nucleic acid encoding the variable heavy and variable light domain, separated by a proteinaceous linker, can be made. In the case of traditional antibodies, the nucleic acid compositions generally include a first nucleic acid

encoding the heavy chain and a second nucleic acid encoding the light chain, which will, upon expression in a cell, spontaneously assemble into the "traditional" tetrameric format of two heavy chains and two light chains.

[00264] As is known in the art, the nucleic acids encoding the components of the invention can be incorporated into expression vectors as is known in the art, and depending on the host cells, used to produce the heterodimeric antibodies of the invention. As will be appreciated by those in the art, these two nucleic acids can be incorporated into a single expression vector or into two different expression vectors. Generally, the nucleic acids are operably linked to any number of regulatory elements (promoters, origin of replication, selectable markers, ribosomal binding sites, inducers, etc.). The expression vectors can be extra-chromosomal or integrating vectors.

[00265] The nucleic acids and/or expression vectors of the invention are then transformed into any number of different types of host cells as is well known in the art, including mammalian, bacterial, yeast, insect and/or fungal cells, with mammalian cells (e.g. CHO cells), finding use in many embodiments.

[00266] The antibodies of the invention are made by culturing host cells comprising the expression vector(s) as is well known in the art. Once produced, traditional antibody purification steps are done.

XI. Biological and Biochemical Assays

[00267] The anti-pSer413 tau antibodies of the invention can be assayed for efficacy in several ways. As a preliminary matter, the antibodies can be tested for binding to human tau protein or peptides that are phosphorylated at position S413 using techniques known in the art such as ELISA techniques (see for example Example 2(1)), Octet, Biacore or FACS, where binding to phosphorylated peptides (e.g. SEQ ID NO:8) is compared to binding to non-phosphorylated peptides (e.g. SEQ ID NO:69) as outlined herein.

[00268] In some embodiments, the binding efficacies of the antibodies disclosed herein are measured by Biacore assay. In certain embodiments, the K_D value is measured with a phosphorylated Tau protein. In some embodiments, the K_D value is measured with a phosphorylated Tau peptide. In one embodiment, the phosphorylated Tau peptide is phosphorylated at only one residue (*e.g.*, SEQ ID NO:75). In another embodiment, the phosphorylated peptide is phosphorylated at multiple residues (*e.g.*, SEQ ID NO:76 or SEQ ID

NO:78). In some embodiments, the K_D value is measured with the antigen (*e.g.*, the phosphorylated Tau protein or the phosphorylated Tau peptide) immobilized, in which cases the affinity measurement includes an avidity component, *i.e.*, in a bivalent binding mode. In other embodiments, the K_D value is measured with the antibody (*e.g.*, the mouse parent antibody, the chimeric antibody, or the humanized antibody variants) immobilized, in which case the affinity measurement does not include an avidity component, *i.e.*, in a monovalent binding mode. In yet other embodiments, the K_D value is measured with the antibody immobilized and with the phosphorylated Tau protein as the analyte. In still other embodiments, the K_D value is measured with the antibody immobilized and with the phosphorylated peptide as the analyte. In certain embodiments, the K_D value is measured in a bivalent binding mode. In other embodiments, the K_D value is measured in a monovalent binding mode.

[00269] In addition, the anti-pSer413 tau antibodies of the invention can also be assayed for binding affinities in human AD brain homogenates, as well as assayed in neutralization studies of AD-derived au seeds in a HEC293 Tau P301L aggregation assay, and/or in AD-derived tau seeding and spreading models using human iPSC neurons in microfluidic chambers.

[00270] Additionally, the anti-pSer413 tau antibodies of the invention can also be used in immunohistochemical assays of AD patient brain samples

[00271] Additionally, the anti-pSer413 tau antibodies of the invention can be assayed for efficacy in animal models of cognitive disorders including Alzeheimer's Disease (AD). Animals for research on therapeutic or prophylactic agents for cognitive disorders of the invention include animal models for cognitive disorders, specifically animal models of tauopathies. Animal models for tauopathies are animals expressing normal-type or mutant tau in the brain, and particularly animal models exhibiting impairment in cognitive function. Such animals expressing normal-type or mutant tau in the brain can be prepared by genetic engineering, a typical example being transgenic mice (Tg mice). Animal models such as Tg mice that express mutant tau are useful as models of genetic familial tauopathies, but for examination of the effect of a therapeutic agent or prophylactic agent for sporadic tauopathies that constitute the majority of cases among humans, preferably an effect is exhibited in Tg mice expressing normal-type tau. Most suitable as Tg mice expressing normal-type tau are mice prepared in the production examples of the present invention, but there may also be used the Tg mice reported by Kambe et al. (Neurobiology of Disease, Vol.42, P.404-414, 2011) and Kimura et al. (The EMBO J. vol.26. P.5143-5152, 2007), both of which are incorporated herein by reference in their entirety and

specifically for the creation and use of transgenic mice. However, although cognitive function impairment is seen in the mice of Kambe et al. and Kimura et al., it appears after 14 months of age and 20 months of age, respectively, and therefore the onset is well into senescence and aging effects may also be contributing factors, while the effects and labor of long-term breeding are also issues.

[00272] The preferred method of examining the effect of a therapeutic agent or prophylactic agent for cognitive disorders according to the invention in an animal model is a method of testing cognitive function, such as a memory learning test. Such a method may be a Morris water maze test, a step-through learning test or a novel object recognition test, but preferably it is a combination of behavioral measurement tests such as an Open Field Test, in order to take into account the conditions of behavior quantity and animal anxiety.

[00273] As methods for examining the effect of a therapeutic agent or prophylactic agent for cognitive disorders according to the invention, it is possible to use methods of examining the levels of tau protein or phosphorylated tau in brain tissue, during administration to an animal model of the cognitive disorder. In AD and other neurodegenerative diseases, expression levels of tau protein or increased abnormal phosphorylated tau are associated with pathology (Khalid Iqbal et al., Curr. Alzheimer Res., Vol.7, p654-664, 2010). It is also well known that reducing tau expression and abnormal phosphorylated tau levels in some pathological model animals leads to improvement in cognitive function and motor function (K. Santa Cruz et al., Science, 30, Vol.9, p.476-481, 2005; Sylvie Le Corre et al., Proc. Nat. Acad. Sci. USA, Vol.103, p.9673-9678, 2006). As a method of examining changes in tau protein or phosphorylated tau, this can be accomplished by a method such as Western blotting using a brain tissue homogenate, as described in the examples, but a person skilled in the art can select another appropriate method such as ELISA (Xiyun Chai et al., J. Biol. Chem., Vol.286, p.34457-34467, 2011) or an immunohistochemical method (David J. Irwin et al., BRAIN, Vol.135, p.807-818, 2012).

XII. Formulations

[00274] Formulations of the antibodies used in accordance with the present invention are prepared for storage by mixing an antibody having the desired degree of purity with optional pharmaceutically acceptable carriers, excipients or stabilizers (as generally outlined in Remington's Pharmaceutical Sciences 16th edition, Osol, A. Ed. [1980]), in the form of lyophilized formulations or aqueous solutions.

[00275] The therapeutic or prophylactic agent for cognitive disorders according to the present invention may be formulated in the form of a pharmaceutical composition containing, in addition to the humanized anti-phosphorylated tau antibody according to the present invention, a pharmaceutically acceptable carrier and/or other excipients. Formulation using a pharmaceutically acceptable carrier and/or other excipients can be carried out using the method described in, e.g., University of the Sciences in Philadelphia, "Remington: The Science and Practice of Pharmacy, 20th EDITION", Lippincott Williams & Wilkins, 2000. Such a therapeutic or prophylactic agent may be provided as a liquid formulation prepared by dissolving, suspending, or emulsifying the ingredients into sterile aqueous medium or oily medium, or as a lyophilized formulation thereof. A medium or solvent for preparing such a formulation may be an aqueous medium, examples of which include distilled water for injection and physiological saline solution, which may optionally be used with addition of an osmoregulating agent (e.g., D-glucose, D-sorbitol, D-mannitol, and sodium chloride), and/or in combination with a suitable dissolving aid such as an alcohol (e.g., ethanol), a polyalcohol (e.g., propylene glycol or polyethylene glycol), or a nonionic surfactant (e.g., polysorbate 80 or polyoxyethylene hydrogenated castor oil 50). Such a formulation can also be prepared with an oily medium or solvent, examples of which include sesame oil and soybean oil, which can optionally be used in combination with a dissolving aid such as benzyl benzoate and benzyl alcohol. Such liquid drugs may often be prepared using appropriate additives such as buffering agents (e.g., phosphate buffering agents and acetate buffering agents), soothing agents (e.g., benzalkonium chloride and procaine hydrochloride), stabilizers (e.g., human serum albumin and polyethylene glycol), preservatives (e.g., ascorbic acid, erythorbic acid, and their salts), coloring agents (e.g., copper chlorophyll β-carotene, Red #2 and Blue #1), antiseptic agents (e.g., paraoxybenzoic acid esters, phenol, benzethonium chloride and benzalkonium chloride), thickeners (e.g., hydroxypropyl cellulose, carboxymethyl cellulose, and their salts), stabilizers (e.g., human serum albumin mannitol and sorbitol), and odor correctives (e.g., menthol and citrus aromas). Different forms of therapeutic or prophylactic agents include solid formulations such as powders, tablets, granules, capsules, pills, suppositories, and lozenges. For a solid formulation to be administered in oral form, there may be used additives such as excipients (e.g., crystalline cellulose, lactose and starch), lubricants (e.g., magnesium stearate and talc), binders (hydroxypropyl cellulose, hydroxypropyl methyl cellulose, macrogol and the like), and disintegrators (e.g., starch and carboxymethyl cellulose calcium). If necessary, additives such as antiseptic agents (e.g., benzyl alcohol, chlorobutanol, methyl paraoxybenzoate and propyl

paraoxybenzoate), antioxidants, coloring agents, sweeteners and the like may be used. Other alternative forms include therapeutic agents or prophylactic agent for application onto mucous membranes, such formulations often containing additives such as pressure-sensitive adhesives, pressure-sensitive enhancers, viscosity regulators, thickening agents and the like (e.g., mucin, agar, gelatin, pectin, carrageenan, sodium alginate, locust bean gum, xanthan gum, tragacanth gum, gum arabic, chitosan, pullulan, waxy starch, sucralfate, cellulose and its derivatives (such as hydroxypropyl methyl cellulose), polyglycerol fatty acid esters, acrylic acid-alkyl (meth)acrylate copolymers, or their salts and polyglycerol fatty acid esters), primarily for the purpose of imparting mucosal adsorption or retention properties. However, the form, solvent and additives for the therapeutic agent or prophylactic agent to be administered to the body are not limited to these, and appropriately selection may be made by a person skilled in the art.

[00276] The therapeutic or prophylactic agent for cognitive disorders according to the present invention may contain, in addition to the humanized anti-phosphorylated tau antibody according to the present invention, other known drugs having the effect of treating or preventing cognitive disorders, and in particular drugs having the effect of inhibiting the progression of cognitive disorders. Alternatively, the therapeutic or prophylactic agent for cognitive disorders according to the present invention containing the humanized anti-phosphorylated tau antibody according to the present invention may be combined with another therapeutic or prophylactic agent containing a known drug having the effect of treating or preventing cognitive disorders, and in particular a drug having the effect of inhibiting the progression of cognitive disorders, to be made in the form of a kit. Ingredients having the effects of inhibiting the progression of cognitive disorders include, but not limited to, Donepezil, Galantamine, Memantine, and Rivastigmine. The dosage of the ingredient having the effect of inhibiting the progression of cognitive disorders and the dosage of the therapeutic or prophylactic agent containing the ingredient having the effect of inhibiting the progression of cognitive disorders may be within the scope of dosages used for normal therapy, but can be increased or decreased depending on the conditions.

[00277] While the present inventors indicated experiment results, in WO2013/180238A (Patent Document 4), which demonstrate that the antibody exhibits a drug effect by acting on the brain through the blood-brain barrier even when administered peripherally by intraperitoneal administration, it is also possible to prepare a formulation that efficiently supplies the humanized anti-phosphorylated tau antibody according to the present invention, which is contained in the therapeutic or prophylactic agent for cognitive disorders according to the

present invention, to the brain tissue. Such formulations are also encompassed by the therapeutic or prophylactic agent for cognitive disorders according to the invention. Methods for efficiently supplying antibodies or peptides to brain tissue through the blood-brain barrier are known, such as methods of adding targeting substances or methods of encapsulating in liposomes or nanoparticles. Substances to be used for targeting include those that undergo total or partial change in charge characteristics by binding with antibody, or peptides, proteins or other compounds having a property of binding with a specific receptor or transporter. Examples of peptides, proteins or other compounds having a property of binding with a specific receptor or membrane protein include ligands that bind to receptor ligands or membrane proteins, and their analogs, and antibodies, agonist compounds/antagonist compounds/allosteric modulators that bind to receptor ligands or membrane proteins, and their analog compounds. Examples of receptor ligands or membrane proteins as targets for a peptide, protein or other compound having the property of binding to a specific receptor or transporter include transferrin receptor (TfR), insulin receptor (IR), insulin-like growth factor receptor (IGFR), LDL receptor-related protein (LRP) and diphtheria toxin receptor (HB-EGF), with no particular limitation to these (Angela R. Jones et al., Pharm. Res., 2007, Vol.24, No.9, pp.1759-1771). A substance for targeting may be chemically added to the antibody to be used for the therapeutic or prophylactic agent for cognitive disorders according to the invention, the method being one that can be appropriately carried out by a person skilled in the art with reference to a known method such as described in, for example, Hermanson et al., Bioconjugate techniques, USA, Academic Press, 1996. The substance for targeting may also be bound to liposomes or nanoparticles encapsulating the antibody or peptide (Sonu Bhaskar et al., Particle and Fibre Toxicology, 2010, 7:3). In addition, when the substance for targeting is a peptide or protein, it can be produced as an appropriate fusion protein by a person skilled in the art using genetic engineering techniques, either by producing a fusion peptide by peptide chemical synthesis, or by combining a nucleic acid comprising a nucleotide sequence coding for the amino acid sequence of the peptide or protein with a nucleic acid comprising a nucleotide sequence coding for the amino acid sequence for the antibody or peptide to be used.

[00278] The therapeutic or prophylactic agent according to the present invention may be administered to a patient in need thereof orally or parenterally, for the purpose of improving symptoms. For oral administration, a dosage form such as granules, powder, tablets, capsules, liquid drug, syrup, emulsion, suspending agent or elixir may be selected. For parenteral administration, a transnasal agent may be prepared, and a liquid drug, suspension or solid

formulation may be selected. An injection may be prepared as a different form of parenteral administration, the injection being selected as subcutaneous injection, intravenous injection, infusion, intramuscular injection, intracerebroventricular injection or intraperitoneal injection. Other formulations used for parenteral administration include suppositories, sublingual agents, percutaneous agents and transmucosal administration agents other than transnasal agents. In addition, intravascular local administration is possible by a mode of addition or coating onto a stent or intravascular obturator.

[00279] The dose for an agent for treatment or prevention according to the invention will differ depending on the patient age, gender, body weight and symptoms, the therapeutic effect, the method of administration, the treatment time, or the types of active ingredients in the medical composition, but normally it may be administered in the range of 0.1 mg to 1 g and preferably in the range of 0.5 mg to 200 mg of active compound per administration for adults. However, since the dose will vary depending on a variety of conditions, lower doses than those mentioned above may be sufficient, or doses exceeding these ranges may be necessary. The agent for treatment or prevention according to the invention can exhibit an effect within a short administration period.

[00280]

XIII. Administration of Antibodies

[00281] Once made, the antibodies of the invention find use in the treatment of cognitive disorders such as tauopathies. As discussed herein, the intracellular accumulation of tau protein has been demonstrated in a variety of neuropathological conditions. The diseases caused by such intracellular accumulation of tau are collectively referred to as "tauopathies" (see Arai, supra, hereby incorporated by reference in its entirety and for the disorders outlined therein). Tauopathies include neurodegenerative diseases such as Alzheimer's disease (AD), corticobasal degeneration (CBD) or corticobasal syndrome (CBS), progressive supranuclear palsy, Pick's disease, argyrophilic grain dementia (argyrophilic grain disease), multiple system tauopathy with dementia (MSTD), chromosome 17-linked frontotemporal dementia with Parkinsonism (FTDP-17), neurofibrillary tangle dementia, diffuse neurofibrillaty tangles with calcification (DNTC), white matter tauopathy with globular glial inclusions (WMT-GGI), frontotemporal lobar degeneration with tau-positive inclusions (FTLD-tau). Tauopathies also include non-neurodegenerative diseases including: infectious diseases such as Economo's encephalitis

sequela and subacute sclerosing panencephalitis; and trauma-induced conditions such as boxer's encephalopathy.

[00282] The cognitive disorders are defined as a type of intellectual impairment which involves a state where once developed or acquired intellectual function has declined ("New Psychiatry (Nankodo's Essential Well-Advanced Series)" (Japanese book), edited by Kunitoshi Kamijima and Shin-ichi Niwa, Nankodo Co., Ltd., 2008, pp.69-70), but are deemed in a broad sense to be diseases exhibiting intellectual impairment and/or memory impairment. In the diagnosis of neurodegenerative diseases such as AD, whether the condition is "neurodegenerative" is determined by, e.g., analyzing as to whether neurofibrillary tangle (NFT) exists via histological analysis after death, while physicians diagnose cognitive disorders, especially as neurodegenerative diseases, by using various means, e.g.: neuropsychological examinations via inquiries such as Hasegawa's dementia scale for revised (HDS-R) and mini-mental state examination (MMSE); neuropsychological examinations via observations such as clinical dementia rating (CDR) or functional assessment staging (FAST); biochemical examinations based on, e.g., increase in the levels of tau and phosphorylated tau or increase in the level of Aβ in cerebrospinal fluid; and image inspection based on information obtained via, e.g., head CT, head MRI, SPECT, or PET. The therapeutic or prophylactic agent for cognitive disorders according to the present invention is administered to a patient whom a physician has diagnosed as suffering from a cognitive disorder, and has the effect of improving the condition of the patient compared to the state before administration, controlling the progression of the condition via administration, or maintaining the condition at, or recovering the condition to, the state before administration, based on at least one of the diagnosis indexes for neurodegenerative disease

[00283] The therapeutic or prophylactic agent for cognitive disorders according to the present invention may also have the effect of improving cognitive function, or inhibiting a decline in cognitive function, or maintaining cognitive function, in a human or non-human animal. Examples of such non-human animals should preferably be those expressing tau having high homology with human tau, and include, although not limited to: chimpanzee, macaque, horse, pig, dog, mouse, rabbit, rat, and cat.

XIV. Examples

A. Example 1: Production of Antibody

1. Mouse antibody (parent antibody)

[00284] Mouse antibody Ta1505 was used as a parent antibody of a chimeric antibody and a humanized antibody. The details of mouse antibody Ta1505 are described in the examples of International Publication No. WO2013/180238A. The amino acid sequence of the light chain (L chain) of mouse antibody Ta1505 is defined in SEQ ID NO: 44, and the nucleotide sequence encoding it is defined in SEQ ID NO: 45. The amino acid sequence of the heavy chain (H chain) of mouse antibody Ta1505 is defined in SEQ ID NO: 46, and the nucleotide sequence encoding it is defined in SEQ ID NO: 47.

2. Chimeric antibody

[00285] The chimeric antibody was produced by combining the variable regions of the light chain (L chain) and the heavy chain (H chain) of mouse antibody Ta1505 with, respectively, the constant regions of the L chain and the H chain of human immunoglobulin (Ig) G1(κ). The amino acid sequence of the light chain (L chain) of the chimeric antibody is defined in SEQ ID NO: 48, and the nucleotide sequence encoding it is defined in SEQ ID NO: 49. The amino acid sequence of the heavy chain (H chain) of the chimeric antibody is defined in SEQ ID NO: 50, and the nucleotide sequence encoding it is defined in SEQ ID NO: 51. Throughout the specification, this chimeric antibody is referred to as "the chimeric antibody" or "chimeric antibody Ta1505".

3. Humanized antibody

a. Design of VL and VH

[00286] Primary sequences having high homology were selected from the human germline, and humanization was carried out by complementarity determining region (CDR) grafting to design a variety of humanized antibodies.

[00287] In detail, human frameworks having high homology were selected from a database; the CDR region of mouse antibody Ta1505 was inserted into each of the frameworks; and amino acid substitutions were carried out for, e.g., reducing immunogenicity while maintaining the activity. Amino acid sequences of various light-chain variable regions (VLs) and heavy-chain variable regions (VHs) were thereby designed. The SEQ ID Numbers of the amino acid sequences of VLs and the SEQ ID Numbers of the nucleotide sequences of genes encoding them are shown in Table 2A, and the SEQ ID Numbers of the amino acid sequences of VHs and the nucleotide sequences of genes encoding them are shown in Table 2B. The

alignments of the amino acid sequences of VLs and VHs are shown in Fig. 2A and Fig. 2B, respectively.

Table 2A: Light-chain variable region (VL)

Code name	Amino acid sequence	Nucleotide sequence
VL15	SEQ ID NO: 32	SEQ ID NO: 33
VL36	SEQ ID NO: 34	SEQ ID NO: 35
VL46	SEQ ID NO: 36	SEQ ID NO: 37
VL47	SEQ ID NO: 38	SEQ ID NO: 39
VL48	SEQ ID NO: 40	SEQ ID NO: 41
VL50	SEQ ID NO: 42	SEQ ID NO: 43

Table 2B: Heavy-chain variable region (VH)

Code name	Amino acid sequence	Nucleotide sequence
VH11	SEQ ID NO: 18	SEQ ID NO: 19
VH12	SEQ ID NO: 20	SEQ ID NO: 21
VH47	SEQ ID NO: 22	SEQ ID NO: 23
VH61	SEQ ID NO: 24	SEQ ID NO: 25
VH62	SEQ ID NO: 26	SEQ ID NO: 27
VH64	SEQ ID NO: 28	SEQ ID NO: 29
VH65	SEQ ID NO: 30	SEQ ID NO: 31

b. Selection of combination of VL and VH based on immunogenicity score

[00288] The VLs and VHs designed by the above-described procedure were combined arbitrarily, and the immunogenicity score (DRB1 score) of each combination was calculated by in vitro assay software Epibase (Lonza). The combinations of VL and VH to be used in the subsequent experiments were selected from the combinations showing low immunogenicity with immunogenicity scores equal to or lower than a predetermined value (DRB1 score: 1500). The selected combinations of VL and VH are shown in Table 3A, and the immunogenicity scores (DRB1 scores) of these combinations are shown in Table 3B.

Table 3A: Selected combinations of VL and VH

		Heavy chain variable region (VH)						
		H11	H12	H47	H61	H62	H64	H65
Light chain variable region (VL)	L15	L15H11						
	L36	L36H11						
	L46	L46H11						
	L47	L47H11					L47H64	L47H65
	L48	L48H11	L48H12	L48H47	L48H61	L48H62	L48H64	
	L50	L50H11						

Table 3B: Immunogenicity score (DRB1 score)

	Heavy chain variable region (VH)						
	H11	H12	H47	H61	H62	H64	H65

Light chain variable region (VL)	L15	1495						
	L36	1437						
	L46	1463						
	L47	1333					1382	1426
	L48	1417	1352	1433	1383	1433	1466	
	L50	1475						

c. Expression culture and purification of humanized antibody

[00289] For each combination of VL and VH selected by the above-described procedure, genes encoding the amino acid sequences of the VL and the VH were prepared. The VL gene was fused in-frame to a human immunoglobulin kappa L-chain constant region (CL) gene (the amino acid sequence and the nucleotide sequence thereof are defined in SEQ ID NO: 52 and SEQ ID NO: 53, respectively), and the VH gene was fused in-frame to a human immunoglobulin gamma-1 H-chain constant region (CH) gene (the amino acid sequence and the nucleotide sequence thereof are defined in SEQ ID NO: 54 and SEQ ID NO: 55, respectively), such that no mutation or defect should occur at each binding site. The 5'-ends of the VL gene and the VH gene were fused in-frame to, respectively, a signal sequence derived from the human immunoglobulin kappa L-chain gene (the amino acid sequence and the nucleotide sequence thereof are defined in SEQ ID NO: 56 and SEQ ID NO: 57, respectively) and a signal sequence derived from the human immunoglobulin gamma-1 H-chain gene (the amino acid sequence and the nucleotide sequence thereof are defined in SEQ ID NO: 58 and SEQ ID NO: 59, respectively), such that no mutation or defect should occur at each binding site.

[00290] The nucleic acid constructs encoding, respectively, the signal sequence-fused L and H chains designed by the above-described procedure were each incorporated into an expression vector for animal cell expression, and then cultured using ExpiCHO (manufactured by Thermo Fisher Scientific K.K.) according to the standard protocol to express antibodies. Each culture was clarified by centrifugation, and the resultant culture supernatant was applied to a protein A affinity column to purify the humanized antibody. The fraction eluted with an acidic solution having a pH of 2.8 to 3.5 was rapidly neutralized, concentrated, and applied to a gel filtration column to remove aggregate. The buffer was changed to phosphate buffered saline (PBS) to collect the main fraction, which was diluted with a solvent into an appropriate concentration and then used in the following tests.

[00291] In the following descriptions, each humanized antibody produced by the above-described procedure may be abbreviated using a combination of the code names of its VL and

VH. For example, a humanized antibody produced using L15 as the VL and H11 as the VH is referred to as L15H11.

[00292] In the following tests, 3% BSA/PBS was used as a solvent for adjusting the concentration of an antibody, without otherwise specified.

B. Example 2: ELISA analysis of antigen peptide binding affinity

1. ELISA analysis of binding affinity to antigen peptide

[00293] Some of the humanized antibodies having combinations of VL and VH shown in Table 3A were analyzed for the binding affinity to an antigen peptide by enzyme-linked immunosorbent assay (ELISA).

[00294] A non-phosphorylated tau peptide PD17(Ser413) and a phosphorylated tau peptide PD17P(pSer413), in which Ser413 was phosphorylated, were each diluted with cooled PBS to 1 μg/mL, and the resultant solutions were each dispensed to a plate at 50 μL/well and allowed to stand at 4°C overnight. The solution was removed, and a blocking buffer (3% bovine serum albumin (BSA)-PBS) was then dispensed at 270 μL/well, and the plate was allowed to stand at 4°C overnight. After the solution was removed, an antibody solution was diluted in a stepwise fashion with 3% BSA-PBS was then added to the plate at 50 μL/well to allow a reaction at room temperature for 90 minutes. The amino acid sequences of non-phosphorylated tau peptide PD17(Ser413) and phosphorylated tau peptide PD17P(pSer413) are defined in SEQ ID NO: 60 and SEQ ID NO: 8, respectively.

[00295] Each well was washed with Tris-buffered saline with Tween 20 (TBS-T), which contains 0.05% of Tween 20. A goat anti-human IgG-alkaline phosphatase label (manufactured by Sigma-Aldrich Co. LLC.) diluted by 2000-fold with a dilution buffer (3% BSA-PBS) was added to the plate at 50 μL/well to allow a reaction at room temperature for 60 minutes. After washing with TBS-T, a chromogenic solution (1 mg/mL p-nitrophenylphosphate (pNPP) solution) was added to the plate at 100 μL/well for color development for 40 minutes. The absorbance (optical density (OD) value) was measured at a wavelength of 405 nm.

[00296] The reactivity was evaluated by determining the ratio of the OD value of each antibody to the OD value of 0.25 nM chimeric antibody, and rating the resultant ratio on the following three-point scale:

[00297] +: sufficient reactivity ($0.6 \leq$ OD ratio),

[00298] ±: very slight reactivity ($0.15 <$ OD ratio < 0.6), and

[00299] -: no reactivity (OD ratio ≤ 0.15).

[00300] Table 4A shows the evaluation results of the reactivity of the antibodies with phosphorylated tau peptide PD17P. Table 4B shows the evaluation results of the reactivity of the antibodies with non-phosphorylated tau peptide PD17. Compared to the chimeric antibody, all the humanized antibodies exhibited higher reactivity with phosphorylated tau peptide PD17P, but lower reactivity with non-phosphorylated tau peptide PD17. These results demonstrate that the humanized antibodies have selective reactivity with the phosphorylated tau peptide.

Table 4A: ELISA analysis of reactivity with phosphorylated peptide PD17P

		Heavy chain variable region (VH)						
		VH11	VH12	VH47	VH61	VH62	VH64	VH65
Light chain variable region (VL)	VL15	+						
	VL36	+						
	VL46	+						
	VL47							+
	VL48	+	+	+	+	+	+	
	VL50	+						

Table 4B: ELISA analysis of reactivity with non-phosphorylated peptide PD17

		Heavy chain variable region (VH)						
		VH11	VH12	VH47	H61	VH62	VH64	VH65
Light chain variable region (VL)	VL15	-						
	VL36	-						
	VL46	-						
	VL47							-
	VL48	-	-	-	-	-	-	
	VL50	-						

2. ELISA analysis of selective binding affinity to phosphorylated tau peptide

[00301] Some of the humanized antibodies having combinations of VL and VH shown in Table 3A were analyzed for the selective binding affinity of the anti-tau humanized antibodies to a phosphorylated tau peptide by enzyme-linked immunosorbent assay (ELISA), using chimeric antibody Ta1505 as a reference antibody.

[00302] A solution containing 1 μg/mL of non-phosphorylated peptide PD17 or phosphorylated peptide PD17P was dispensed to a plate at 50 μL/well, and allowed to stand at 4°C overnight. After the solution was removed, a blocking buffer (3% bovine serum albumin (BSA)-PBS) was dispensed at 270 μL/well, and the plate was allowed to stand at 4°C overnight, to thereby produce plates in which non-phosphorylated peptide PD17 or phosphorylated peptide PD17P was immobilized on each well.

[00303] A series of five concentrations of each antibody were prepared by 4-fold serial dilution, starting from an initial concentration of 0.6 μg/mL (4 nM). Each solution was added to a well of the plates at 50 μL/well to allow a reaction at room temperature for one hour. After washing process, a goat anti-human IgG-alkaline phosphatase label (manufactured by Sigma-Aldrich Co. LLC.) was diluted by 2000-fold, and then added to each well at 50 μL/well to further allow a reaction for one hour.

[00304] After washing process, 1 mg/mL p-nitrophenylphosphate (pNPP) solution was added to each well at 100 μL/well to allow a reaction for 20 minutes. The absorbance (OD) was measured at a wavelength of 405 nm.

[00305] The data of the absorbance OD at the respective concentrations of each prepared antibody were analyzed with SoftMax Pro software Ver.6.5 (Molecular Devices Corporation) to determine the following numeric values A to D, which were assigned to the four parameter logistic regression curve shown below:

[00306] $$y = D + \frac{A - D}{1 + \left(\frac{x}{C}\right)^{B}}$$ (Equation I)

where

x represents the concentration of the antibody, and

y represents the absorbance (OD value).

[00307] The absorbance OD value of the non-phosphorylated peptide PD17 at a concentration of 1 nM was input to the expression to calculate the concentration x of each antibody corresponding to the OD value. Since the concentration of PD17 was 1 nM, the scale factor of the binding affinity to phosphorylated tau peptide PD17P based on the binding affinity to PD17 for each peptide was calculated by 1/x, and used as an indicator of selective binding affinity to a phosphorylated tau peptide.

[00308] The results are shown in the graph of Fig. 3. The binding affinity of chimeric antibody Ta1505 to phosphorylated tau peptide PD17P was 30 times as strong as that to non-phosphorylated peptide PD17. In contrast, the selective binding affinity in humanized antibodies exhibited high selectivity with a scale factor of 40 to 210 times. The results demonstrate that the humanized antibodies have specific binding affinity to the phosphorylated peptide.

3. ELISA analysis of selective binding affinity to Ser413 phosphorylation site using phosphorylated tau peptide

[00309] The humanized antibodies having combinations of VL and VH shown in Table 3A were each analyzed for its ability to selectively bind to the Ser413 phosphorylation site among the main phosphorylation sites of the protein, by means of ELISA using a variety of phosphorylated peptides.

[00310] Twelve phosphorylated peptides were used as shown in Table 5. The column named "Epitope" indicates the phosphorylated site(s) of 4R2N-type tau protein (for example, "pS46" in Peptide No. 1 indicates that the site of Ser46 is phosphorylated epitope, and descriptions of multiple epitopes indicate that one peptide has two or three phosphorylated sites), with the exception that Peptide No. 12 has an epitope including Ser413 site but is not phosphorylated (PD17).

Table 5: Peptide for analysis of specific binding to tau protein Ser413 phosphorylation site

Peptide No.	Epitope	Amino acid sequence (one-letter code)	SEQ ID NO:
1	pS46	H-GLKE(pS)PLQT-OH	61
2	pS199	H-SGYS(pS)PGSPGC-OH	62
3	pS202	H-SSPG(pS)PGTPC-OH	63
4	pT212/pS214	H-GCGSPGTPGSRSR(pT)P(pS)LPTPPTREPK-OH	64
5	pT217	H-GC-GSRSRTPSLP(pT)PPTREPKKVAVV-OH	65
6	pT231	H-KVAVVR(pT)PPKSPS-OH	66
7	pS396/pS400 /pS404	H-GC-RENAKAKTDHGAEIVYK(pS)PVV(pS)GDT (pS)PRHL-OH	67
8	pS412	H-NV(pS)STGSC-OH	68
9	pS412/pS413	H-PRHLSNV(pS)(pS)TGSIDMVD-OH	7
10	pS413	H-PRHLSNVS(pS)TGSIDMVD-OH (PD17P)	8
11	pS409/pS412 /pS413	H-PRHL(pS)NV(pS)(pS)TGSIDMVD-OH	9
12	S413	H-PRHLSNVSSTGSIDMVD-OH (PD17)	69

[00311] The twelve peptides shown in Table 5, i.e., Peptides Nos. 1 to 12, or conjugates of these peptides with bovine serum albumin (BSA) or keyhole limpet hemocyanin (KLH), were each diluted with PBS cooled to 4°C to 1 μg/mL, and the resultant solution was dispensed to a plate at 50 μL/well and allowed to stand at 4°C overnight. The solution was removed, and a blocking buffer (3% BSA-PBS) was then dispensed at 270 μL/well, and the plate was allowed to stand at 4°C overnight. After the solution was removed, the purified antibody was diluted with 3% BSA-PBS to 150 ng/mL, and then added to the plate at 50 μL/well to allow a reaction at room temperature for 90 minutes. Each well was washed with Tris-buffered saline (TBS-T) containing 0.05% Tween 20. A goat anti-human IgG (H+L)-alkaline phosphatase label

(manufactured by Sigma-Aldrich Co. LLC.) diluted by 2000-fold with a dilution buffer (3% BSA-PBS) was added to the plate at 50 μL/well to allow a reaction at room temperature for 60 minutes. After washing with TBS-T, a chromogenic solution (1 mg/mL pNPP solution) was added to the plate at 100 μL/well for color development for 40 minutes. The absorbance (OD value) was measured at a measurement wavelength of 405 nm and a reference wavelength of 550 nm.

[00312] The reactivity was evaluated on the following three-point scale:

+: sufficient reactivity (1.0 ≤ OD value),

±: very slight reactivity (0.5 ≤ OD value < 1.0), and

-: no reactivity (OD value < 0.5).

[00313] The results are shown in Table 6. All the humanized antibodies showed strong binding affinity to only Peptide No. 9 (pSer412/pSer413: PRHLSNV(pS)(pS)TGSIDMVD), Peptide No. 10 (pSer413: PRHLSNVS(pS)TGSIDMVD), and Peptide No. 11 (pSer409/pSer412/pSer413: PRHL(pS)NV(pS)(pS)TGSIDMVD), but did not bind to the other peptides, of which the site corresponding to Ser413 of tau protein was not phosphorylated. The pattern and the strength of the binding were substantially the same as those of the chimera (chimeric antibody Ta1505). These results indicate that the humanized antibodies have binding affinity specific to tau protein having a phosphorylated Ser413 site.

Table 6: Results of ELISA analysis of reactivity with various phosphorylated tau peptides

Antibody	Peptide No. in Table 5											
	1	2	3	4	5	6	7	8	9	10	11	12
Chimera	-	-	-	-	-	-	-	-	+	+	+	-
L15H11	-	-	-	-	-	-	-	-	+	+	+	-
L36H11	-	-	-	-	-	-	-	-	+	+	+	-
L46H11	-	-	-	-	-	-	-	-	+	+	+	-
L47H11	-	-	-	-	-	-	-	-	+	+	+	-
L48H11	-	-	-	-	-	-	-	-	+	+	+	-
L50H11	-	-	-	-	-	-	-	-	+	+	+	-
L48H12	-	-	-	-	-	-	-	-	+	+	+	-
L48H47	-	-	-	-	-	-	-	-	+	+	+	-
L48H61	-	-	-	-	-	-	-	-	+	+	+	-
L48H62	-	-	-	-	-	-	-	-	+	+	+	-
L47H64	-	-	-	-	-	-	-	-	+	+	+	-
L48H64	-	-	-	-	-	-	-	-	+	+	+	-
L47H65	-	-	-	-	-	-	-	-	+	+	+	-

C. Example 3: ELISA analysis of binding affinity to antigen protein

[00314] Some of the humanized antibodies having combinations of VL and VH shown in Table 3A were analyzed for the binding affinity to an antigen protein by ELISA.

[00315] In order to prepare a phosphorylated tau protein pTau, tau protein 4R2N was expressed in insect cells using a baculovirus system. In addition, in order to prepare a hyperphosphorylated tau protein hpTau, a recombinant baculovirus capable of simultaneously expressing a tau protein and GSK3β was produced.

[00316] In the production of a recombinant baculovirus, pFastBac1 and pFastBac Dual vectors were used. For the phosphorylated tau protein pTau, a cDNA encoding tau protein 4R2N having a His-tagged C-terminal was inserted into pFastBac1. For the hyperphosphorylated tau protein hpTau, a cDNA encoding tau protein 4R2N having a His-tagged C-terminal and a cDNA encoding GSK3β were inserted into pFastBac Dual. The amino acid sequence and the nucleotide sequence of His-tagged tau protein 4R2N are defined in SEQ ID NOs: 70 and 71, respectively. The amino acid sequence and the nucleotide sequence of GSK3β are defined in SEQ ID NOs: 72 and 73, respectively.

[00317] The resultant vectors were each introduced into cell line Sf9 by lipofection, and the cells were cultured to prepare recombinant baculoviruses. The resultant recombinant baculoviruses were then used to infect cell line Sf9 or Tn5, to thereby express the phosphorylated tau protein (pTau) and the hyperphosphorylated tau protein (hpTau).

[00318] The cells expressing the desired protein were collected, and subjected to sonication and centrifugation to obtain a cell lysate, which was then applied to a Ni-NTA column to purify the tau protein. The concentration of the purified tau protein was determined by bicinchoninic acid (BCA) assay, using bovine serum albumin (BSA) as a standard sample.

[00319] The resultant hyperphosphorylated tau protein (hpTau) and phosphorylated tau protein (pTau) were diluted with cooled PBS to 1 μg/mL, and the diluted solutions were dispensed to a plate at 50 μL/well and allowed to stand at 4°C overnight. After the solutions were removed, a blocking buffer (3% BSA-PBS) was then dispensed at 270 μL/well, and the plate was allowed to stand at 4°C overnight. After removal of the buffer solution, a series of antibody solutions prepared via serial dilution with 3% BSA-PBS was added to the plate at 50 μL/well to allow a reaction at room temperature for 90 minutes.

[00320] The wells were then washed with Tris-buffered saline (TBS-T) containing 0.05% Tween 20. A goat anti-human IgG-alkaline phosphatase label (manufactured by Sigma-Aldrich Co. LLC.) diluted by 2000-fold with a dilution buffer (3% BSA-PBS) was added to the plate at

50 μL/well to allow a reaction at room temperature for 60 minutes. After washing with TBS-T, a chromogenic solution (1 mg/mL pNPP solution) was added to the plate at 100 μL/well for color development for 40 minutes. The absorbance (OD value) was measured at a wavelength of 405 nm.

[00321] The reactivity was evaluated by determining the ratio of the OD value of each antibody to the OD value of 0.25 nM chimeric antibody, and rating the resultant ratio on the following three-point scale:

+: sufficient reactivity ($0.6 \leq$ OD ratio),

±: very slight reactivity ($0.15 <$ OD ratio < 0.6), and

-: no reactivity (OD ratio ≤ 0.15).

[00322] Table 7A shows the results of the evaluated reactivity of the humanized antibodies with hyperphosphorylated tau protein hpTau. Table 7B shows the results of the evaluated reactivity of the humanized antibodies with phosphorylated tau protein pTau. The results demonstrate that all the humanized antibodies have high reactivity with both hyperphosphorylated tau protein hpTau and phosphorylated tau protein pTau, compared to that of the chimeric antibody.

Table 7A: Result of ELISA analysis of reactivity with hyperphosphorylated tau protein hpTau

	VH11	VH12	VH47	VH61	VH62	VH64	VH65
VL15	+						
VL36	+						
VL46	+						
VL47							+
VL48	+	+	+	+	+	+	
VL50	+						

Table 7B: Result of ELISA analysis of reactivity with phosphorylated tau protein pTau

	VH11	VH12	VH47	VH61	VH62	VH64	VH65
VL15	+						
VL36	+						
VL46	+						
VL47							+
VL48	+	±	+	+	+	+	
VL50	+						

D. Example 4: Analysis of binding affinity by SPR

[00323] Some of the humanized antibodies having combinations of VL and VH shown in Table 3A were subjected to binding affinity analysis by surface plasmon resonance (SPR) using chimeric antibody Ta1505 as a reference antibody.

[00324] The hyperphosphorylated tau protein 4R2N (hpTau: see Example 3) was used as an antigen protein, and a non-phosphorylated tau protein produced by *Escherichia coli* (Tau: His-tagged non-phosphorylated tau protein 4R0N: ATGen Co. Ltd., Cat. No.: ATGP0795) was used as a negative control. Antigen peptides used were a monophosphorylated tau peptide (1×P), in which only Ser413 was phosphorylated, and a triphosphorylated tau peptide (3×P), in which Ser409 and Ser412 were further phosphorylated in addition to Ser413. The amino acid sequences of these peptides are shown in Table 8. A negative control peptide used was a non-phosphorylated tau peptide (Non P), whose amino acid sequence is also shown in Table 8. The amino acid sequence of the His-tagged non-phosphorylated tau protein 4R0N is defined in SEQ ID NO: 74. The amino acid sequences of the monophosphorylated tau peptide 1×P, the triphosphorylated tau peptide 3×P, and the non-phosphorylated tau peptide Non P are defined in SEQ ID NOs: 75, 76, and 77, respectively.

Table 8: Antigen peptide used in SPR

Code name	Amino acid sequence (one-letter code)	SEQ ID NO:
1×P	TSPRHLSNVS(pS)TGSIDMVDSPC	75
3×P	TSPRHL(pS)NV(pS)(pS)TGSIDMVDSPC	76
Non P	TSPRHLSNVSSTGSIDMVDSPC	77

[00325] The binding affinities of the hyperphosphorylated tau protein 4R2N (hpTau), non-phosphorylated tau protein 4R0N, monophosphorylated tau peptide 1×P, triphosphorylated tau peptide 3×P, and non-phosphorylated tau peptide Non P with each antibody were measured with an SPR system Biacore T200 (GE Healthcare Japan), in accordance with the instruction manual for evaluation attached to the system.

[00326] The binding affinity was measured by a method including: preparing an NTA sensor chip (including a carboxymethyl dextran layer to which nitrilotriacetic acid (NTA) was already immobilized: code No. BR-1005-32); immobilizing each of the above-mentioned peptides fused with His-tag and each of the antigen peptides shown in Table 8 on the sensor chip by covalent bonds through an amine coupling reaction using an amine coupling kit (GE Healthcare Japan, code No. BR-1006-33); and measuring the binding kinetics of antibodies to the immobilized proteins and peptides.

[00327] The His-tagged hpTau or Tau proteins were each immobilized on a sensor chip using an HBS-N buffer (code No. BR-1003-69) as the immobilization reaction solution and allowing a nickel chloride solution to react with the NTA sensor chip for bonding Ni to NTA. Subsequently, the sensor chip was activated with a mixed solution of N-ethyl-N'-(3-dimethylaminopropyl)-carbodiimide hydrochloride (1-ethyl-3-(3-dimethylaminopropyl)carbodiimide hydrochloride: EDC) and N-hydroxysuccinimide (NHS). A solution of each of the His-tagged proteins, adjusted to a concentration of 100 to 500 ng/mL with a 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid (HEPES) buffer (0.01 M HEPES, 150 mM KCl, 2 mM DTT, 1 mM EDTA, pH 7.2), was applied to the sensor chip, whereby the protein was immobilized on the sensor chip through covalent bonds with the Ni-NTA. The remaining active NHS was blocked with ethanolamine, and Ni ions were removed with an EDTA solution (Ni-affinity amine coupling: see International Publication No. WO2005/022156). The antigen peptides (1×P, 3×P, and Non P) were each immobilized on a sensor chip by activating the NTA sensor chip with a mixed solution of N-ethyl-N'-(3-dimethylaminopropyl)-carbodiimide (EDC) hydrochloride and N-hydroxysuccinimide (NHS), and then applying a solution of the antigen peptide diluted with a 10 mM sodium acetate buffer (pH: 4 to 4.5) to 1 to 10 μM to the sensor chip, whereby the peptide was covalently bound to the sensor chip. The remaining active NHS was blocked with ethanolamine. Thus, sensor chips immobilized respectively with the proteins and peptides mentioned above were produced.

[00328] A CAPS buffer (pH 10.5) was applied to these sensor chips at 37°C to stabilize the protein- or peptide-immobilized surfaces of the sensor chips. A solution of each antibody, prepared with a HEPES buffer (pH 7.2) as a specific binding reaction solution, was applied to the sensor chip surface at concentrations ranging from 0.2 to 1 nM (around the estimated binding dissociation constant [KD]) for 150 seconds (uniform in the same evaluation) for a reaction, and the binding kinetics were measured. The resultant data were analyzed using Biacore analysis software (Biacore T200 Evaluation Software, Version 3.0).

[00329] The binding kinetics of each antibody was corrected by subtracting the binding kinetics of the non-phosphorylated protein 4R0N to the sensor chip as a negative control from the binding kinetics of the antigen protein 4R2N (hpTau) to the sensor chip. The binding kinetics of each antigen peptide was corrected by subtracting the binding kinetics of the non-phosphorylated peptide Non P to the sensor chip as a negative control from the binding kinetics of each of the antigen peptides 1×P and 3×P. No binding of each antibody to the non-

phosphorylated tau protein 4R0N or the non-phosphorylated peptide Non P was observed within the range of sample concentrations subjected to measurement.

[00330] Table 9A shows the binding activities of humanized antibodies to the phosphorylated tau protein 4R2N (hpTau). Table 9B shows the results of binding activities of humanized antibodies to the monophosphorylated tau peptide 1×P and the triphosphorylated tau peptide 3×P. The results demonstrate that each humanized antibody exhibited high binding activity to all of the hyperphosphorylated tau protein hpTau, monophosphorylated tau peptide 1×P, and triphosphorylated tau peptide 3×P.

Table 9A: Binding activity of humanized antibodies to phosphorylated tau protein (hpTau) (1:1 binding model)

Sample	hpTau KD(M)
Chimera	1.70E-10
L15H11	1.50E-10
L36H11	1.40E-10
L46H11	1.30E-10
L47H11	1.80E-10
L48H12	1.60E-10
L48H47	1.40E-10
L48H64	1.00E-10
L47H65	1.50E-10
L48H11	1.40E-10
L48H61	1.70E-10
L48H62	1.30E-10
L50H11	1.50E-10

Table 9B: Binding activity of humanized antibodies to phosphorylated peptides (1×P, 3×P) (1:1 binding model)

Sample	1×P KD(M)	3×P KD(M)
Chimera	4.60E-11	2.20E-11
L15H11	3.90E-11	1.90E-11
L36H11	4.70E-11	1.90E-11
L46H11	4.80E-11	1.70E-11
L47H11	6.20E-11	2.20E-11
L48H12	6.50E-11	2.70E-11
L48H47	5.80E-11	2.20E-11
L48H64	5.00E-11	1.50E-11
L47H65	5.40E-11	1.90E-11
L48H11	6.00E-11	2.00E-11
L48H61	1.80E-10	2.40E-11
L48H62	5.20E-11	2.00E-11
L50H11	4.80E-11	1.90E-11

E. Example 5: Binding affinity analysis of pSer413-Tau in AD patient brain homogenate

[00331] Among the humanized antibodies having combinations of VL and VH shown in Table 3A, L15H11, L46H11, and L47H65 were evaluated for binding affinity to Ser413 phosphorylated tau protein (pSer413-Tau) derived from an Alzheimer's disease (AD) patient clinical sample in a liquid phase, using a mouse antibody Ta1505 and a chimeric antibody as reference antibodies.

[00332] The humanized antibodies, mouse antibody, and chimeric antibody were each biotinylated using N-hydroxysuccinimide-LC-biotin (NHS-LC-biotin) (Thermo Fisher Scientific K.K.), followed by dialysis with PBS.

[00333] Frozen hippocampus tissue (160 mg) from the brain of an AD patient at Braak stage V/VI was added to 0.8 mL of TBS-I (Tris-buffered saline, protease inhibitor cocktail, phosphatase inhibitor cocktail) and sonicated in iced water. The sonicated solution was centrifuged at 3000×g at 4°C for 10 minutes, and the supernatant was collected and further ultracentrifuged at 100000×g at 4°C for 15 minutes to obtain a brain homogenate. Innotest pTau (pT181) kit (Fijirebio Inc.) was used as an ELISA system of Ser413 phosphorylated tau protein (pSer413-Tau), but the biotinylated antibody included in the kit (labelled as CONJ1) was replaced with the biotinylated antibodies produced above.

[00334] Each of the biotinylated antibodies produced above was made into a solution at a concentration of 0.1 nM, and added to an HT7 antibody immobilized MT plate (included in the kit), along with a 1000-fold dilution of the brain homogenate. The resultant sample was mixed and incubated at 4°C overnight. On the following day, the plate was washed, and an HRP-labeled streptavidin (included in the kit as CONJ2) was added to the plate, followed by incubation for one hour. After washing, a color reagent TMB was added, followed by incubation under light-shielding at room temperature for 30 minutes. The reaction was then stopped with a reaction terminator solution (included in the kit as STOP solution), and absorbance at a wavelength of 450 nm was measured.

[00335] The results are shown in Fig. 4. As shown in the graph of Fig. 4, humanized antibodies L15H11, L46H11, and L47H65 all exhibited high binding affinity to the Ser413 phosphorylated tau protein (pSer413-Tau) derived from the AD patient clinical sample.

F. Example 6: Test for kinetics in blood

[00336] Five initial humanized antibody variants were tested for pharmacokinetics in normal mice, in comparison with a commercially available antibody Herceptin (Genentech, south San Francisco, CA) and the chimeric Ta1505 antibody. The light chain and heavy chain of each humanized antibody variant is summarized in **Table 10** below.

[00337] Table 10. Light Chain and Heavy Chain of Some Initial Humanized Antibody Variants

Antibody	LC	HC
Variant 2	L1 (SEQ ID NO:130)	H2 (SEQ ID NO:134)
Variant 5	L2 (SEQ ID NO:131)	H1 (SEQ ID NO:133)
Variant 6	L2 (SEQ ID NO:131)	H2 (SEQ ID NO:134)
Variant 9	L3 (SEQ ID NO:132)	H1 (SEQ ID NO:133)
Variant 10	L3 (SEQ ID NO:132)	H2 (SEQ ID NO:134)

[00338] The antibodies were intraperitoneally injected at a dose of 10 mg/kg to 11-week-old male C57BL/6J mice (Charles River Laboratories Japan, Inc.). Plasma was obtained from the blood collected 1, 3, 8, 24, and 72 hours post dose. The sample size was 2 animals per antibody.

[00339] Sandwich ELISA with anti-human IgG polyclonal antibodies was used to determine plasma antibody concentrations. A calibration curve was plotted by serial dilution of each antibody with mouse plasma.

[00340] Plasma was diluted by a factor of 1,000 and the antibody concentration was measured.

[00341] A 1 μg/mL PBS solution of goat anti-human IgG (Fc) polyclonal antibodies was pipetted into a 96-well plate (MaxSorp (NUNC)) and allowed to stand at 4°C overnight. Then, blocking was accomplished with 3% BSA/PBS to prepare a plate with immobilized goat anti-human IgG (Fc) polyclonal antibodies. Mouse plasma was used to serially dilute the antibodies and obtain the standard substance. The plasma and standard substance were diluted with 3% BSA/PBS and pipetted into the plate with immobilized goat anti-human IgG (Fc) polyclonal antibodies at 50 μL/well. The mixtures were allowed to react at room temperature for 1.5 hours, and the plate was then washed with TBS-T (TBS, 0.05% Tween 20). Then, a solution prepared by diluting alkaline phosphatase-labeled anti-human IgG (H+L) polyclonal antibodies (Southern Biotech, Cat #2087-04) by a factor of 2000 with 3% BSA/PBS was pipetted at 50 μL/well, and the reaction was allowed to proceed for 1 hour at room temperature. Next, the plate was washed with TBS-T, the coloring reagent was added at 100 μL/well, and the plate was incubated for 1

hour at room temperature. Thereafter, a plate reader was used to measure absorbance at 405 nm and 550 nm.

[00342] A calibration curve was plotted with standard substance and used to calculate plasma antibody concentrations. The results are shown in Figure 5A.

[00343] The initial humanized variants tested (Variant 2, 5, 6, 9, and 10) showed a rapid decrease of plasma concentration at 8 hours after administration, compared to the level of Herceptin and the chimeric antibody, which stayed high and stable up to 3 days after injection. These initial humanized antibodies presented a shorter half-life and poor PK profile in comparison with the chimeric antibody, which is likely attributable to the high pI values of these variants. Further improvement was required to reach the PK of the chimeric antibody.

[00344]

[00345] Among the humanized antibodies having combinations of VL and VH shown in Table 3A, L15H11, L46H11, and L47H65 were subjected to a test for kinetics in blood. The reference antibodies used were chimeric antibody Ta1505 and known humanized antibodies used in clinical trial studies for AD treatment, which were produced by recombinant expression based on the amino acid sequence information disclosed in patent information or database information. Specifically, the database of the international ImMunoGeneTics information system (registered trademark) (IMGT) (http://www.imgt.org) was referred to for Solanezumab (anti-A□ antibody, Eli Lilly and Company); the amino acid sequence of VH2Vk3 disclosed in WO2014/200921 A1 was used for IPN007 (anti-Tau N-terminal antibody, Bristol-Myers Squibb iPierian); and the amino acid sequence of VH32VL21 disclosed in WO2015/091656A1 was used for MAb3221 (anti-Tau-pS422 antibody, Roche Diagnostics K.K.).

[00346] Each of the antibodies was intraperitoneally administered to 11-week old male C57BL/6J mice (Charles River Laboratories Japan, Inc.) at a concentration of 10 mg/kg each. Blood was drawn at 1, 3, 8, and 24 hours, and subsequently on the 3rd and 7th day after the administration to collect plasma. Each antibody was tested in triplicate (N = 3).

[00347] The concentration of the antibody in plasma was measured by sandwich ELISA using an anti-human polyclonal antibody. Specifically, a PBS solution containing 1 μg/mL goat anti-human IgG (Fc) polyclonal antibody (Southern Biotech) was added to a 96-well plate (Max Sorp (NUNC)) for immobilization at 4°C overnight, followed by blocking with 3% BSA-PBS to thereby produce a goat anti-human IgG (Fc) polyclonal antibody-immobilized plate.

[00348] Separately, a series of standard antibody samples of known concentrations were prepared by serial dilution of each antibody with plasma taken from a mouse to which the antibody was not administered, and measured for preparation of a calibration curve.

[00349] The plasma samples and the standard samples to be measured were each diluted by 10000-fold, and added to a goat anti-human IgG (Fc) polyclonal antibody-immobilized plate at 50 μL/well for reaction at room temperature for one hour and 30 minutes, and the plate was then washed with TBS-T (Tris-buffered saline, 0.05% Tween 20). Subsequently, a solution of alkaline phosphatase-labeled anti-human IgG (H+L) polyclonal antibody (Southern Biotech) diluted by 2000-fold with 3% BSA-PBS was added to the plate at 50 μL/well to allow a reaction at room temperature for one hour. After washing with TBS-T, a chromogenic solution (1 mg/mL pNPP solution) was added to the plate at 100 μL/well, followed by incubation at room temperature for one hour. The absorbance was then measured with a plate reader at a measurement wavelength of 405 nm and a reference wavelength of 550 nm. A calibration curve was prepared based on the measurement results of the standard samples, and the antibody concentration in each plasma sample was calculated using the calibration curve.

[00350] Fig. 5B is a graph showing changes in concentrations of antibodies in plasma over time. As obvious from the graph, humanized antibodies L15H11 (LC SEQ ID NO:32 HC SEQ ID NO:18), L46H11 (LC SEQ ID NO:36, HC SEQ ID NO:18), and L47H65 (LC SEQ ID NO:38, HC SEQ ID NO:30) (all using human IgG1 (SEQ ID NO:135) and human kappa (SEQ ID NO:79) showed substantially the same kinetics in blood as that of chimeric antibody Ta1505. The kinetics of these humanized antibodies L15H11, L46H11, and L47H65 in blood were comparable to those of known humanized antibodies IPN007 and MAb3221, and significantly superior to those of known humanized antibody Solanezumab.

G. Example 7: Analysis of intracerebral migration

[00351] A study was conducted to analyze the brain concentration of the humanized variants with improved plasma pharmacokinetics.

[00352] Blood was collected from the mice injected with the parent mouse antibody, the chimeric antibody, or the humanized antibody variants (L15H11, L36H11, L46H11, L48H12, L48H47, L48H64, L47H65, or L48H11) 1 week after injection of the antibodies. Next, the animals were anesthetized with a 3-anesthetic cocktail, laparotomized, and exsanguinated via the abdominal aorta. Then, brain tissue was collected. This collected brain tissue was divided into left and right hemispheres, frozen on dry ice/ethanol, and stored at -80°C. Each frozen

hemisphere was weighed and transferred to a 2 mL tube. Then, 0.8 mL of TBS-I (Tris Buffered Saline, protease inhibitor cocktail and phosphatase inhibitor cocktail) was added, and the mixture was sonicated in ice water. The sonicated mixture was centrifuged at 3000×g and 4°C for 10 minutes, and the supernatant was collected. The supernatant was further centrifuged at 100,000×g and 4°C for 15 minutes to obtain a brain homogenate.

[00353] A 10 μg/mL PBS solution of goat anti-human IgG (Fc) polyclonal antibodies was pipetted into a 96-well plate (MaxSorp (NUNC)) and allowed to stand at 4°C overnight. Then, blocking was accomplished with 3% BSA/PBS to prepare a plate with immobilized goat anti-human IgG (Fc) polyclonal antibodies. Brain homogenate obtained from mice not injected with antibodies was used to serially dilute the antibodies and to obtain the standard substance.

[00354] The brain homogenates and standard substance were diluted by a factor of 10 and pipetted into the plate with immobilized goat anti-human IgG (Fc) polyclonal antibodies at 50 μL/well. The mixtures were allowed to react at room temperature for 2 hours, and the plate was then washed with TBS-T (TBS, 0.025% Tween 20). Then, a solution prepared by diluting alkaline phosphatase-labeled anti-human IgG (H+L) polyclonal antibodies (Sigma, Cat #SAB3701337-1MG) by a factor of 2000 with 3% BSA/PBS was pipetted at 50 μL/well, and the reaction was allowed to proceed for 1 hour at room temperature. Next, the plate was washed with TBS-T, the coloring reagent pNPP (Sigma, Cat #P7998) was added at 100 μL/well, and the plate was incubated for 1 hour at room temperature. Thereafter, a plate reader was used to measure absorbance at 405 nm and 550 nm.

[00355] A calibration curve was plotted with standard substance and used to calculate the antibody concentrations in the brain homogenate. The antibody concentration per brain weight (whole brain volume) was determined and the ratio to the plasma concentration was calculated.

[00356] **FIG. 6A** demonstrates that the humanized antibody variants with good pharmacokinetics (*e.g.*, L15H11, L46H11, and L47H65 according to FIG. 5B) had improved concentration in the brain.

[00357] Intracerebral migration of each of the humanized antibodies L15H11, L46H11, and L47H65 was analyzed, using chimeric antibody Ta1505 and known humanized antibodies Solanezumab, IPN007, and MAb3221, which were used in clinical trial studies for AD treatment, as reference antibodies.

[00358] One week after the administration of each antibody in Example 6, blood was drawn from mice, which were then subjected to laparotomy under anesthesia by three types of

mixed anesthetic agents. After death from exsanguination from the abdominal vena cava, the brain tissue was collected. The collected brain tissue was divided into right and left hemispheres, which were frozen in dry ice ethanol and stored at -80°C. Each of the frozen hemispheres was weighed and transferred to a 2-mL tube, to which 0.8 mL of TBS-I (Tris-buffered saline, protease inhibitor cocktail, and phosphatase inhibitor cocktail) was added. Each hemisphere was then sonicated in iced water. The sonicated solution was centrifuged at 3000×g at 4°C for 10 minutes, and the supernatant was collected and further ultracentrifuged at 100000×g at 4°C for 15 minutes to obtain a brain homogenate.

[00359] The antibody level in the brain homogenate was measured by antigen ELISA using an anti-human polyclonal antibody. Specifically, a PBS solution containing 10 μg/mL of goat anti-human IgG Fc was added to a 96-well plate (Max Sorp (NUNC)) for immobilization at 4°C overnight, followed by blocking with 3% BSA-PBS to thereby produce an immobilized plate.

[00360] Separately, a series of antibody standard samples of known concentrations were prepared by serial dilution of each antibody with a brain homogenate taken from a mouse to which the antibody was not administered, and measured for preparation of a calibration curve.

[00361] The brain homogenates and the standard samples to be measured were each diluted by 10-fold with 0.1% skimmed milk-3% BSA-PBS, and added to the PD17(P)-immobilized plate at 50 μL/well for reaction at room temperature for 2 hours. The plate was then washed with TBS-T (TBS, 0.05% Tween 20). Subsequently, a solution of alkaline phosphatase-labeled anti-human IgG (H+L) polyclonal antibody (Sigma-Aldrich Co. LLC., Cat No. SAB3701337: 1 mg) diluted by 2000-fold with 0.1% skimmed milk-3% BSA-PBS was added to the plate at 50 μL/well to allow a reaction at room temperature for one hour. After washing of the plate with TBS-T, pNPP (Sigma-Aldrich Co. LLC., Cat No. P7998: 100 mL) was added as a chromogenic substrate at 100 μL/well, followed by incubation at room temperature for one hour. The absorbance was then measured with a plate reader at a measurement wavelength of 405 nm and a reference wavelength of 550 nm. A calibration curve was prepared based on the measurement results of the standard samples, and the antibody level in each brain homogenate sample was determined using the calibration curve, and evaluated as the antibody level in the brain.

[00362] In addition, the antibody level in plasma was determined using blood collected before the laparotomy in the same manner as in Example 6, and the ratio of the antibody level in the brain to that in the plasma was calculated.

[00363] Fig. 6B is a graph showing the ratio of the level of each antibody in the brain to the level of the corresponding antibody in the plasma. The ratios of the antibody concentrations in the brain to the antibody concentrations in the plasma of humanized antibodies L15H11, L46H11, and L47H65 were high compared to those of chimeric antibody Ta1505 and known humanized antibodies IPN007, MAb3221, and Solanezumab. The results indicate that these humanized antibodies have high migration to the brain.

[00364]

Example 8: Further Development of Selective Humanized Antibodies

Further mutations were introduced to the CDR1 sequence of VL46 to remove a deamidation hotspot and/or to revert the CDR sequence to the parent mouse sequence. **Table 11** summarizes the mutations introduced to VL46 CDR1.

Table 11. Mutations Introduced in VL46 CDR1

Mutation	Purpose
VL46_G34A (SEQ ID NO:105)	Remove deamidation
VL46_G34S (SEQ ID NO:106)	Remove deamidation
VL46_G34T (SEQ ID NO:107)	Remove deamidation
VL46_N33Q (SEQ ID NO:108)	Remove deamidation
VL46_N33Q_G34A (SEQ ID NO:109)	Remove deamidation
VL46_N33D (SEQ ID NO:110)	Mimic full deamidation
VL46_N33S (SEQ ID NO:111)	Remove deamidation
VL46_N33T (SEQ ID NO:112)	Remove deamidation

VL46_S28N (SEQ ID NO:113)	Revert LC CDR1 to mouse
VL46_G34A_S28N (SEQ ID NO:114)	Remove deamidation and revert LC CDR1 to mouse

In addition, different IgG isotypes including IgG1 and IgG4 with S228P mutation were tested in the newly generated humanized antibody variants. **Table 12** summarizes the light chain and the heavy chain of the newly generated humanized antibody variants along with the parent mouse antibody and the chimeric antibody.

Table 12. Light Chain and Heavy Chain of Specific Antibodies

Mouse x [MAPT_H] mAb (Ta1505) IgG2a / Kappa (HY)	**ms IgG2a**
Mouse Human Chimera x [MAPT_H] mAb (Ta1505) IgG1 / Kappa (CX)	**chim. IgG1**
Mouse Human Chimera x [MAPT_H] mAb (Ta1505) IgG4 S228P / Kappa (CX)	**chim. IgG4**
Humanized x [MAPT_H] mAb (Ta1505 VL46 / VH11) IgG1 / Kappa (CX)	**hu VL46 / VH11 IgG1**
Humanized x [MAPT_H] mAb (Ta1505 VL46 / VH11) IgG4 S228P / Kappa (CX)	**hu VL46 / VH11 IgG4**
Humanized x [MAPT_H] mAb (Ta1505-VL46_G34A / VH11) IgG1 / Kappa (CX)	**hu VL46 / VH11 G34A IgG1**
Humanized x [MAPT_H] mAb (Ta1505-VL46_G34A / VH11) IgG4 S228P / Kappa (CX)	**hu VL46 / VH11 G34A IgG4**
Humanized x [MAPT_H] mAb (Ta1505-VL46_G34S / VH11) IgG4 S228P / Kappa (CX)	**hu VL46 / VH11 G34S IgG4**
Humanized x [MAPT_H] mAb (Ta1505-VL46_G34T / VH11) IgG4 S228P / Kappa (CX)	**hu VL46 / VH11 G34T IgG4**
Humanized x [MAPT_H] mAb (Ta1505-VL46_N33Q / VH11) IgG4 S228P / Kappa (CX)	**hu VL46 / VH11 N33Q IgG4**
Humanized x [MAPT_H] mAb (Ta1505-VL46_N33Q_G34A / VH11) IgG4 S228P / Kappa (CX)	**hu VL46 / VH11 N33Q G34A IgG4**
Humanized x [MAPT_H] mAb (Ta1505-VL46_N33D / VH11) IgG4 S228P / Kappa (CX)	**hu VL46 / VH11 N33D IgG4**
Humanized x [MAPT_H] mAb (Ta1505-VL46_N33S / VH11) IgG4 S228P / Kappa (CX)	**hu VL46 / VH11 N33S IgG4**
Humanized x [MAPT_H] mAb (Ta1505-VL46_N33T / VH11) IgG4 S228P / Kappa (CX)	**hu VL46 / VH11 N33T IgG4**
Humanized x [MAPT_H] mAb (Ta1505-VL46 / VH11 A64D) IgG4 S228P / Kappa (CX)	**hu VL46 / VH11 A64D IgG4**
Humanized x [MAPT_H] mAb (Ta1505-VL46 S28N / VH11) IgG4 S228P / Kappa (CX)	**hu VL46 / VH11 S28N IgG4**
Humanized x [MAPT_H] mAb (Ta1505 VL47 / VH65) IgG1 / Kappa (CX)	**hu VL47 / VH65 IgG1**
Humanized x [MAPT_H] mAb (Ta1505-VL46_G34A_S28N / VH11) IgG1 / Kappa (CX)	**hu VL46 / VH11 G34A S28N IgG1**
Humanized x [MAPT_H] mAb (Ta1505-VL46_G34A_S28N / VH11) IgG4 S228P / Kappa (CX)	**hu VL46 / VH11 G34A S28N IgG4**

H. Example 9: Biacore™ binding analysis of the newly generated humanized antibody variants

Binding affinities of the newly generated humanized antibody variants were measured using Biacore™ T200 and 4000 biosensors (GE Healthcare, Chicago, IL). The following running buffer: 10 mM HEPES (GE Healthcare, BR100671), 150 mM NaCl (GE Healthcare, BR100671), 0.05% v/v Surfactant P20 (GE Healthcare, BR100671), 2 mM DTT (Sigma, 10708984001, St. Louis, MO), and 1 mM EDTA (GE Healthcare, 28995043), was used for immobilization, sample dilution and data collection unless otherwise noted below.

Protein immobilized refers to the recombinant human phosphorylated Tau protein immobilized on the chip; 1xP peptide immobilized refers to the phosphorylated peptide

(TSPRHLSNVS(pS)TGSIDMVDSPC, SEQ ID NO:75) immobilized on the chip. Both of these methods have an avidity component to the affinity measurement. 4xP peptide analyte has the antibody captured on the chip and the 4x phosphorylated peptide (GAEIVYK(pS)PVVSGDT(pS)PRHLSNVS(pS)TGSIDMVD(pS)PQLATLADEVSASLAKQG L, SEQ ID NO:78) in solution.

Antibody binding to immobilized, phosphorylated Tau protein was measured using Series S Sensor Chips NTA (GE Healthcare, BR100034). The running buffer for the immobilization was 10 mM HEPES, 150 mM $NaCl_2$, 0.05% v/v Surfactant P20, pH 7.4 (GE Healthcare, BR100671) and the flow rate was 10 μL/minute. 350 mM EDTA was injected for 1 minute to clean the chip, followed by a 2 minute injection of 0.5 mM $NiCl_2$ (GE Healthcare, NTA Reagent Kit) to prepare the chip to capture his-tagged protein. The chip was activated according to the manufacturer's instructions using an amine coupling kit (GE Healthcare, BR100633), then 300 nM phosphorylated Tau protein (80AWB) was injected until the desired amount of protein had been immobilized. The chip was blocked with ethanolamine from the amine coupling kit. Each experiment used multiple levels of immobilized phosphorylated Tau protein. The lowest level varied from 54 resonance units (RU) to 452 RU, and the highest level varied from 463 RU to 1020 RU. To measure the affinity of Ta1505 antibody binding to the immobilized phosphorylated Tau protein, a 5-membered, 3-fold dilution series of each antibody was prepared, resulting in concentrations of 0.37 nM to 30 nM. Each concentration and several buffer blanks were injected for 3 minutes at a flowrate of 45 μl/minute. The dissociation of the antibody was monitored for 15 minutes, then the surface was regenerated with a 30 second to 1 minute injection of 100 mM CAPS (Sigma, C6070), 1 M KCl (Sigma, P9541), 1 mM EDTA, 2 mM DTT, pH 10.5.

Antibody binding to immobilized, phosphorylated Tau peptide was measured using a Series S Sensor Chip CM3 (GE Healthcare, BR100536). The chip was activated using an amine coupling kit, then 30 μg/mL phosphorylated Tau peptide (SEQ ID NO:75) in 10 mM sodium acetate, pH 5.0 was injected until 51 RU had been immobilized. The chip was blocked with ethanolamine. A negative control reference surface was prepared similarly, using non-phosphorylated Tau peptide (TSPRHLSNVSSTGSIDMVDSPC, SEQ ID NO:77). To measure the affinity of Ta1505 antibody binding to the immobilized phosphorylated Tau peptide, a 5-membered, 3-fold dilution series of each antibody was prepared, resulting in concentrations of 0.37 nM to 30 nM. Each concentration and several buffer blanks were injected for 3 minutes at a flowrate of 45 μL/minute. The dissociation of the antibody was monitored for 15 minutes. The

surface was regenerated with a 30 second injection of 100 mM hydrochloric acid (Fisher Scientific, SA56-1, Waltham, MA).

Phosphorylated Tau peptide binding to immobilized antibody was measured using Series S Sensor Chips CM5 (GE Healthcare, 29149603). The chip was activated using an amine coupling kit, then 1 to 3 μg/ml antibody in 10 mM sodium acetate, pH 5.0 (Ge Healthcare, BR100351) was injected until 280 to 9100 RU of antibody had been immobilized. The chip was blocked with ethanolamine. To measure the affinity of phosphorylated Tau peptide (SEQ ID NO:78) binding to the immobilized Ta1505 antibodies, a 6-membered 2.5-fold dilution series of the peptide was prepared, resulting in concentrations of 5.1 nM to 500 nM. Samples and several buffer blanks were injected at 30 to 50 μL/minute for 3 minutes, and dissociation was monitored for 15 minutes. The surface was regenerated with a 30 second injection of 20 mM sodium acetate, pH 4.5, either unadjusted, or with the pH adjusted to 3.5 with hydrochloric acid.

The data were processed and fit using Biacore™ T200 Evaluation Software version 2.0 or Biacore™ 4000 Evaluation Software version 1.1 (GE Healthcare). The data were "double referenced" by subtracting the response from a negative control flowcell and subtracting the response from a buffer injection or the average response from two buffer injections. The data were then fit with the "1:1 Binding" model to determine the association rate constant, k_a ($M^{-1}s^{-1}$, where "M" equals molar and "s" equals seconds) and the dissociation rate constant, k_d (s^{-1}). These rate constants were used to calculate the equilibrium dissociation constant, K_D (M) = k_d/k_a. **Table 13** summarizes the K_D values of the newly generated humanized antibody variants in comparison with those of the parent mouse antibody and the chimeric antibody.

Table 13. K_D (nM) Values of Antibodies to Phosphorylated Tau Protein or Peptide

	Protein Immobilized	1xP peptide immobilized	4xP peptide analyte
ms IgG2a	0.43	0.03	17.5
chim. IgG1	0.46	0.04	27.2
chim. IgG4	0.58	0.06	28.5
hu VL46 (SEQ ID NO:163)/ VH11 IgG1 (SEQ ID NO:144)	0.46	0.06	24.3
hu VL46 (SEQ ID NO:163)/ VH11 IgG4 (SEQ ID NO:152)	0.70	0.09	39.3

hu VL46 G34A (SEQ ID NO:166)/ VH11 IgG1 (SEQ ID NO:144)	0.28	ND	ND
hu VL46 G34A (SEQ ID NO:166) / VH11 IgG4 (SEQ ID NO:151)	0.52	0.09	36.2
hu VL46 G34S (SEQ ID NO:168) / VH11 IgG4(SEQ ID NO:151)	NB	NB	ND
hu VL46 G34T (SEQ ID NO:170)/ VH11 IgG4(SEQ ID NO:151)	0.75	0.13	ND
hu VL46 N33Q (SEQ ID NO:172)/ VH11 IgG4(SEQ ID NO:151)	1.27	0.14	ND
hu VL46 N33Q G34A (SEQ ID NO:174) / VH11 IgG4 (SEQ ID NO:151)	3.40	0.31	ND
hu VL46 (SEQ ID NO:163)/ VH11 IgG4(SEQ ID NO:151)	NB	1.78	ND
hu VL46 N33S (SEQ ID NO:178)/ VH11 IgG4 (SEQ ID NO:151)	2.31	0.29	ND
hu VL46 N33T (SEQ ID NO:180)/ VH11 IgG4 (SEQ ID NO:151)	1.31	0.18	ND
hu VL46 A64D / VH11 IgG4 (SEQ ID NO:151)	0.77	0.16	ND
hu VL46 S28N (SEQ ID NO:183)/ VH11 IgG4 (SEQ ID NO:151)	0.42	0.10	32.5

hu VL47 (SEQ ID NO:164)/ VH65 IgG1 (SEQ ID NO:153)	0.45	0.06	ND
hu VL46 G34A S28N (SEQ ID NO:184)/ VH11 IgG1 (SEQ ID NO:144)	0.43	ND	ND
hu VL46 G34A S28N (SEQ ID NO:184)/ VH11 IgG4 (SEQ ID NO:151)	0.80	ND	ND

I. Example 10: ELISA binding analysis of the parent mouse antibody, the chimeric antibody, and some humanized antibody variants

Binding of the parent mouse antibody, the chimeric antibody, and the newly generated humanized antibody variants to a phosphorylated peptide (PRHLSNVS(pS)TGSIDMVD, SEQ ID NO:79) and the corresponding non-phosphorylated peptide (PRHLSNVSTGSIDMVD, SEQ ID NO:80) was analyzed by ELISA.

Fifty microliters of 1 μg/ml phosphorylated or non-phosphorylated peptide in PBS were added to each well of ELISA plates and the plates were incubated at 4°C overnight. On the next day, the plates were washed 3 times and blocked with 200 μL of superblock at 4°C overnight. On the third day, the plates were washed 3 times. Then 50 μL of antibodies in 1:3 serial dilution in the ELISA buffer, starting at 10 μg/mL, were added to each well of the plate, incubated at room temperature for 1 hour, then washed 3 times. Fifty microliters of 1:3000 dilution of goat anti-mouse-HRP (Southern Biotech, 1030-05) were added for measuring the parent mouse antibody. Fifty microliters of 1:3000 dilution of goat anti-human-HRP (Jackson Immunologics, 109-036-098) were added for measuring the chimeric antibody and humanized antibodies. The ELISA plates were incubated at room temperature for 45 minutes, washed 3 times, then developed with ABTS at room temperature for 5 minutes. Thereafter, a plate reader was used to measure absorbance at 405 nm.

FIG. 8A and 8B show that the parent antibody generated by hybridoma or recombinant method bound to the phosphorylated peptide (**FIG. 8A**) but not the non-phosphorylated peptide (**FIG. 8B**). Similarly, the chimeric antibodies (with either IgG1 or IgG4 backbone) bound to the phosphorylated peptide (**FIG. 8C**) but not the non-phosphorylated peptide (**FIG. 8D**).

FIGS. 9A-9C demonstrate that most of the newly generated humanized antibody variants bound to the phosphorylated peptide with affinity comparable to the chimeric IgG4 antibody whereas a few variants lost binding affinity to the phosphorylated peptide. Particularly, in **FIG. 9B**, the huVL46/VH11_N33D_IgG4 variant, which mimics full deamidation of amino acid N33 to D, had vastly reduced binding to the phosphorylated peptide, suggesting that the fully deamidated antibody would lose its binding affinity to the phosphorylated Tau protein dramatically. Thus, reduction of deamidation at N33 in the humanized antibody variants is imperative.

XV. Example 11: Antigen binding analysis of the newly generated humanized antibody variants in brain homogenates of Alzheimer's disease patients

The parent mouse antibody, the chimeric antibody, and selected humanized antibody variants were evaluated for their binding potency to Ser413 phosphorylated Tau protein (pSer413-Tau) derived from an Alzheimer's disease (AD) patient clinical samples in a liquid phase.

As described in **FIGS. 10A-10E**, antibody occupancy on pSer413-Tau was determined by the difference between total and free antigen binding detected using mouse biotinylated-Ta1505 IgG2a antibody after incubation of the AD brain samples with increasing concentration of control human IgG (Sigma, Cat# I2511) and selected antibody variants, respectively.

The detection with the same mouse biotinylated-Ta1505 IgG2a antibody allows a direct comparison of binding potency between the antibodies variants. Mouse Ta1505 IgG2a antibody was biotinylated using N-hydroxysuccinimide-LC-biotin (NHS-LC-biotin) (Thermo Fisher Scientific K.K.), followed by dialysis with PBS.

Frozen pre-frontal cortex tissue (100 mg) from the brain of an AD patient was added to 1 mL of TBS-I (Tris-buffered saline, Cat# BP2471-1, Thermo Fisher Scientific) containing a protease and phosphatase inhibitor cocktail (Thermo Fisher Scientific, Cat# 1861281) and lysed on iced water using Qiagen Tissue Lyzer II. The homogenized sample was centrifuged at 27,000g (TLA-55 rotor) at 4°C for 20 minutes in Beckman Coulter Optima Max-XP Ultracentrifuge, and the supernatant was collected and further centrifuged at 150,000×g (TLA-55 rotor) at 4°C for 20 minutes in Beckman Coulter Optima Max-XP Ultracentrifuge to obtain a pellet called P2 fraction. The P2 fraction was re-suspended in homogenization buffer TBS-I by sonication. Protein concentration of the P2 fraction was determined using Pierce™ BCA Protein Assay Kit (Thermo Fisher Scientific, Cat# 23225) per manufacturer's instructions. Protein concentration was adjusted to 4 μg/mL.

INNOTEST® pTau (pT181) kit (Fijirebio Inc., Cat#81581) was used as an ELISA system for Ser413 phosphorylated Tau protein (pSer413-Tau), but the biotinylated antibody included in the kit (labelled as CONJ1) was replaced with the biotinylated mouse Ta1505 IgG2a antibody as produced above.

The parent mouse antibody, the chimeric antibody, the humanized antibody variants, or the control human IgG were diluted to 200 nM from stocks in sample diluent buffer (provided by INNOTEST® kit). Antibody solutions were then serially diluted and incubated with AD brain P2 fractions previously diluted at 1/1000 in assay plate for 4 hours at room temperature. The antibody-antigen mixture was then added to an HT7 antibody immobilized MT plate (included in the kit), along with diluted biotinylated antibody mouse Ta1505 IgG2a (1/10 in CONJ DIL 1, provided by INNOTEST® kit). The resultant sample was mixed and incubated at 4°C overnight. On the following day, the plate was washed, and an HRP-labeled streptavidin (included in the kit as CONJ2) was added to the plate, followed by incubation for one hour. After washing, a color reagent TMB was added, followed by incubation under light-shielding at room temperature for 30 minutes. The reaction was then stopped with a reaction terminator solution (included in the kit as STOP solution), and absorbance at a wavelength of 450 nm was measured.

Non-linear analysis of the concentration-response curve illustrated in **FIGS. 10A-10E** allows determination of the concentration of each antibody required for the 50% occupancy of Tau pSer413 in AD brain homogenates, as well as the percentage of maximum occupancy level for each antibody. **FIGS. 10A-10E** demonstrate comparable binding characteristics, including *in vitro* level of occupancy and maximum binding of pSer413 Tau antigen, for the parent mouse antibody, the chimeric antibody, and selected humanized antibody variants in brain homogenates of AD patients.

XVI. Example 12: Stability and purity analysis of the newly generated humanized antibody variants

Stability of various antibodies was determined by measuring the melting temperature (Tm1) and aggregation temperature (Tagg) via nano-DSF. Purity of each antibody was measured by SEC and non-reduced capillary SDS (NR-cSDS).

Determination of Tm and Tagg: Tm and Tagg was determined by nano-DSF using a Prometheus NT.48 Differential Scanning Fluorimeter (Nanotemper Technologies) controlled by PR.ThermControl v2.0.4 software. Excitation power was 40% and temperature was increased from 20°C to 95°C at a rate of 1°C/min. Tm and Tagg were automatically measured. Samples

were prepared by diluting to 1 mg/mL in 20 mM sodium acetate, pH 5.5, buffer and drawn by capillary action into a Prometheus glass capillary (PR-L002).

Determination of purity by SEC: SEC was carried out on an ACQUITY UPLC H-Class system. Column used was an ACQUITY UPLC Protein BEH SEC column (Part No. 186005225, 1.7 µm, 200A, 4.6 mm x150 mm) from Waters (Milford, MA). Column temperature was 25°C and 10 µL sample at 1 mg/mL was injected using a system flow rate of 0.5 mL/min. Mobile phase was 100 mM sodium phosphate, 200 mM sodium chloride, and 0.02 % sodium azide, pH 7.0. Data were quantified at both 214 and 280 nm and analyzed using Empower 3 software. A BEH200 SEC Protein Standard Mix (Part No. 186006518) from Waters (Milford, MA) was injected at 10 µg and USP Resolution, Theoretical Plates, and Tailing were measured.

Determination of purity by NR-cSDS: To assess purity by NR-cSDS, 5 µL of each sample at 1 mg/mL was mixed in a 96 well plate with 35 µL of loading buffer (HT Protein Express Sample Buffer (Perkin Elmer)) containing 50 mM iodoacetamide. The plate was incubated at 70°C for 20 minutes and 75 µL of water was added to each well. Each sample was analyzed on a LabChip GXII System (Perkin Elmer) using an HT Protein Express Chip (Perkin Elmer). Electropherograms were collected by measuring the fluorescence of the sample over time, and integrated using the LabChip GX software V4.1.1619.0 SP1 (Perkin Elmer).

Table 14 summarizes the stability and purity of the tested antibodies.

Table 14. Stability and Purity of Various Antibodies

	Stability		**Purity**	
	Tm1	**Tagg**	**% SEC purity**	**%NR-cSDS Purity**
ms IgG2a	65.0	ND	99.1	100
chim. IgG1	ND	ND	96.2	ND
chim. IgG4	ND	ND	95.3	ND
hu VL46 / VH11 IgG1	68.5	69.5	99.0	100
hu VL46 / VH11 IgG4 (ref)	66.8	66.0	98.4	100
hu VL46 / VH11 G34A IgG1	65.9	66.2	100	100

hu VL46 / VH11 G34A IgG4	64.0	63.8	100	98.0
hu VL46 / VH11 G34A IgG4	65.3	66.6	100	100
hu VL46 / VH11 G34S IgG4	ND	ND	ND	100.0
hu VL46 / VH11 G34T IgG4	65.8	66.5	ND	99.3
hu VL46 / VH11 N33Q IgG4	65.2	67.1	99.6	100
hu VL46 / VH11 N33Q G34A IgG4	ND	ND	ND	100
hu VL46 / VH11 N33D IgG4	ND	ND	ND	100
hu VL46 / VH11 N33S IgG4	ND	ND	ND	99.8
hu VL46 / VH11 N33T IgG4	ND	ND	ND	100
hu VL46 / VH11 A64D IgG4	ND	ND	ND	100
hu VL46 / VH11 S28N IgG4	68.1	67.8	99.6	100
hu VL47 / VH65 IgG1	59.6	60.2	99.9	100
hu VL46 / VH11 G34A S28N IgG1	65.5	66.7	100	97.0
hu VL46 / VH11 G34A S28N IgG4	63.8	65.0	100	97.0

All of the newly generated humanized antibody variants maintained or improved stability and purity compared to the parent mouse antibody or the chimeric antibodies. One of the original humanized variant huVL47/VH65 _IgG1 stood out as having reduced stability as measured by Tm1/Tagg.

XVII. Example 13: Deamidation analysis of the newly generated humanized antibody variants

Deamidation level of amino acid N33 in the light chain CDR1 of various antibodies were determined. Stress conditions, such as 50°C or pH 10 incubation, were tested. A 4°C incubation was performed as a control.

4°C and 50°C incubation: Samples formulated in 20 mM sodium acetate, pH 5.5, at 2 mg/ml were held at 50°C in a temperature controlled stability chamber for one week. Samples were held at 4°C for the 4°C control for comparison purposes.

pH 10 incubation: Samples formulated in 20 mM sodium acetate, pH 5.5, at 2 mg/mL were adjusted to pH 10 using 0.5 M NaOH and held at 25°C for one week in a temperature controlled stability chamber. Afterwards, samples were then exchanged into 20 mM sodium acetate, pH 5.5, buffer using Zeba Spin desalting columns (7K MWCO, Thermo Fisher 2 mL, 89890).

Deamidation Analysis by Peptide Mapping: For peptide mapping by mass spectrometry, 100 µg of each sample was denatured with 30 µL of 8 M guanidine/1 M Tris hydrochloride solution (15:1), reduced with 2 µL of 1 M DTT for 30 minutes at 60°C and alkylated with 5 µL of 1 M iodoacetamide for 45 minutes in dark. Before digestion, samples were buffer exchanged into 50 mM ammonium bicarbonate using 7 kilo Dalton molecular weight cut off ZEBA cartridges. Samples were split into different tubes and digested in parallel with 2 µg of trypsin and chymotrypsin for 2 hours at 37°C. Digestion was quenched by the addition of 3 µL of 5 M hydrochloride to each sample. 2 µL of sample was injected on the Peptide BEH-C18 – 1x50 mm Waters UPLC column (Part # 186005592) maintained at 40°C. Data were acquired in a Dionex/QE plus MS using a linear gradient over 50 min from 2% to 36% acetonitrile in 0.1% formic acid. Samples were analyzed using PEAKS DB (Bioinformatics Solutions Inc.) for database searching as well as PepFinder (Thermo Fisher Scientific) and manual verification for the percent change assessment. **Table 15** summarizes deamidation percentage of amino acid N33 in the light chain CDR1 of the tested antibodies.

Table 15. Deamidation Percentage of N33 in Light Chain CDR1 under Various Conditions

	4°C control	50°C	pH 10
ms IgG2a	41.1	36.9	31.7
hu VL46 / VH11 IgG1	33.0	33.9	31.8
hu VL46 / VH11 IgG4	32.0	31.0	37.9
hu VL46 / VH11 G34A IgG4	<1%	ND	<1%
hu VL46 / VH11 G34T IgG4	<1%	ND	<1%
hu VL46 / VH11 N33Q IgG4	<1%	ND	<1%
hu VL46 / VH11 G34A S28N IgG1	<1%	1.2	1%
hu VL46 / VH11 G34A S28N IgG4	<1%	2.1	1%

Compared to the parent mouse antibody and the original humanized variant (VL46 / VH11) IgG1 or IgG4 antibody, the newly generated humanized antibody variants exhibited significant reduction in the deamidation level of N33 in the light chain CDR1.

CLAIMS

What Is Claimed Is:

1. An anti-pSer413 tau antibody comprising:

a) a heavy variable domain selected from SEQ ID NO:116 and SEQ ID NO:117; and

b) a light variable domain selected from SEQ ID NO:114, SEQ ID NO:104, SEQ ID NO:105, SEQ ID NO:106, SEQ ID NO:107, SEQ ID NO:108, SEQ ID NO:109, SEQ ID NO:110, SEQ ID NO:111, SEQ ID NO:112, SEQ ID NO:113 and SEQ ID NO:103.

2. An anti-pSer413 tau antibody comprising:

a) a heavy variable domain selected from SEQ ID NO:116 and SEQ ID NO:117; and

b) a light variable domain selected from SEQ ID NO:114, SEQ ID NO:104, SEQ ID NO:105, SEQ ID NO:106, SEQ ID NO:107, SEQ ID NO:108, SEQ ID NO:109, SEQ ID NO:110, SEQ ID NO:111, SEQ ID NO:112, SEQ ID NO:113 and SEQ ID NO:103; wherein the ratio of said antibody's binding affinity to the phosphorylated peptide of SEQ ID NO:8 to said antibody's binding affinity to the non-phosphorylated peptide of SEQ ID NO:69 is at least about 40.

3. The anti-pSer413 tau antibody according to any previous claim wherein said antibody comprises a heavy constant domain selected from SEQ ID NO:135, SEQ ID NO:136, SEQ ID NO:137, SEQ ID NO:138, SEQ ID NO:139, SEQ ID NO:140, SEQ ID NO:141, SEQ ID NO:142 and SEQ ID NO:144.

4. The anti-pSer413 tau antibody according to any previous claim wherein said antibody comprises a light constant domain selected from SEQ ID NO:79 and SEQ ID NO:80.

5. The anti-pSer413 tau antibody according to any previous claim wherein said variable light domain has SEQ ID NO:114 and said variable heavy domain has SEQ ID NO:116.

6. The anti-pSer413 tau antibody according to claim 5 wherein the light chain of said antibody has SEQ ID NO:184 and the heavy chain of said antibody has SEQ ID NO:144.

7. The anti-pSer413 tau antibody according to claim 5 wherein said light chain has SEQ ID NO:184 and the heavy chain of said antibody has SEQ ID NO:152.

8. The anti-pSer413 tau antibody according to any previous claim wherein said heavy chain and said light chain are selected from the pairs LC SEQ ID NO:184 and HC SEQ ID

NO:144; LC SEQ ID NO:184 and HC SEQ ID NO:145; LC SEQ ID NO:184 and HC SEQ ID NO:146; LC SEQ ID NO:184 and HC SEQ ID NO:147; LC SEQ ID NO:184 and HC SEQ ID NO:148; LC SEQ ID NO:184 and HC SEQ ID NO:149; LC SEQ ID NO:184 and HC SEQ ID NO:150; LC SEQ ID NO:184 and HC SEQ ID NO:151; LC SEQ ID NO:184 and HC SEQ ID NO:152; LC SEQ ID NO:184 and HC SEQ ID NO:153; LC SEQ ID NO:184 and HC SEQ ID NO:154; LC SEQ ID NO:184 and HC SEQ ID NO:155; LC SEQ ID NO:184 and HC SEQ ID NO:156; LC SEQ ID NO:184 and HC SEQ ID NO:157; LC SEQ ID NO:184 and HC SEQ ID NO:158; LC SEQ ID NO:184 and HC SEQ ID NO:159; LC SEQ ID NO:184 and HC SEQ ID NO:160; LC SEQ ID NO:184 and HC SEQ ID NO:161; LC SEQ ID NO:185 and HC SEQ ID NO:144; LC SEQ ID NO:185 and HC SEQ ID NO:145; LC SEQ ID NO:185 and HC SEQ ID NO:146; LC SEQ ID NO:185 and HC SEQ ID NO:147; LC SEQ ID NO:185 and HC SEQ ID NO:148; LC SEQ ID NO:185 and HC SEQ ID NO:149; LC SEQ ID NO:185 and HC SEQ ID NO:150; LC SEQ ID NO:185 and HC SEQ ID NO:151; LC SEQ ID NO:185 and HC SEQ ID NO:152; LC SEQ ID NO:185 and HC SEQ ID NO:153; LC SEQ ID NO:185 and HC SEQ ID NO:154; LC SEQ ID NO:185 and HC SEQ ID NO:155; LC SEQ ID NO:185 and HC SEQ ID NO:156; LC SEQ ID NO:185 and HC SEQ ID NO:157; LC SEQ ID NO:185 and HC SEQ ID NO:158; LC SEQ ID NO:185 and HC SEQ ID NO:159; LC SEQ ID NO:185 and HC SEQ ID NO:160 and LC SEQ ID NO:185 and HC SEQ ID NO:161.

9. A nucleic acid composition comprising:

a) a first nucleic acid encoding a light chain selected from SEQ ID NO:162, SEQ ID NO:163, SEQ ID NO:164, SEQ ID NO:165, SEQ ID NO:166, SEQ ID NO:167, SEQ ID NO:168, SEQ ID NO:169, SEQ ID NO:170, SEQ ID NO:171, SEQ ID NO:172, SEQ ID NO:173, SEQ ID NO:174, SEQ ID NO:175, SEQ ID NO:176, SEQ ID NO:177, SEQ ID NO:178, SEQ ID NO:179, SEQ ID NO:180, SEQ ID NO:181, SEQ ID NO:182, SEQ ID NO:183, SEQ ID NO:184 and SEQ ID NO:185; and

b) a second nucleic acid encoding a heavy chain selected from SEQ ID NO:144; SEQ ID NO:145; SEQ ID NO:146; SEQ ID NO:147; SEQ ID NO:148; SEQ ID NO:149; SEQ ID NO:150; SEQ ID NO:151; SEQ ID NO:152; SEQ ID NO:153; SEQ ID NO:154; SEQ ID NO:155; SEQ ID NO:156; SEQ ID NO:157; SEQ ID NO:158; SEQ ID NO:159; SEQ ID NO:160 and SEQ ID NO:161.

10. An expression vector composition comprising the nucleic acid composition according to claim 9, wherein said first nucleic acid is contained in a first expression vector and said second nucleic acid is contained in a second expression vector.

11. An expression vector composition comprising the nucleic acid composition according to claim 9, wherein said first nucleic acid and said second nucleic acid is contained in an expression vector.

12. A host cell comprising the expression vector composition of claim 10 or 11.

13. A method of making an anti-pSer413 tau antibody comprising culturing said host cell of claim 12 under conditions wherein said antibody is expressed and recovering said antibody.

14. A method of treating a tauopathy in a subject comprising administering to the subject the anti-pSer413 tau antibody of any one of claims 1 to 8.

15. An anti-pSer413 tau antibody comprising:

a) a heavy chain variable domain comprising a vhCDR1 comprising SEQ ID NO:86, a vhCDR2 comprising SEQ ID NO:115 and a vhCDR3 comprising SEQ ID NO:88; and

b) a light chain variable domain comprising a set of vlCDRs selected from the group consisting of:

i) a vlCDR1 comprising SEQ ID NO:102, a vlCDR2 comprising SEQ ID NO:82 and a vlCDR3 comprising SEQ ID NO:83;

ii) a vlCDR1 comprising SEQ ID NO:91, a vlCDR2 comprising SEQ ID NO82: and a vlCDR3 comprising SEQ ID NO:83;

iii) a vlCDR1 comprising SEQ ID NO:92, a vlCDR2 comprising SEQ ID NO:82 and a vlCDR3 comprising SEQ ID NO:83;

iv) a vlCDR1 comprising SEQ ID NO:93, a vlCDR2 comprising SEQ ID NO82: and a vlCDR3 comprising SEQ ID NO:83;

v) a vlCDR1 comprising SEQ ID NO:94, a vlCDR2 comprising SEQ ID NO:82 and a vlCDR3 comprising SEQ ID NO:83;

vi) a vlCDR1 comprising SEQ ID NO:95, a vlCDR2 comprising SEQ ID NO:82 and a vlCDR3 comprising SEQ ID NO:83;

vii) a vlCDR1 comprising SEQ ID NO:96, a vlCDR2 comprising SEQ ID NO:82 and a vlCDR3 comprising SEQ ID NO:83;

viii) a vlCDR1 comprising SEQ ID NO:97, a vlCDR2 comprising SEQ ID NO:82 and a vlCDR3 comprising SEQ ID NO:83;

ix) a vlCDR1 comprising SEQ ID NO:98, a vlCDR2 comprising SEQ ID NO:82 and a vlCDR3 comprising SEQ ID NO:83;

x) a vlCDR1 comprising SEQ ID NO:99, a vlCDR2 comprising SEQ ID NO:82 and a vlCDR3 comprising SEQ ID NO:83;

xi) a vlCDR1 comprising SEQ ID NO:100, a vlCDR2 comprising SEQ ID NO:82 and a vlCDR3 comprising SEQ ID NO:83; and

xii) a vlCDR1 comprising SEQ ID NO:101, a vlCDR2 comprising SEQ ID NO:82 and a vlCDR3 comprising SEQ ID NO:83.

16. An anti-pSer413 tau antibody comprising:

a) a heavy chain variable domain comprising a vhCDR1 comprising SEQ ID NO:86, a vhCDR2 comprising SEQ ID NO:115 and a vhCDR3 comprising SEQ ID NO:88; and

b) a light chain variable domain comprising a set of vlCDRs selected from the group consisting of:

i) a vlCDR1 comprising SEQ ID NO:102, a vlCDR2 comprising SEQ ID NO:82 and a vlCDR3 comprising SEQ ID NO:83;

ii) a vlCDR1 comprising SEQ ID NO:91, a vlCDR2 comprising SEQ ID NO82: and a vlCDR3 comprising SEQ ID NO:83;

iii) a vlCDR1 comprising SEQ ID NO:92, a vlCDR2 comprising SEQ ID NO:82 and a vlCDR3 comprising SEQ ID NO:83;

iv) a vlCDR1 comprising SEQ ID NO:93, a vlCDR2 comprising SEQ ID NO82: and a vlCDR3 comprising SEQ ID NO:83;

v) a vlCDR1 comprising SEQ ID NO:94, a vlCDR2 comprising SEQ ID NO:82 and a vlCDR3 comprising SEQ ID NO:83;

vi) a vlCDR1 comprising SEQ ID NO:95, a vlCDR2 comprising SEQ ID NO:82 and a vlCDR3 comprising SEQ ID NO:83;

vii) a vlCDR1 comprising SEQ ID NO:96, a vlCDR2 comprising SEQ ID NO:82 and a vlCDR3 comprising SEQ ID NO:83;

viii) a vlCDR1 comprising SEQ ID NO:97, a vlCDR2 comprising SEQ ID NO:82 and a vlCDR3 comprising SEQ ID NO:83;

ix) a vlCDR1 comprising SEQ ID NO:98, a vlCDR2 comprising SEQ ID NO:82 and a vlCDR3 comprising SEQ ID NO:83;

x) a vlCDR1 comprising SEQ ID NO:99, a vlCDR2 comprising SEQ ID NO:82 and a vlCDR3 comprising SEQ ID NO:83;

xi) a vlCDR1 comprising SEQ ID NO:100, a vlCDR2 comprising SEQ ID NO:82 and a vlCDR3 comprising SEQ ID NO:83; and

xii) a vlCDR1 comprising SEQ ID NO:101, a vlCDR2 comprising SEQ ID NO:82 and a vlCDR3 comprising SEQ ID NO:83;

wherein the ratio of said antibody's binding affinity to the phosphorylated peptide of SEQ ID NO:8 to said antibody's binding affinity to the non-phosphorylated peptide of SEQ ID NO:69 is at least about 40.