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(54) Title: ANTIBODIES SPECIFIC TO TAU PHOSPHORYLATED AT SERINE 422 AND USES FOR THE TREATMENT AND DIAGNOSIS OF TAUOPATHIES

(57) Abstract: The present invention relates to antibodies or fragments thereof that specifically bind to Tau phosphorylated at serine 422 (p S422), and to their use for treating and diagnosing Alzheimer's disease, Tauopathies and related Tau disorders.



ANTIBODIES SPECIFIC TO TAU PHOSPHORYLATED AT SERINE 422 AND USES FOR THE TREATMENT AND DIAGNOSIS OF TAUOPATHIES

FIELD OF THE INVENTION:

The present invention relates to antibodies or fragments thereof that specifically bind to Tau phosphorylated at serine 422 (pS422), and to their use for treating and diagnosing Tauopathies.

BACKGROUND OF THE INVENTION:

Human Tau is a neuronal microtubule-associated protein found predominantly in axons and functions to promote tubulin polymerization and stabilize microtubules. Six isoforms (isoform A, B, C, D, E, F, G, fetal-Tau) are found in the human brain, the longest isoform comprising 441 amino acids (isoform F, Uniprot P10636-8). Tau and its properties are also described by Reynolds, C. H. et al., J. Neurochem. 69 (1997) 191-198. Tau, in its hyperphosphorylated form, is the major component of paired helical filaments (PHF), the building block of neurofibrillary lesions in Alzheimer's disease (AD) brain. Tau can be phosphorylated at its serine or threonine residues by several different kinases including GSK3beta, cdk5, MARK and members of the MAP kinase family

Protein misfolded aggregates affect a number of different neurodegenerative diseases including Alzheimer's disease, Parkinsonism and other Tauopathies. Several studies have provided the hypothesis that protein aggregates spread in predictable sequences between anatomically related brain regions (Delacourte A, David J-P, Sergeant N, Buée L, Wattez A, Vermersch P, Ghazali F, Fallet-Bianco C, Pasquier F, Lebert F, Petit H, DiMenza C. The biochemical pathway of neurofibrillary degeneration in aging and Alzheimer's disease. Neurology, 52 (1999) 1158-1165.; Verny, M., C. Duyckaerts, Y. Agid, and J. J. Hauw. "The Significance of Cortical Pathology in Progressive Supranuclear Palsy. Clinico-Pathological Data in 10 Cases." Brain 119 (Pt 4) (Aug 1996): 1123-36. Saito, Y., N. N. Ruberu, M. Sawabe, T. Arai, N. Tanaka, Y. Kakuta, H. Yamanouchi, and S. Murayama. "Staging of Argyrophilic Grains: An Age-Associated Tauopathy." J Neuropathol Exp Neurol 63, no. 9 (Sep 2004):911-8.). Such spreading of the Tau pathology strongly suggests an extracellular Tau component (Goedert M, Clavaguera F, Tolnay M. The propagation of prion-like protein inclusions in neurodegenerative diseases. Trends Neurosci. 2010 Jul;33(7):317-25 ; Frost B, Diamond MI. Prion-like mechanisms in neurodegenerative diseases. Nat Rev Neurosci. 2010

Mar;11(3):155-9). Thus new approaches based on antibody therapies may act by blocking the « spread » of the pathological process. Vaccination of mice in experimental models of Tauopathy (for review Gu and Sigurdsson, 2011) and Synucleinopathy (Masliah and al., 2005) has previously been reported to ameliorate pathology. Despite any adverse effect reported in these studies, passive immunotherapy may be preferred. Indeed, vaccination usually involves delivery of a strong adjuvant to boost antibody production and may induce an undesirable immune response as in the AN-1792 clinical trial (Orgogozo and al., 2003). Passive immunotherapy uses antibodies that are specific and then, safer than vaccination. Passive immunization has been shown to be effective in reducing A β (for review Jicha, 2009) and more recently intracellular protein like synuclein (Masliah and al., 2011) and Tau (Boutajangout and al., 2011; Chai and al., 2011). As for vaccination, the choice of the epitope of Tau to target is very important. Indeed, targeting non-phosphorylated Tau has been shown to be deleterious (Rosenmann and al., 2006).

Bussiere, T. et al. (Acta Neuropathol. 97 (1999) 221-230) describes that phosphorylated serine 422 on Tau proteins is a pathological epitope found in several diseases with neurofibrillary degeneration. Augustinack, J. C. et al. describe phosphorylated serine 422 as correlating with the severity of neuronal pathology in Alzheimer's disease (Acta Neuropathol 103 (2002) 26-35). Guillozet-Bongaarts, A. (J. Neurochem 97 (2006) 1005-1014) describe the phosphorylation serine 422 on Tau protein as being part of the maturation process of PHFs. Phosphorylation serine 422 on Tau protein is also found in association with developing pathology in various transgenic mouse models of Alzheimer's disease. Thus, Deters, N. et al. mention in Biochem. Biophys. Res. Commun. 379 (2009) 400-405 that double-transgenic Dom5/pR5 mice showed 7-fold increased numbers of hippocampal neurons that contain Tau specifically phosphorylated the pathological S422 epitope. Goetz, J. et al. (Science 293 (2001) 1491-1495) reported the appearance of Tau phosphorylated at serine 422 in the brains of Tau P301L transgenic mice injected with Abeta42 fibrils. Due to involvement of Tau phosphorylated at Serine 422 in Tau pathology, various assays for its detection and quantification in view of the diagnosis and monitoring of these disorders have been proposed

WO2010142423 describes antibodies binding to Tau that is phosphorylated at serine 422 (pS422). Monoclonal antibodies against Tau pS422 are also described in EP 1 876 185. Polyclonal antibodies against Tau pS422 are commercially available (e.g. ProSci Inc. and Biosource International). However, antibodies directed against Tau phosphorylated at Ser422, that are able to reduce the pathological Tau species, to correct the cognitive impairment produced by Tauopathy pathology and accelerate the subsequent central

degradation/clearance of Tau phosphorylated at Ser422 correlated with increased of Tau in the plasma have not yet been described in the prior art

SUMMARY OF THE INVENTION:

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The inventors provide an antibody that specifically binds to Tau phosphorylated at serine 422 (pS422) and demonstrate that said antibody is able to reduce the pathological Tau aggregation, to accelerate the subsequent central degradation of Tau phosphorylated at Ser422 correlated with increased of Tau in the plasma and finally to correct the cognitive impairment produced by Tauopathy pathology. The present invention thus provides a publicly available source for said antibody This monoclonal antibody, hereinafter designated "2H9" is indeed produced by the hybridoma deposited in accordance with the terms of Budapest Treaty, at the CNCM (Collection Nationale de Cultures de Microorganismes, Institut Pasteur, 25 rue du Docteur Roux, 75724 Paris Cedex 15, France), on August 7, 2012, under the deposit number
15 CNCM-I-4666.

DETAILED DESCRIPTION OF THE INVENTION:

Definitions:

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The term "Tau" according to the invention refers to all Tau isoforms derived from the MAPT gene. All numbering is given from the longest brain isoform of human Tau, comprising 441 amino acids (isoform F, Uniprot P10636-8). The term "Tau phosphorylated at serine 422 (pS422)" thus refers to the Tau protein phosphorylated on the serine residue at
25 position 422 on the 441 Tau isoform but this Ser residue is found in all Tau isoforms.

The term "tauopathy" has its general meaning in the art and refers to a disease characterized by an abnormal hyperphosphorylation of Tau (Iqbal, K. et al. Biochimica et Biophysica Acta (BBA) 1739 (2005) 198-210). Tauopathies include among others, Alzheimer's Disease, Down syndrome; Guam parkinsonism dementia complex; Dementia pugilistica and other chronic traumatic encephalopathies; myotonic dystrophies; Niemann-Pick disease type C; Pick disease; argyrophilic grain disease; Frontotemporal dementia; Cortico-basal degeneration; Pallido-ponto-nigral degeneration; Progressive supranuclear palsy; and Prion disorders such as Gerstmann-Sträussler-Scheinker disease with tangles.

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According to the present invention, “antibody” or “immunoglobulin” have the same meaning, and will be used equally in the present invention. The term “antibody” as used herein refers to immunoglobulin molecules and immunologically active portions of immunoglobulin molecules, i.e., molecules that contain an antigen binding site that immunospecifically binds an antigen. As such, the term antibody encompasses not only whole antibody molecules, but also antibody fragments as well as variants (including derivatives) of antibodies and antibody fragments. In natural antibodies, two heavy chains are linked to each other by disulfide bonds and each heavy chain is linked to a light chain by a disulfide bond. There are two types of light chain, lambda (l) and kappa (k). There are five main heavy chain classes (or isotypes) which determine the functional activity of an antibody molecule: IgM, IgD, IgG, IgA and IgE. Each chain contains distinct sequence domains. The light chain includes two domains, a variable domain (VL) and a constant domain (CL). The heavy chain includes four domains, a variable domain (VH) and three constant domains (CH1, CH2 and CH3, collectively referred to as CH). The variable regions of both light (VL) and heavy (VH) chains determine binding recognition and specificity to the antigen. The constant region domains of the light (CL) and heavy (CH) chains confer important biological properties such as antibody chain association, secretion, trans-placental mobility, complement binding, and binding to Fc receptors (FcR). The Fv fragment is the N-terminal part of the Fab fragment of an immunoglobulin and consists of the variable portions of one light chain and one heavy chain. The specificity of the antibody resides in the structural complementarity between the antibody combining site and the antigenic determinant. Antibody combining sites are made up of residues that are primarily from the hypervariable or complementarity determining regions (CDRs). Occasionally, residues from nonhypervariable or framework regions (FR) influence the overall domain structure and hence the combining site. Complementarity Determining Regions or CDRs refer to amino acid sequences which together define the binding affinity and specificity of the natural Fv region of a native immunoglobulin binding site. The light and heavy chains of an immunoglobulin each have three CDRs, designated VL-CDR1, VL-CDR2, VL-CDR3 and VH-CDR1, VH-CDR2, VH-CDR3, respectively. An antigen-binding site, therefore, includes six CDRs, comprising the CDR set from each of a heavy and a light chain V region. Framework Regions (FRs) refer to amino acid sequences interposed between CDRs.

The monoclonal antibody, hereinafter designated “2H9” is produced by the hybridoma deposited in accordance with the terms of Budapest Treaty, at the CNCM (Collection Nationale de Cultures de Microorganismes, Institut Pasteur, 25 rue du Docteur Roux, 75724 Paris Cedex 15, France), on August 7, 2012, under the deposit number CNCM-I-4666.

The inventors have cloned and sequenced the variable domain (VL) of the light chain, and the variable domain (VH) of the heavy chain of the monoclonal antibody 2H9. The location of the sequences encoding the complementarity determining regions (CDRs) of said antibody have been determined with reference to other antibody sequences (Kabat EA et al., 1991).

These sequences are described below in Table 1 (for the heavy chain) and Table 2 (for the light chain).

TABLE 1

mAb 2H9 Domains	DNA Sequence	Aminoacid Sequence
VH	CAGGTCCAAGTCTGCAGCCTGGGACT GAACTGGTGAAGCCTGGGGCTTCAGTG AAGCTGTCCTGCAAGGCTTCTGGCTACA CCTTCACCAGCTACTGGATGCACTGGG TGAAGCAGAGGCCTGGACAAGGCCTTG AGTGGATTGGAAATATCAATCCTAACAA TGGTGGTACTAACTACAATGAGAAAGTTC AAGAACAAGGCCACACTGACTGTAGAC AAATCCTCCAGTACAGCCTACATGCACC TCAGCAGCCTGACATCTGAGGACTCTG CGGTCTATTATTGTGCAGGGGGGAGAA ATTACGATGTCTGGGGCACAGGGACCA CGGTCACCGTCTCCTCA (SEQ ID NO:3)	QVQLLPQGTSLVPGASVKLSCKASG YTFTSYWMHWVKRPGQGLEWIGNI NPNNGGTNYNEKFKNKATLTVDKSS STAYMHLSSLTSEDSAVYYCAGGRN YDVWGTGTTVTSS (SEQ ID NO:11)
VH-CDR1	GGATTCACCTTTCAGTAGATATAGC (SEQ ID NO:4)	GYTFTSYWMH (SEQ ID NO:12)
VH-CDR2	AATATCAATCCTAACAAATGGTGGTACTA ACTACAATGAGAAGTTCAAGAAC (SEQ ID NO:5)	NINPNNGGTNYNEKFKN (SEQ ID NO:13)
VH-CDR3	GGGAGAAATTACGATGTC (SEQ ID NO:6)	GRNYDV (SEQ ID NO:14)

TABLE 2

mAb 2H9 Domains	DNA Sequence	Aminoacid Sequence
VL	GATGTTTTGATGACCCAACTCCACTCT CCCTGCCTGTCAGTCTTGGAGATCAAG CCTCCATCTCTTGCAGATCTAGTCAGAA CATTGTATATGGTAATGGAAACACCTAT TTAGAATGGTACCTGCAGAGACCAGGC CAGTCTCCAAAGCTCCTGATCTACAAAG TTTCCAACCGATTTTCTGGGGTCCCAGA CAGGTTCAAGTGGCAGTGGATCAGGGAC AGATTTCACTCAAGATCACCAGAGTG GAGGCTGAGGATCTGGGAGTTTATTACT GCTTTCAAGGTTACATGTTCCGCTCAC GTTCCGGTGCTGGGACCAAGCTGGAGCT GAAA (SEQ ID NO:7)	DVLMQTPLSLPVSLGDQASISCRSSQNI VYNGNTYLEWYLQRPQSPKLLIYKVS NRFSGVPDRFSGSGSGTDFTLKTRVEAE DLGVYYCFQGSHVPLTFGAGTKLELK (SEQ ID NO:15)
VL-CDR1	AGATCTAGTCAGAACATTGTATATGGTA ATGGAAACACCTATTAGAA (SEQ ID NO:8)	RSSQNIYVNGNTYLE (SEQ ID NO:16)
VL-CDR2	AAAGTTTCCAACCGATTTTCT (SEQ ID NO:9)	KVSNRFS (SEQ ID NO:17)
VL-CDR3	TTTCAAGGTTACATGTTCCGCTCACG (SEQ ID NO:10)	FQGSHPVLT (SEQ ID NO:18)

The term "chimeric antibody" refers to an antibody which comprises a VH domain and a VL domain of an antibody, and a CH domain and a CL domain of a human antibody.

According to the invention, the term "humanized antibody" refers to an antibody having variable region framework and constant regions from a human antibody but retains the

5 CDRs of a previous non human antibody.

The term "antibody fragment" refers to a fragment of an antibody which contain the variable domains comprising the CDRs of said antibody. The basic antibody fragments include Fab, Fab', F(ab')₂ Fv, scFv, dsFv. For example of antibody fragment see also for review, Holliger et al Nature Biotechnology 23, issue 9 1126 - 1136 (2005), which are
10 includes herein by reference.

The term "Fab" denotes an antibody fragment having a molecular weight of about 50,000 and antigen binding activity, in which about a half of the N-terminal side of H chain and the entire L chain, among fragments obtained by treating IgG with a protease, papaine, are bound together through a disulfide bond.

15 The term "F(ab')₂" refers to an antibody fragment having a molecular weight of about 100,000 and antigen binding activity, which is slightly larger than the Fab bound via a disulfide bond of the hinge region, among fragments obtained by treating IgG with a protease, pepsin.

The term "Fab' " refers to an antibody fragment having a molecular weight of about
20 50,000 and antigen binding activity, which is obtained by cutting a disulfide bond of the hinge region of the F(ab')₂.

A single chain Fv ("scFv") polypeptide is a covalently linked VH::VL heterodimer which is usually expressed from a gene fusion including VH and VL encoding genes linked by a peptide-encoding linker. "dsFv" is a VH::VL heterodimer stabilised by a disulfide bond.

25 Divalent and multivalent antibody fragments can form either spontaneously by association of monovalent scFvs, or can be generated by coupling monovalent scFvs by a peptide linker, such as divalent sc(Fv)₂.

The term "diabodies" "tribodies" or "tetrabodies" refers to small antibody fragments with multivalent antigen-binding sites (2, 3 or four), which fragments comprise a heavy-chain variable domain (VH) connected to a light-chain variable domain (VL) in the same polypeptide chain (VH-VL). By using a linker that is too short to allow pairing between the
30 two domains on the same chain, the domains are forced to pair with the complementary domains of another chain and create two antigen-binding sites.

By "purified" and "isolated" it is meant, when referring to an antibody according to the invention or to a nucleotide sequence, that the indicated molecule is present in the substantial absence of other biological macromolecules of the same type. The term "purified" as used herein preferably means at least 75% by weight, more preferably at least 85% by weight, more preferably still at least 95% by weight, and most preferably at least 98% by weight, of biological macromolecules of the same type are present. An "isolated" nucleic acid molecule which encodes a particular polypeptide refers to a nucleic acid molecule which is substantially free of other nucleic acid molecules that do not encode the polypeptide; however, the molecule may include some additional bases or moieties which do not deleteriously affect the basic characteristics of the composition.

Antibodies of the invention:

The present invention relates to an antibody characterized in that it is capable of binding human Tau phosphorylated at serine 422 and having a variable light chain (VL) comprising the VL-CDR1, VL-CDR2 and VL-CDR3 of the VL chain and a variable heavy chain (VH) comprising the VH-CDR1, VH-CDR2 and VH-CDR3 of the VH chain of the antibody 2H9 obtainable from hybridoma deposited as CNCM-I-4666.

In a particular embodiment, the antibody of the invention which is characterized in that it is capable of binding human Tau phosphorylated at serine 422, has the following 6 CDRs:

	Aminoacid sequence
VH-CDR1	GYTFTSYWMH (SEQ ID NO:12)
VH-CDR2	NINPNNGGTNYNEKFKN (SEQ ID NO:13)
VH-CDR3	GRNYDV (SEQ ID NO:14)
VL-CDR1	RSSQNIVYGNGNTYLE (SEQ ID NO :16)
VL-CDR2	KVSNRFS (SEQ ID NO :17)
VL-CDR3	FQGSHVPLT (SEQ ID NO :18)

In a particular embodiment, said antibody is selected from :

- a) murine monoclonal antibody 2H9 obtainable from the hybridoma CNCM I 4666
- b) chimeric or humanized antibodies obtained from the antibody 2H9;
- c) antibodies which comprises the VL chain and the VH chain of the antibody 2H9;
- d) the fragments of the antibodies a) to c) above

In a particular embodiment, said antibody is selected from the group consisting of, chimeric antibodies, and humanized antibodies.

In another embodiment the antibody of the invention comprises the VL chain and the VH chain of the antibody 2H9 obtainable from hybridoma deposited as CNCM-I-4666.

In another embodiment, the antibody of the invention is a chimeric antibody, which comprises the variable chains (VL and VH) of the antibody 2H9 obtainable from hybridoma deposited as CNCM-I-4666.

In another embodiment, the antibody of the invention is a humanized antibody comprising the CDRs of the antibody 2H9 obtainable from hybridoma deposited as CNCM-I-4666.

A further aspect of the invention thus relates to a murine monoclonal antibody (2H9) obtainable from the hybridoma available under CNCM deposit number I-4666.

The invention further provides fragments of said antibodies which include but are not limited to Fv, Fab, F(ab')₂, Fab', dsFv, scFv, sc(Fv)₂ diabodies, tribodies and tetrabodies.

In a preferred embodiment, the antibody of the invention is able to reduce the brain level of the Tau phosphorylated at Ser422 and its aggregation.

The ability to reduce the level of this pathological Tau species and its aggregation can easily be tested using, for instance, immunohistochemical or biochemical brain analyses in a Tauopathy animal model after passive administration of the tested antibody as described in Troquier et al., 2012, Boutajangout et al., 2007;2010;2011; Chai et al., 2011).

Methods of producing antibodies of the invention:

The antibodies of the invention may be produced by any technique known in the art, such as, without limitation, any chemical, biological, genetic or enzymatic technique, either alone or in combination.

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Knowing the amino acid sequence of the desired sequence, one skilled in the art can readily produce said antibodies, by standard techniques for production of polypeptides. For instance, they can be synthesized using well-known solid phase method, preferably using a commercially available peptide synthesis apparatus (such as that made by Applied Biosystems, Foster City, California) and following the manufacturer's instructions. Alternatively, antibodies of the invention can be synthesized by recombinant DNA techniques well-known in the art. For example, antibodies can be obtained as DNA expression products after incorporation of DNA sequences encoding the antibodies into expression vectors and introduction of such vectors into suitable eukaryotic or prokaryotic hosts that will express the desired antibodies, from which they can be later isolated using well-known techniques.

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Accordingly, a further object of the invention relates to a nucleic acid sequence encoding an antibody according to the invention.

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In a particular embodiment, the invention relates to a nucleic acid sequence encoding the VH domain of the antibody of the invention (e.g. the antibody obtainable from hybridoma deposited as CNCM-I-4666 (2H9)) and/ or the VL domain of the antibody of the invention (e.g. the antibody obtainable from hybridoma deposited as CNCM-I-4666 (2H9)).

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Typically, said nucleic acid is a DNA or RNA molecule, which may be included in any suitable vector, such as a plasmid, cosmid, episome, artificial chromosome, phage or a viral vector.

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The terms "vector", "cloning vector" and "expression vector" mean the vehicle by which a DNA or RNA sequence (e.g. a foreign gene) can be introduced into a host cell, so as to transform the host and promote expression (e.g. transcription and translation) of the introduced sequence.

So, a further object of the invention relates to a vector comprising a nucleic acid of the invention. Such vectors may comprise regulatory elements, such as a promoter, enhancer, terminator and the like, to cause or direct expression of said antibody upon administration to a subject. Examples of promoters and enhancers used in the expression vector for animal cell
5 include early promoter and enhancer of SV40, LTR promoter and enhancer of Moloney mouse leukemia virus, promoter and enhancer of immunoglobulin H chain and the like. Examples of plasmids include replicating plasmids comprising an origin of replication, or integrative plasmids, such as for instance pUC, pcDNA, pBR, and the like. Examples of viral vector include adenoviral, retroviral, herpes virus and AAV vectors. Such recombinant
10 viruses may be produced by techniques known in the art, such as by transfecting packaging cells or by transient transfection with helper plasmids or viruses..

A further object of the present invention relates to a host cell which has been transfected, infected or transformed by a nucleic acid and/or a vector according to the
15 invention and expressing an antibody according to the invention.

Accordingly such recombinant host cells can be used for the production of antibodies of the invention

The term "transformation" means the introduction of a "foreign" (i.e. extrinsic or extracellular) gene, DNA or RNA sequence to a host cell, so that the host cell will express the
20 introduced gene or sequence to produce a desired substance, typically a protein or enzyme coded by the introduced gene or sequence. A host cell that receives and expresses introduced DNA or RNA has been "transformed".

The nucleic acids of the invention may be used to produce an antibody of the
25 invention in a suitable expression system. The term "expression system" means a host cell and compatible vector under suitable conditions, e.g. for the expression of a protein coded for by foreign DNA carried by the vector and introduced to the host cell. Common expression systems include E. coli host cells and plasmid vectors, insect host cells and Baculovirus vectors, and mammalian host cells and vectors. Other examples of host cells include, without
30 limitation, prokaryotic cells (such as bacteria) and eukaryotic cells (such as yeast cells, mammalian cells, insect cells, plant cells, etc.). Specific examples include E.coli, Kluyveromyces or Saccharomyces yeasts, mammalian cell lines (e.g., Vero cells, CHO cells, 3T3 cells, COS cells, etc.) as well as primary or established mammalian cell cultures (e.g., produced from lymphoblasts, fibroblasts, embryonic cells, epithelial cells, nervous cells,

adipocytes, etc.). Examples also include mouse SP2/0-Ag14 cell (ATCC CRL1581), mouse P3X63-Ag8.653 cell (ATCC CRL1580), CHO cell in which a dihydrofolate reductase gene (hereinafter referred to as "DHFR gene") is defective (Urlaub G et al; 1980), rat YB2/3HL.P2.G11.16Ag.20 cell (ATCC CRL1662, hereinafter referred to as "YB2/0 cell"), and the like.

The present invention also relates to a method of producing an antibody according to the invention, said method comprising the steps of: (i) introducing in vitro or ex vivo a recombinant nucleic acid or a vector as described above into a competent host cell, (ii) culturing in vitro or ex vivo the recombinant host cell obtained (iii), recovering the expressed antibody.

In another particular embodiment, the method comprises the steps of:

- (i) culturing the hybridoma deposited as CNCM-I-4666 under conditions suitable to allow expression of 2H9 antibody; and
- (ii) recovering the expressed antibody.

Antibodies of the invention are suitably separated from the culture medium by conventional immunoglobulin purification procedures such as, for example, protein A-Sepharose, hydroxylapatite chromatography, gel electrophoresis, dialysis, or affinity chromatography.

In a particular embodiment, the human chimeric antibody of the present invention can be produced by obtaining nucleic sequences encoding VL and VH domains as previously described, constructing a human chimeric antibody expression vector by inserting them into an expression vector for animal cell having genes encoding human antibody CH and human antibody CL, and expressing the coding sequence by introducing the expression vector into an animal cell. As the CH domain of a human chimeric antibody, it may be any region which belongs to human immunoglobulin, but those of IgG class are suitable and any one of subclasses belonging to IgG class, such as IgG1, IgG2, IgG3 and IgG4, can also be used. Also, as the CL of a human chimeric antibody, it may be any region which belongs to Ig, and those of kappa class or lambda class can be used. Methods for producing chimeric antibodies involve conventional recombinant DNA and gene transfection techniques are well known in the art.

The humanized antibody of the present invention may be produced by obtaining nucleic acid sequences encoding CDR domains, as previously described, constructing a humanized antibody expression vector by inserting them into an expression vector for animal
5 cell having genes encoding (i) a heavy chain constant region identical to that of a human antibody and (ii) a light chain constant region identical to that of a human antibody, and expressing the genes by introducing the expression vector into an animal cell.

The humanized antibody expression vector may be either of a type in which a gene encoding an antibody heavy chain and a gene encoding an antibody light chain exists on
10 separate vectors or of a type in which both genes exist on the same vector (tandem type). In respect of easiness of construction of a humanized antibody expression vector, easiness of introduction into animal cells, and balance between the expression levels of antibody H and L chains in animal cells, humanized antibody expression vector of the tandem type is preferred. Methods for producing humanized antibodies based on conventional recombinant DNA and
15 gene transfection techniques are well known in the art. Antibodies can be humanized using a variety of techniques known in the art including, for example, CDR-grafting (EP 239,400; PCT publication WO91/09967; U.S. Pat. Nos. 5,225,539; 5,530,101; and 5,585,089), veneering or resurfacing (EP 592,106; EP 519,596), and chain shuffling (U.S. Pat. No.5,565,332). The general recombinant DNA technology for preparation of such antibodies
20 is also known (see European Patent Application EP 125023 and International Patent Application WO 96/02576).

The Fab of the present invention can be obtained by treating an antibody which specifically reacts with human HER3 with a protease, papain. Also, the Fab can be produced
25 by inserting DNA encoding Fab of the antibody into a vector for prokaryotic expression system, or for eukaryotic expression system, and introducing the vector into a procaryote or eucaryote (as appropriate) to express the Fab.

The F(ab')₂ of the present invention can be obtained treating an antibody which
30 specifically reacts with human HER3 with a protease, pepsin. Also, the F(ab')₂ can be produced by binding Fab' described below via a thioether bond or a disulfide bond.

The Fab' of the present invention can be obtained treating F(ab')₂ which specifically reacts with human HER3 with a reducing agent, dithiothreitol. Also, the Fab' can be produced

by inserting DNA encoding Fab' fragment of the antibody into an expression vector for prokaryote, or an expression vector for eukaryote, and introducing the vector into a prokaryote or eukaryote (as appropriate) to perform its expression.

5 The scFv of the present invention can be produced by obtaining cDNA encoding the VH and VL domains as previously described, constructing DNA encoding scFv, inserting the DNA into an expression vector for prokaryote, or an expression vector for eukaryote, and then introducing the expression vector into a prokaryote or eukaryote (as appropriate) to express the scFv. To generate a humanized scFv fragment, a well known technology called CDR
10 grafting may be used, which involves selecting the complementary determining regions (CDRs) from a donor scFv fragment, and grafting them onto a human scFv fragment framework of known three dimensional structure (see, e. g., W098/45322; WO 87/02671; US5,859,205; US5,585,089; US4,816,567; EP0173494).

15 Amino acid sequence modification(s) of the antibodies described herein are contemplated. For example, it may be desirable to improve the binding affinity and/or other biological properties of the antibody. It is known that when a humanized antibody is produced by simply grafting only CDRs in VH and VL of an antibody derived from a non-human animal in FRs of the VH and VL of a human antibody, the antigen binding activity is reduced
20 in comparison with that of the original antibody derived from a non-human animal. It is considered that several amino acid residues of the VH and VL of the non-human antibody, not only in CDRs but also in FRs, are directly or indirectly associated with the antigen binding activity. Hence, substitution of these amino acid residues with different amino acid residues derived from FRs of the VH and VL of the human antibody would reduce of the binding
25 activity. In order to resolve the problem, in antibodies grafted with human CDR, attempts have to be made to identify, among amino acid sequences of the FR of the VH and VL of human antibodies, an amino acid residue which is directly associated with binding to the antibody, or which interacts with an amino acid residue of CDR, or which maintains the three-dimensional structure of the antibody and which is directly associated with binding to
30 the antigen. The reduced antigen binding activity could be increased by replacing the identified amino acids with amino acid residues of the original antibody derived from a non-human animal.

Modifications and changes may be made in the structure of the antibodies of the present invention, and in the DNA sequences encoding them, and still obtain a functional molecule that encodes an antibody with desirable characteristics.

In making the changes in the amino sequences, the hydropathic index of amino acids may be considered. The importance of the hydropathic amino acid index in conferring interactive biologic function on a protein is generally understood in the art. It is accepted that the relative hydropathic character of the amino acid contributes to the secondary structure of the resultant protein, which in turn defines the interaction of the protein with other molecules, for example, enzymes, substrates, receptors, DNA, antibodies, antigens, and the like. Each amino acid has been assigned a hydropathic index on the basis of their hydrophobicity and charge characteristics these are: isoleucine (+4.5); valine (+4.2); leucine (+3.8) ; phenylalanine (+2.8); cysteine/cystine (+2.5); methionine (+1.9); alanine (+1.8); glycine (-0.4); threonine (-0.7); serine (-0.8); tryptophane (-0.9); tyrosine (-1.3); proline (-1.6); histidine (-3.2); glutamate (-3.5); glutamine (-3.5); aspartate (-3.5); asparagine (-3.5); lysine (-3.9); and arginine (-4.5).

A further object of the present invention also encompasses function-conservative variants of the antibodies of the present invention.

"Function-conservative variants" are those in which a given amino acid residue in a protein or enzyme has been changed without altering the overall conformation and function of the polypeptide, including, but not limited to, replacement of an amino acid with one having similar properties (such as, for example, polarity, hydrogen bonding potential, acidic, basic, hydrophobic, aromatic, and the like). Amino acids other than those indicated as conserved may differ in a protein so that the percent protein or amino acid sequence similarity between any two proteins of similar function may vary and may be, for example, from 70 % to 99 % as determined according to an alignment scheme such as by the Cluster Method, wherein similarity is based on the MEGALIGN algorithm. A "function-conservative variant" also includes a polypeptide which has at least 60 % amino acid identity as determined by BLAST or FASTA algorithms, preferably at least 75 %, more preferably at least 85%, still preferably at least 90 %, and even more preferably at least 95%, and which has the same or substantially similar properties or functions as the native or parent protein to which it is compared. Two amino acid sequences are "substantially homologous" or "substantially similar" when greater than 80 %, preferably greater than 85 %, preferably greater than 90 % of the amino acids are

identical, or greater than about 90 %, preferably greater than 95 %, are similar (functionally identical) over the whole length of the shorter sequence. Preferably, the similar or homologous sequences are identified by alignment using, for example, the GCG (Genetics Computer Group, Program Manual for the GCG Package, Version 7, Madison, Wisconsin)

5 pileup program, or any of sequence comparison algorithms such as BLAST, FASTA, etc.

For example, certain amino acids may be substituted by other amino acids in a protein structure without appreciable loss of activity. Since the interactive capacity and nature of a protein define the protein's biological functional activity, certain amino acid substitutions can
10 be made in a protein sequence, and, of course, in its DNA encoding sequence, while nevertheless obtaining a protein with like properties. It is thus contemplated that various changes may be made in the antibodies sequences of the invention, or corresponding DNA sequences which encode said antibodies, without appreciable loss of their biological activity.

15 It is known in the art that certain amino acids may be substituted by other amino acids having a similar hydrophobic index or score and still result in a protein with similar biological activity, i.e. still obtain a biological functionally equivalent protein.

As outlined above, amino acid substitutions are generally therefore based on the
20 relative similarity of the amino acid side-chain substituents, for example, their hydrophobicity, hydrophilicity, charge, size, and the like. Exemplary substitutions which take various of the foregoing characteristics into consideration are well known to those of skill in the art and include: arginine and lysine; glutamate and aspartate; serine and threonine; glutamine and asparagine; and valine, leucine and isoleucine.

25

Accordingly, the invention also provides an antibody comprising a heavy chain and a light chain wherein the variable domain comprises:

- a VH-CDR1 having at least 90% or 95% identity with the VH-CDR1 of of the VH chain of the antibody obtainable from hybridoma deposited as CNCM-I-4666,

30 - a VH-CDR2 having at least 90% or 95% identity with the VH-CDR2 of of the VH chain of the antibody obtainable from hybridoma deposited as CNCM-I-4666,

- a VH-CDR3 having at least 90% or 95% identity with the VH-CDR3 of of the VH chain of the antibody obtainable from hybridoma deposited as CNCM-I-4666,

- a VL-CDR1 having at least 90% or 95% identity with the VL-CDR1 of of the VL chain of the antibody obtainable from hybridoma deposited as CNCM-I-4666,

- a VL-CDR2 having at least 90% or 95% identity with the VL-CDR2 of of the VL chain of the antibody obtainable from hybridoma deposited as CNCM-I-4666,

5 - a VL-CDR3 having at least 90% or 95% identity with the VL-CDR3 of of the VL chain of the antibody obtainable from hybridoma deposited as CNCM-I-4666,

-that specifically binds to tau phosphorylated at serine 422 (pS422) with substantially the same affinity as an antibody having a variable light chain (VL) comprising the VL-CDR1, VL-CDR2 and VL-CDR3 of the VL chain and a variable heavy chain (VH) comprising the
10 VH-CDR1, VH-CDR2 and VH-CDR3 of the VH chain of the antibody 2H9 obtainable from hybridoma deposited as CNCM-I-4666, and more preferably with substantially the same affinity and the same biological activities as the murine antibody 2H9.

The biological activities of the antibody of the invention, are, for example, to reduce the phosphorylated Tau level and its pathological aggregation as described above. The
15 evaluation of the phosphorylated Tau level and its pathological aggregation allows to determine the therapeutic properties of the antibody such as the correction of cognitive impairment produced by Tauopathy.

Said antibodies may be assayed for specific binding by any method known in the art.
20 Many different competitive binding assay format(s) can be used for epitope binding. The immunoassays which can be used include, but are not limited to, competitive assay systems using techniques such western blots, radioimmunoassays, ELISA, "sandwich" immunoassays, immunoprecipitation assays, precipitin assays, gel diffusion precipitin assays, immunoradiometric assays, fluorescent immunoassays, protein A immunoassays, and
25 complement-fixation assays. Such assays are routine and well known in the art (see, e.g., Ausubel et al., eds, 1994 Current Protocols in Molecular Biology, Vol. 1, John Wiley & sons, Inc., New York). For example, the BIAcore® (GE Healthcare, Piscataway, NJ) is one of a variety of surface plasmon resonance assay formats that are routinely used to epitope bin panels of monoclonal antibodies. Additionally, routine cross-blocking assays such as those
30 described in Antibodies, A Laboratory Manual, Cold Spring Harbor Laboratory, Ed Harlow and David Lane, 1988, can be performed.

Another type of amino acid modification of the antibody of the invention may be useful for altering the original glycosylation pattern of the antibody. By "altering" is meant

deleting one or more carbohydrate moieties found in the antibody, and/or adding one or more glycosylation sites that are not present in the antibody. Glycosylation of antibodies is typically N-linked. "N-linked" refers to the attachment of the carbohydrate moiety to the side chain of an asparagine residue. The tripeptide sequences asparagine-X-serine and asparagines-X-threonine, where X is any amino acid except proline, are the recognition sequences for enzymatic attachment of the carbohydrate moiety to the asparagine side chain. Thus, the presence of either of these tripeptide sequences in a polypeptide creates a potential glycosylation site. Addition of glycosylation sites to the antibody is conveniently accomplished by altering the amino acid sequence such that it contains one or more of the above-described tripeptide sequences (for N-linked glycosylation sites).

Another type of covalent modification involves chemically or enzymatically coupling glycosides to the antibody. These procedures are advantageous in that they do not require production of the antibody in a host cell that has glycosylation capabilities for N-or O-linked glycosylation. Depending on the coupling mode used, the sugar(s) may be attached to (a) arginine and histidine, (b) free carboxyl groups, (c) free sulfhydryl groups such as those of cysteine, (d) free hydroxyl groups such as those of serine, threonine, or hydroxyproline, (e) aromatic residues such as those of phenylalanine, tyrosine, or tryptophan, or (f) the amide group of glutamine.

Removal of any carbohydrate moieties present on the antibody may be accomplished chemically or enzymatically. Chemical deglycosylation requires exposure of the antibody to the compound trifluoromethanesulfonic acid, or an equivalent compound. This treatment results in the cleavage of most or all sugars except the linking sugar (N-acetylglucosamine or N-acetylgalactosamine), while leaving the antibody intact. Chemical deglycosylation is described by Sojahn H. et al. (1987) and by Edge, AS. et al. (1981). Enzymatic cleavage of carbohydrate moieties on antibodies can be achieved by the use of a variety of endo- and exoglycosidases as described by Thotakura, NR. et al. (1987).

Another type of covalent modification of the antibody comprises linking the antibody to one of a variety of non proteinaceous polymers, eg., polyethylene glycol, polypropylene glycol, or polyoxyalkylenes, in the manner set forth in US Patent Nos. 4,640, 835; 4,496, 689; 4,301, 144; 4,670, 417; 4,791, 192 or 4,179,337.

Immunoconjugates:

An antibody of the invention can be conjugated with a detectable label to form an immunoconjugate. Suitable detectable labels include, for example, a radioisotope, a fluorescent label, a chemiluminescent label, an enzyme label, a bioluminescent label or colloidal gold. Methods of making and detecting such detectably-labeled immunoconjugates are well-known to those of ordinary skill in the art, and are described in more detail below.

The detectable label can be a radioisotope that is detected by autoradiography. Isotopes that are particularly useful for the purpose of the present invention are ^3H , ^{125}I , ^{131}I , ^{35}S and ^{14}C .

Immunoconjugates can also be labeled with a fluorescent compound. The presence of a fluorescently-labeled antibody is determined by exposing the immunoconjugate to light of the proper wavelength and detecting the resultant fluorescence. Fluorescent labeling compounds include fluorescein isothiocyanate, rhodamine, phycoerytherin, phycocyanin, allophycocyanin, o-phthaldehyde and fluorescamine.

Alternatively, immunoconjugates can be detectably labeled by coupling an antibody to a chemiluminescent compound. The presence of the chemiluminescent-tagged immunoconjugate is determined by detecting the presence of luminescence that arises during the course of a chemical reaction. Examples of chemiluminescent labeling compounds include luminol, isoluminol, an aromatic acridinium ester, an imidazole, an acridinium salt and an oxalate ester.

Similarly, a bioluminescent compound can be used to label immunoconjugates of the present invention. Bioluminescence is a type of chemiluminescence found in biological systems in which a catalytic protein increases the efficiency of the chemiluminescent reaction. The presence of a bioluminescent protein is determined by detecting the presence of luminescence. Bioluminescent compounds that are useful for labeling include luciferin, luciferase and aequorin.

Alternatively, immunoconjugates can be detectably labeled by linking a monoclonal antibody to an enzyme. When the enzyme conjugate is incubated in the presence of the appropriate substrate, the enzyme moiety reacts with the substrate to produce a chemical moiety which can be detected, for example, by spectrophotometric, fluorometric or visual means. Examples of enzymes that can be used to detectably label polyspecific immunoconjugates include β -galactosidase, glucose oxidase, peroxidase and alkaline phosphatase.

An antibody of the invention may be labelled with a metallic chemical element such as lanthanides. Lanthanides offer several advantages over other labels in that they are stable isotopes, there are a large number of them available, up to 100 or more distinct labels, they are relatively stable, and they are highly detectable and easily resolved between detection channels when detected using mass spectrometry. Lanthanide labels also offer a wide dynamic range of detection. Lanthanides exhibit high sensitivity, are insensitive to light and time, and are therefore very flexible and robust and can be utilized in numerous different settings. Lanthanides are a series of fifteen metallic chemical elements with atomic numbers 57-71. They are also referred to as rare earth elements. Lanthanides may be detected using CyTOF technology. CyTOF is inductively coupled plasma time-of-flight mass spectrometry (ICP-MS). CyTOF instruments are capable of analyzing up to 1000 cells per second for as many parameters as there are available stable isotope tags.

Those of skill in the art will know of other suitable labels which can be employed in accordance with the present invention. The binding of marker moieties to monoclonal antibodies can be accomplished using standard techniques known to the art.

Moreover, the convenience and versatility of immunochemical detection can be enhanced by using monoclonal antibodies that have been conjugated with avidin, streptavidin, and biotin.

Diagnostic and therapeutic methods of the invention:

Antibodies of the present invention and immunoconjugates can be used for detecting human Tau phosphorylated at serine 422 (e.g. Fibrillary aggregates comprising thereof), and/or evaluating its amount in a biological sample, in particular a culture medium sample, a whole blood sample, a serum sample, a plasma sample, a cerebrospinal fluid sample, or a brain tissue sample. Therefore they can be used for diagnosing all diseases associated with abnormal Tau phosphorylated at serine 422 levels, whether they are associated with Tau phosphorylated at serine 422 excess.

Accordingly, the method of detection of the invention is consequently useful for the *in vitro* diagnosis of tauopathy.

An object of the invention is a method for detecting human Tau phosphorylated at serine 422, and/or evaluating its amount in a biological sample, wherein said method comprises contacting said sample with an antibody or immunoconjugate of the invention under conditions allowing the formation of an immune complex between human Tau

phosphorylated at serine 422 and said antibody/immunoconjugate, and detecting or measuring the immune complex formed.

The immune complex formed can be detected or measured by a variety of methods using standard techniques, including, by way of non-limitative examples, enzyme-linked
5 immunosorbent assay (ELISA) or other solid phase immunoassays, radioimmunoassay, electrophoresis, immunofluorescence, or Western blot.

A further object of the invention is a method for diagnosing a tauopathy, wherein said method comprising evaluating the amount of Tau phosphorylated at serine 422, as indicated
10 above, in a biological sample from a subject to be tested, and comparing the determined amount with a control value of Tau in a normal subject.

Finally, the invention also provides kits comprising at least one antibody of the invention or a fragment thereof. Kits of the invention can contain an antibody coupled to a solid support, e.g., a tissue culture plate or beads (e.g., sepharose beads). Kits can be provided
15 which contain antibodies for detection and quantification of Tau phosphorylated at serine 422 in vitro, e.g. in an ELISA or a Western blot. Such antibody useful for detection may be provided with a label such as a fluorescent or radiolabel.

A further object of the invention relates to a pharmaceutical composition comprising
20 an antibody of the invention or a fragment thereof for use in the treatment of tauopathy.

A further object of the invention relates to a method for treating a tauopathy comprising administering a subject in need thereof with a therapeutically effective amount of an antibody of the invention or a fragment thereof.

By a "therapeutically effective amount" of the antibody of the invention is meant a
25 sufficient amount of the antibody to treat said tauopathy, at a reasonable benefit/risk ratio applicable to any medical treatment. It will be understood, however, that the total daily usage of the antibodies and compositions of the present invention will be decided by the attending physician within the scope of sound medical judgment. The specific therapeutically effective
30 dose level for any particular patient will depend upon a variety of factors including the disorder being treated and the severity of the disorder; activity of the specific antibody employed; the specific composition employed, the age, body weight, general health, sex and diet of the patient; the time of administration, route of administration, and rate of excretion of

the specific antibody employed; the duration of the treatment; drugs used in combination or coincidental with the specific antibody employed; and like factors well known in the medical arts. For example, it is well known within the skill of the art to start doses of the compound at levels lower than those required to achieve the desired therapeutic effect and to gradually
5 increase the dosage until the desired effect is achieved.

For administration, the antibody of the invention or the fragment thereof is formulated as a pharmaceutical composition. A pharmaceutical composition comprising an antibody of the invention or a fragment thereof can be formulated according to known methods to prepare
10 pharmaceutically useful compositions, whereby the therapeutic molecule is combined in a mixture with a pharmaceutically acceptable carrier. A composition is said to be a “pharmaceutically acceptable carrier” if its administration can be tolerated by a recipient patient. Sterile phosphate-buffered saline is one example of a pharmaceutically acceptable carrier. Formulations may further include one or more excipients, preservatives, solubilizers,
15 buffering agents, albumin to prevent protein loss on vial surfaces, etc. The form of the pharmaceutical compositions, the route of administration, the dosage and the regimen naturally depend upon the condition to be treated, the severity of the illness, the age, weight, and sex of the patient, etc. The pharmaceutical compositions of the invention can be formulated for a topical, oral, parenteral, intranasal, intravenous, intramuscular, subcutaneous
20 or intraocular. To prepare pharmaceutical compositions, an effective amount of the antibody may be dissolved or dispersed in a pharmaceutically acceptable carrier or aqueous medium. The pharmaceutical forms include sterile aqueous solutions or dispersions; formulations including sesame oil, peanut oil or aqueous propylene glycol; and sterile powders for the extemporaneous preparation of sterile injectable solutions or dispersions. It must be stable
25 under the conditions of manufacture and storage and must be preserved against the contaminating action of microorganisms, such as bacteria and fungi. An antibody of the invention can be formulated into a composition in a neutral or salt form. Pharmaceutically acceptable salts include the acid addition salts (formed with the free amino groups of the protein) and which are formed with inorganic acids such as, for example, hydrochloric or
30 phosphoric acids, or such organic acids as acetic, oxalic, tartaric, mandelic, and the like. Salts formed with the free carboxyl groups can also be derived from inorganic bases such as, for example, sodium, potassium, ammonium, calcium, or ferric hydroxides, and such organic bases as isopropylamine, trimethylamine, histidine, procaine and the like. The carrier can also be a solvent or dispersion medium containing, for example, water, ethanol, polyol (for

example, glycerol, propylene glycol, and liquid polyethylene glycol, and the like), suitable mixtures thereof, and vegetable oils. The proper fluidity can be maintained, for example, by the use of a coating, such as lecithin, by the maintenance of the required particle size in the case of dispersion and by the use of surfactants. The prevention of the action of microorganisms can be brought about by various antibacterial and antifungal agents, for example, parabens, chlorobutanol, phenol, sorbic acid, thimerosal, and the like. In many cases, it will be preferable to include isotonic agents, for example, sugars or sodium chloride. Prolonged absorption of the injectable compositions can be brought about by the use in the compositions of agents delaying absorption, for example, aluminium monostearate and gelatin.

The antibodies of the invention may be formulated within a therapeutic mixture to comprise about 0.0001 to 1.0 milligrams, or about 0.001 to 0.1 milligrams, or about 0.1 to 1.0 or even about 10 milligrams per dose or so. Multiple doses can also be administered.

The invention will be further illustrated by the following figures and examples. However, these examples and figures should not be interpreted in any way as limiting the scope of the present invention.

FIGURES:

Figure 1.

2H9 staining in the pyramidal cell layer of the CA1 region of a THY-Tau22 mice aged of 6 months and in the occipital cortex of an Alzheimer patients (A, C & D). No staining was observed in the occipital cortex of a control patients (B). Western blot analysis was performed with total fraction from the occipital cortex of AD patients at different Braak stage and hippocampus of THY-Tau22 (E). 2H9 labeled the Tau triplet (Tau 55, 64 and 69) in AD patients while failing to recognize normal Tau proteins in brain homogenates from control case (not shown). In hippocampus homogenates from THY-Tau22 mice, 2H9 recognizes also the hyperphosphorylated forms of Tau (E).

Figure 2.

PBS, 5 or 10mg/Kg 2H9 antibody were intraperitoneally injected on a weekly basis in THY-Tau22 mice (from 3 to 9 months). Behaviour. (A) During exposure, WT and THY-

Tau22 mice equally explored the arms (not shown). In the test phase, WT mice demonstrated a strong preference for the novel arm as compared to the other arm. This preference was reduced in THY-Tau22 mice. Such defect was prevented by passive immunotherapy as THY-Tau22 mice treated with 5mg/kg and 10mg/kg of 2H9 spent more time in the novel arm. (B)

5 Distance to platform of THY-Tau22 and WT mice during acquisition was similar between the groups. Two days following learning, a probe trial was performed. While WT mice spent significantly more time in the platform quadrant vs. other quadrants, THY-Tau22 control mice did not. THY-Tau22 mice injected with 2H9 at 10mg/kg spent significantly more time in the target quadrant supporting a beneficial effect of active immunotherapy.

10

Figure 3. Effects of passive immunotherapy on Tau proteins in THY-Tau22 mice

pS422 and AT100 stained areas were quantified in THY-Tau22 mice injected either with PBS or the antibody (5 or 10mg/Kg).

A. In the multiple injections experiments, abnormally phosphorylated Tau species as revealed by pS422 immunoreactivity are decreased in mice injected with 10 mg/kg as compared to THY-Tau22 controls ($p=0.0447$ using ANOVA). There was a trend in the decreased levels of phosphorylated Tau species as revealed by AT100 immunoreactivity between injected THY-Tau22 mice and PBS-injected THY-Tau22 mice.

15

B. In the experiment with a double injection at 5.5 and 10.5 months, there was a trend in the decreased levels of phosphorylated Tau species as revealed by pS422 and AT100 immunoreactivities between injected THY-Tau22 mice and PBS-injected THY-Tau22 mice.

20

Figure 4

PBS, 5 or 10mg/Kg 2H9 antibody were intraperitoneally injected in THY-Tau22 mice (5.5 months). One week after injection, Tau plasma levels were analyzed and found significantly increased with a dose-dependent effect.

25

Figure 5

2H9 Hybridoma supernatants were analyzed by serial dilution of medium using ELISA as follows: 96-well microtiter plates (Maxisorp F8; Nunc, Inc.) were coated overnight at 4°C with 100ng/well of S422-Tau peptide, pS422-Tau peptide and an irrelevant peptide (ATP synthase alpha chain; NeoMPS, France) in 50 mM NaHCO₃, pH 9.6. After washes, plates were blocked and several dilutions of medium tested by use of goat anti-mouse IgG horseradish peroxidase-conjugated antibody. Tetramethyl was the substrate. Plates were

30

measured with a spectrophotometer (Multiskan Ascent, Thermo Labsystem) at 450 nm. 2H9 antibody only recognizes the phospho-S422 Tau peptide.

Column 1 Irrelevant Peptide

Column 2 S422 Tau peptide

5 Column 3 Phospho S422 Tau peptide

EXAMPLE:

Material & Methods

10

Animals

For this study, we have used heterozygous males THY-Tau22 and littermate wild type mice as controls (Schindowski and al., 2006; Belarbi and al., 2011 ; Van Der Jeugd and al., 2011, Burnouf et al, 2012). All animals were kept in standard animal cages under
15 conventional laboratory conditions (12h/12h light-dark cycle, 22°C), with *ad libitum* access to food and water. All experiments on animals were performed in compliance with, and following the approval of the local Animal Resources Committee, standards for the care and use of laboratory animals and with French and European Community rules (Approval n° AF 06/2010, March 31, 2010).

20

Generation and characterization of monoclonal antibody 2H9

C57Bl6 mice were immunized with phospho-peptide (NeoMPS, France) containing the epitope Ser422 phosphorylated (in bold) and three amino-acids Tyr-Gly-Gly conjugated to KLH emulsified in Freund's adjuvant: Y14T [Tyr-Gly-Gly-**Ile-Asp-Met-Val-Asp-Ser(PO₃H₂)-Pro-Gln-Leu-Ala-Thr**]. Briefly, the lymphocytes from the spleen of the
25 immunized mice were fused with myeloma cells, according to the method described in (Pandey, 2010). Firstly, the hybridoma supernatants were screened in ELISA as described in (Troquier et al., 2012). Then, the selected hybridomas were tested by immunohistochemistry and Western blot in both brain homogenates from AD patients and THY-Tau22 mice. The
30 selected monoclonal antibody was specific for the phosphor-Y14T peptide. It was then produced, purified by protein G affinity chromatography (HiTrap Protein G HP) and controlled for endotoxin levels (2H9 < 0,15U/mg).

SEQ ID N°1 : YGGIDMVDS(P)PQLAT (phospho-peptide used for immunization)

SEQ ID N°2 IDMVDS(P)PQLAT (epitope Ser422 phosphorylated)

Passive immunotherapy

5 THY Tau22 were injected intraperitoneally (i.p.) with 2H9 a monoclonal antibody IgG2a. Two different doses were tested: 5mg/kg (n=8) and 10 mg/kg (n=10). THY-Tau22 controls (n=11) were injected i.p. with phosphate buffered saline (PBS). Animals received their first injection at 3 months of age and then every week until behavioral testing at 8,5-9 months of age. A second set of animals was used for short passive immunotherapy. PBS
10 (n=5), 5 (n=4) or 10mg/Kg (n=9) 2H9 antibody were intraperitoneally injected in 5.5 months old THY-Tau22 mice. One week after injection, Tau plasma levels were analyzed. Five months later, animals received the same treatment and were sacrificed one week later.

Stereotaxic injections

15 THY-Tau22 mice aged of 5 months were anesthetized with ketamine (10 mg/mL) and xylazine (1.5 mg/mL), and then positioned on a stereotaxic frame. Two injections were made with 1µL of monoclonal antibodies (AT8, AT100 (Innogenetics, Gent, Belgium) and 2H9) at 1µg/µL and/or the same volume of PBS buffer or isotype control into the hippocampus in the left and right hemispheres at a rate of 0.2 µL/min. After one week, mice were sacrificed and
20 transcordially perfused sequentially with 0.9% NaCl and 4% paraformaldehyde in 0.1 mol/L phosphate-buffered saline (pH 7.4). Brains were post fixed for 2 days in 4% paraformaldehyde and then incubated in 20% sucrose for 24 hours and finally kept frozen at -80°C until use.

Behavioral testing

Y-Maze

25 Mice were tested for hippocampus-dependent spatial memory using a two-trial Y-maze task. The arms of the maze were 22cm long, 6.4cm wide and 15cm deep. The floor of the maze was covered with sawdust that was mixed after each trial in order to eliminate
30 olfactory cues. Various extra-maze cues were placed on the surrounding walls. Experiments were conducted with an ambient light level of 6 lux. During the exposure phase, mice were assigned to two arms (the "start arm" and the "other arm") that they were allowed to freely explore during 5 min, without access to the third arm of the maze (the "novel arm") blocked by an opaque door. The assignment of arms was counterbalanced within each experimental

group. Mice were then removed from the maze and returned to their home cage for 2 min. During the test phase, mice were placed at the end of the same “start arm” and allowed to freely explore all three arms during 1 min. The amount of time spent in each of the arms was recorded using EthovisionXT (Noldus, Netherlands).

5

Morris Water Maze

Spatial memory abilities were examined in the standard hidden-platform acquisition and retention version of the Morris water maze. A 100-cm circular pool was filled with water, opacified with non-toxic white paint, and kept at 24 ± 1 °C. Before the behavioral test, animals were submitted to a habituation phase, during which they were put on a 10-cm round submerged platform (1 cm below the water surface) and allow to explore the pool during 2 min. During the learning phase, animals were required to locate the submerged platform using only the spatial cues in the test room. The platform remained in the same quadrant throughout the task. Four positions around the edge of the tank were arbitrarily designated 1, 2, 3, and 4; thus dividing the tank into four quadrants. Each mouse was given four swimming trials per day (10 min interval) for five consecutive days. The start position (1, 2, 3, or 4) was pseudo-randomized across trials. Mice that failed to find the submerged platform within 2 min were guided to the platform, where they remained for 15 s before being returned to their cages. Time required to locate the hidden escape platform (escape latency) and distance traveled (path length) were recorded using a video tracking system.

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15
20

Two days following the acquisition phase, a probe trial was conducted. During this probe trial (120 s), the platform was removed, search pattern of the mice was again tracked, and performance measures were calculated that included escape latency, path length, velocity.

25

Tau assays in blood samples from 2H9 injected mice

Tau concentrations in plasma (pg/ml) were determined at different times (6 hours; 24 hours and 1 week) following a single i.p injection using the INNOTEST® hTau Ag (Innogenetics, Belgium) that is a sandwich ELISA microplate assay for the quantitative determination of human Tau antigen in fluids. Capture antibody is the AT120 antibody and biotinylated antibodies HT7 and BT2 are detecting antibodies (Schraen-Maschke and al., 2008).

30

Immunohistochemistry

Animals were sacrificed and brains removed. The right hemisphere were post-fixed for 7 days in 4% paraformaldehyde, then incubated in 20% sucrose for 24 hours and finally kept frozen at -80°C until use. Serial free-floating coronal sections (40µm) were obtained using a cryostat (Leica Microsystems GmbH, Germany). Sections of interest were used for free floating immunohistochemistry using the following antibodies AT100 (Pierce MN-1060, against pT212/S214, 1/400), and anti-Tau pS422 (988, 1/1000) as described, and finally mounted on gelatine slides. Staining was semi-quantified as previously described (Belarbi et al, 2009). Photomicrographs were taken using a Leica digital camera, imported in ImageJ software (Scion) and converted to black and white images. Threshold intensity was set and kept constant and the number of pixel, expressing staining density, was determined for both THY-Tau22 and immunized THY-Tau22- mice. Quantifications were performed blindly by at least two observers and averaged from five to nine animals per group.

Immunofluorescence

For stereotaxic injections, free-floating coronal sections (40µm) were obtained using a cryostat (Leica Microsystems GmbH, Germany). Immunofluorescent labeling was performed using the following primary antibodies: biotinylated goat anti mouse IgG (“Biosciences; 1 :1000) and secondary antibodies coupled to Alexa 488 (Invitrogen) or Streptavidin Texas Red for biotinylated primary antibodies. Sections were counterstained and mounted with Vectashield/DAPI (Vector). DAPI was present in the Vectashield mounting medium for fluorescence (Vector). Slides were analyzed with a Zeiss LSM710 confocal laser scanning microscope (60 magnification). Images were collected in the z direction at 1-µm or 0,8-µm intervals.

Statistics

Data are presented as mean ± standard error of the mean (SEM). Data were analysed using analysis of variance (ANOVA), and where appropriate, followed by post-hoc Tukey’s test. Differences of $p < 0.05$ were considered significant. Data were analyzed by Prism Graphpad (San Diego, CA, USA).

RESULTS

Selection and characterization of novel monoclonal pS422 Tau antibody 2H9

By immunohistochemistry 2H9 labels only neurons of AD brain cortex but not control brain (Fig. 1A-B). In THY-Tau22 brain, 2H9 stain neurons of hippocampus and amygdala at the age of 6 months (Fig. 1C-D). On western blot, 2H9 labeled the Tau triplet (Tau 55, 64 and 69) in AD total cortical brain homogenates, while failing to recognize normal Tau proteins in brain homogenates from control case (Fig. 1E). In hippocampus homogenates from THY-Tau22, 2H9 recognize early pS422-Tau immunoreactive species and also the higher MW variant form of hyperphosphorylated Tau that appears at 6 months of age and increases at 12 months (Fig. 1E).

Repeated passive immunotherapy and cognition. THY-Tau22 mice have deficits in learning and memory in several behavioral testing including the Morris water maze and the Y-Maze at 9-12 months of age (Van der Jeugd et al., 2011; Burnouf et al., 2012). In the present work, THY-Tau22 mice were assessed for behavioral testing at 9 months of age.

Y-Maze. Memory of immunized mice was first assessed using a two-trial Y-maze task. During the exposure phase, no differences were found in the distance moved and duration between the groups (Fig. 2A-B). During the test phase, WT littermate controls (n=8) and 5mg/kg THY-Tau22 mice (n=8) spent a significantly greater proportion of time in the novel arm compare to the other arm whereas controls THY-Tau22 mice (n=7) did not (Fig. 2C). THY-Tau22 injected with 10mg/kg of 2H9 spent also more time in the novel arm (n=9 ; p=0,11). In this test, the behavior of littermate controls was similar to that of immunized THY-Tau22 mice (Fig.2C).

Morris Water Maze. Following the two-trial Y-maze mice were tested in the Morris water maze. During the training phase, all groups performed at comparable levels (Fig. 2D). Two days after the training period, a probe test was performed. At 30 sec, control THY-Tau22 mice did not display a significant preference for the target quadrant compared with controls littermates (p = 0,0134) and 10mg/kg THY-Tau22 mice animals (p = 0.0216; Fig. 2E-F). Although this difference was not significant for 5mg/kg, THY-Tau22 mice seem to spend more time in the target quadrant compared to non-vaccinated animals (Fig. 2E-F).

Passive immunotherapy and Tau pathology. To assess the impact of Tau immunotherapy on the development of Tau pathology, we carried out an immunohistochemical analysis of abnormally phosphorylated (AT100; pSer212/pThr214; pSer422) Tau species in both immunotherapy protocols (repeated and double). In repeated injection protocol, passive transfers significantly reduced the level of pathological Tau species

at the targeted epitope pS422 ($p=0,0447$, 47% of reduction compared to controls) but have less effect on AT100 staining ($p=0,56$; 23% reduction compared to controls, respectively). In a second set of experiments, 2H9 IP injection was performed twice at 5.5 and 10.5 months of age and sufficient to reduce Tau phosphorylation (significant decrease in AT8- and pS422-immunoreactivities and trend on AT100-immunoreactivity)(Figure).

Passive immunotherapy and Tau clearance: peripheral sink or phagocytosis activation

Tau concentrations in plasma were measured one week after a single i.p injection of 2H9 at 5mg/kg – 10mg/kg or PBS in THY-Tau22. Plasma from THY-Tau22 mice exhibited higher Tau concentrations than those of non-vaccinated mice (Fig 4).

We have also injected pathological phospho Tau monoclonal antibodies (2H9, AT100) and phospho Tau antibody (AT8) into the hippocampus of THY-Tau22 mice. All these antibodies enter the neurons contrary to isotype control (not show) but we found most neurons that have internalized AT8.

Hybridoma filing

Hybridoma supernatant from murine monoclonal antibody 2H9, deposited at CNCM under # I-4666, was checked by ELISA about its phosphor-Ser 422 specificity (Figure 5). It also gives similar stainings by immunohistochemistry and western blotting as described in Figure 1.

DISCUSSION

Our previous findings indicate that vaccination with a phospho peptide including the pSer422 residue leads to a reduction of Tau pathology in the hippocampus of THY-Tau22. This decrease was observed by immunohistochemistry and by western blot for the insoluble Tau species (Troquier et al., 2012). Here we investigated the effect of a peripheral administration of a novel monoclonal antibody against pSer422 in the THY-Tau22 model. Recently several passive Tau immunotherapies have reported promising results (Boutajangout et al., 2011; Chai et al., 2011). 2H9 is a monoclonal antibody that recognizes Tau pSer422 in AD brain and in THY-Tau22 brain. This pathological phospho-epitope is found in multiple Tauopathies (Buissière et al., 1999; Augustinack et al., 2002; Guillozet-Bongaarts et al., 2007) and animals models (for review Götz et al., 2010). Phosphorylation of Ser422 increases aggregation propensity (Haase et al., 2004) and correlates with tangle formation in P301L

model (Deters et al., 2008) as in THY Tau22 model (Schindowski et al., 2006). Tau pSer422 is early events that precede caspase cleavage (DeCalignon et al., 2010). Caspases cuts Tau at the aspartate residue at position 421 (Asp421), yielding a truncated Tau protein that has been suggested to be a nucleation agent. Then immunotherapy targeting pSer422 may prevent this cleavage. In the present study, i.p injections of an antibody raised against pS422 delayed cognitive deficits in the THY-Tau22 model as observed in the Y-Maze and the Morris Water Maze tests. Immunohistochemical analysis also revealed a reduction of abnormally phosphorylated Tau proteins in the hippocampus. We are convinced that immunotherapy targeting Tau pS422 has a great potential as treatment for Tauopathies. The preliminary results of a recent passive transfert in TauPS2APP triple transgenic mice with monoclonal antibody raised against pS422 confirms the potential of targeting this particular epitope (Bohrmann et al., 2011). In order to ameliorate vaccine it is necessary to understand the mechanisms of antibody-mediated clearance of Tau aggregates.

In our previous study of vaccination, we have observed an increase in plasmatic Tau concentrations of vaccinated mice suggesting a mechanism of “peripheral sink” like for A β immunotherapy (DeMattos et al., 2001; Lemere et al., 2003). After a single injection of 10 mg/kg of 2H9, we also observed an increase in Tau concentrations in plasma from THY-Tau22, it strongly suggests that circulating antibodies sequester Tau and favor efflux of Tau from the brain. This result is consistent with the reduction of soluble Tau species observed by western blot. It is also known that a small percentage of antibodies is able to cross the blood-brain barrier (Levites et al., 2006). Once in the brain, anti-Tau antibodies may easily recognize extracellular epitopes found in ghost tangles and trigger microglia-related clearance. However, they may also enter the cell through the endosome-lysosome pathway and activate mechanisms of Tau clearance as propose by several groups (Asuni et al., 2007; Krishnamurthy et al., 2011; Boimel et al., 2010).

In summary, these findings show that passive Tau pSer422 immunotherapy can reduces cognitive impairments. The aim of this study was also to elucidate the mechanisms of antibody-mediated clearance. Overall, like for A β immunotherapy, several mechanisms may be proposed. We suggested that the presence of circulating antibodies creates a « peripheral sink » which alters the equilibrium across the blood brain barrier for Tau to favor efflux owing to the reduced free A β concentration in blood. The second hypothesis implicates that antibodies enter the CNS. Then, they may act directly on extracellular Tau and block the spread of Tau pathology (Frost et al., 2009 ; Clavaguera et al., 2010). These antibodies may

also be internalized by the neurons by different processes (for review Sigurdsson, 2008) and activate the autophagic/lysosomal pathways.

REFERENCES:

5

Throughout this application, various references describe the state of the art to which this invention pertains. The disclosures of these references are hereby incorporated by reference into the present disclosure.

Asuni et al. Immunotherapy targeting pathological tau conformers in a tangle mouse
10 model reduces brain pathology with associated functional improvements. *Journal of Neuroscience* (2007) vol. 27 (34) pp. 9115-29

Augustinack et al. Specific tau phosphorylation sites correlate with severity of neuronal cytopathology in Alzheimer's disease. *Acta neuropathologica* (2002) vol. 103 (1) pp. 26-35

15 Belarbi et al. Beneficial effects of exercise in a transgenic mouse model of Alzheimer's disease-like Tau pathology. *Neurobiology of Disease* (2011) vol. 43 (2) pp. 486-94

Belarbi et al. Early Tau pathology involving the septo-hippocampal pathway in a Tau transgenic model: relevance to Alzheimer's disease. *Current Alzheimer research* (2009) vol. 6 (2) pp. 152-7

20 Boimel et al. Efficacy and safety of immunization with phosphorylated tau against neurofibrillary tangles in mice. *Experimental Neurology* (2010) pp. 1-14

Boutajangout et al. Targeting hyperphosphorylated tau protein with a monoclonal antibody at an advanced stage of tau pathology improves cognition in a mouse model. *Alzheimer's and Dementia* (2011) vol. 7 (S) pp. S480-S481

25 Boutajangout et al. Passive immunization targeting pathological phospho-tau protein in a mouse model reduces functional decline and clears tau aggregates from the brain. *Journal of Neurochemistry* (2011)

Boutajangout et al. Immunotherapy Targeting Pathological Tau Prevents Cognitive Decline in a New Tangle Mouse Model. *Journal of Neuroscience* (2010) vol. 30 (49) pp.
30 16559-16566

Bussi re et al. Phosphorylated serine422 on tau proteins is a pathological epitope found in several diseases with neurofibrillary degeneration. *Acta neuropathologica* (1999) vol. 97 (3) pp. 221-30

Cartier et al. AAV gene therapy with cholesterol 24-hydroxylase improves Alzheimer's phenotype of tau mouse model. *Alzheimer's and Dementia* (2011) vol. 7 (S) pp. S651

Chai et al. Passive immunization with anti-tau antibodies in two transgenic models: Reduction of tau pathology and delay of disease progression. *Journal of Biological ...* (2011)

Clavaguera et al. Transmission and spreading of tauopathy in transgenic mouse brain. *Nature cell biology* (2009) vol. 11 (7) pp. 909-13

D'abramo et al. Passive immunization of P301L mice with two different tau monoclonal antibodies. *Alzheimer's and Dementia* (2011) vol. 7 (S) pp. S481

De Calignon et al. Caspase activation precedes and leads to tangles. *Nature* (2010) vol. 464 (7292) pp. 1201-4

De Gassart. Lipid raft-associated protein sorting in exosomes. *Blood* (2003) vol. 102 (13) pp. 4336-4344

DeMattos et al. Peripheral anti-A beta antibody alters SNC and plasma A beta clearance and decreases brain A beta burden in a mouse model of Alzheimer's disease. *Proceedings of the National Academy of Sciences of the United States of America* (2001) vol. 98 (15) pp. 8850-5

Deters et al. Divergent phosphorylation pattern of tau in P301L tau transgenic mice. *European Journal of Neuroscience* (2008) vol. 28 (1) pp. 137-47

Frost et al. Propagation of tau misfolding from the outside to the inside of a cell. *The Journal of biological chemistry* (2009) vol. 284 (19) pp. 12845-52

Guillozet-Bongaarts et al. Phosphorylation and cleavage of tau in non-AD tauopathies. *Acta neuropathologica* (2007) vol. 113 (5) pp. 513-20

Haase et al. Pseudophosphorylation of tau protein alters its ability for self-aggregation. *Journal of Neurochemistry* (2004) vol. 88 (6) pp. 1509-20

Jeugd et al. Hippocampal tauopathy in tau transgenic mice coincides with impaired hippocampus-dependent learning and memory, and attenuated late-phase long-term depression of synaptic transmission. *Neurobiology of Learning and Memory* (2011) vol. 95 pp. 296-304

Jicha. Is passive immunization for Alzheimer's disease 'alive and well' or 'dead and buried?'. *Expert opinion on biological therapy* (2009) vol. 9 (4) pp. 481-91

Krishnamurthy et al. Mechanistic studies of antibody clearance of tau aggregates using an ex vivo brain slice. *Alzheimer's and Dementia* (2010) vol. 6 (S276) pp. 1-1

Lasagna-Reeves et al. Tau oligomers impair memory and induce synaptic and mitochondrial dysfunction in wild-type mice. *Molecular neurodegeneration* (2011) vol. 6 pp. 39

5 Lee et al. Protein aggregate spreading in neurodegenerative diseases: Problems and perspectives. *Neuroscience research* (2011) pp.

Lemere et al. Amyloid-beta immunization in Alzheimer's disease transgenic mouse models and wildtype mice. *Neurochemical research* (2003) vol. 28 (7) pp. 1017-27

Leroy et al. Early axonopathy preceding neurofibrillary tangles in mutant tau transgenic mice. *The American Journal of Pathology* (2007) vol. 171 (3) pp. 976-92

10 Levites et al. Anti-Abeta42- and anti-Abeta40-specific mAbs attenuate amyloid deposition in an Alzheimer disease mouse model. *The Journal of clinical investigation* (2006) vol. 116 (1) pp. 193-201

Masliah et al. Passive Immunization Reduces Behavioral and Neuropathological Deficits in an Alpha-Synuclein Transgenic Model of Lewy Body Disease. *PloS one* (2011)

15 Masliah et al. Effects of alpha-synuclein immunization in a mouse model of Parkinson's disease. *Neuron* (2005) vol. 46 (6) pp. 857-68

Orgogozo et al. Subacute meningoencephalitis in a subset of patients with AD after Abeta42 immunization. *Neurology* (2003) vol. 61 (1) pp. 46-54

20 Pandey. Hybridoma technology for production of monoclonal antibodies. *Hybridoma* (2010) vol. 1 (2) pp. 017

Rabchevsky et al. Peripheral injections of Freund's adjuvant in mice provoke leakage of serum proteins through the blood-brain barrier without inducing reactive gliosis. *Brain research* (1999) vol. 832 (1-2) pp. 84-96

25 Rosenmann et al. Detection of circulating antibodies against tau protein in its unphosphorylated and in its neurofibrillary tangles-related phosphorylated state in Alzheimer's disease and healthy subjects. *Neuroscience letters* (2006) vol. 410 (2) pp. 90-3

Schindowski et al. Alzheimer's disease-like tau neuropathology leads to memory deficits and loss of functional synapses in a novel mutated tau transgenic mouse without any motor deficits. *The American journal of pathology* (2006) vol. 169 (2) pp. 599-616

30 Schraen-Maschke et al. Tau as a biomarker of neurodegenerative diseases. *Biomarkers in medicine* (2008) vol. 2 (4) pp. 363-84

Sigurdsson. Immunotherapy targeting pathological tau protein in Alzheimer's disease and related tauopathies. *Journal of Alzheimer's disease : JAD* (2008) vol. 15 (2) pp. 157-68

CLAIMS:

1. An antibody characterized in that it is capable of binding human Tau phosphorylated at serine 422, and in that it comprises the VH-CDR1, VH-CDR2 and VH-CDR3 of the heavy chain and the VL-CDR1, VL-CDR2 and VL-CDR3 of the light chain of the antibody 2H9 produced by the hybridoma CNCM I-4666.
2. An antibody characterized in that it is capable of binding human Tau phosphorylated at serine 422, wherein said antibody has the following 6 CDRs:

	Aminoacid sequence
VH-CDR1	GYTFTSYWMH (SEQ ID NO:12)
VH-CDR2	NINPNNGGTNYNEKFKN (SEQ ID NO:13)
VH-CDR3	GRNYDV (SEQ ID NO:14)
VL-CDR1	RSSQNIVYGNGNTYLE (SEQ ID NO :16)
VL-CDR2	KVSNRFS (SEQ ID NO :17)
VL-CDR3	FQGSHVPLT (SEQ ID NO :18)

3. An antibody according to claims 1 to 2, characterized in that it is selected from :
- murine monoclonal antibody 2H9 obtainable from the hybridoma CNCM I-4666
 - chimeric or humanized antibodies obtained from the antibody 2H9;
 - antibodies which comprises the VL chain and the VH chain of the antibody 2H9;
 - the fragments of the antibodies a) to c) above.
4. An antibody according to any of claims 1 to 3, where is the fragment are selected from Fv, Fab, F(ab')₂, Fab', dsFv, scFv, sc(Fv)₂ diabodies tribodies and tetrabodies.

5. An antibody according to any of claims 1 to 4, characterized in that it is able to reduce the brain level of Tau phosphorylated at Ser422 and its aggregation.
6. A nucleotide acid sequence encoding an antibody according to any of claims 1 to 5.
- 5 7. A vector comprising a nucleotide acid sequence of claim 5.
8. A host-cell transfected, infected or transformed by a nucleotide acid sequence of claim 6 and/or a vector of claim 6 and expressing an antibody according to any of claims 1 to 5.
9. A host-cell of claim 7, which is the hybridoma CNCM I-4666.
- 10 10. A method for preparing an antibody according to any of claims 1 to 5, characterized in that it comprises the steps of:
 - (i) introducing in vitro or ex vivo a recombinant nucleotide acid sequence of claim 6 and/or a vector of claim 7 into a competent host cell as claimed in any one of claims 8 to 9,
 - 15 (ii) culturing in vitro or ex vivo the recombinant host cell obtained,
 - (iii) recovering said antibody from said culture.
11. A method for detecting human Tau phosphorylated at serine 422, and/or evaluating its amount in a biological sample, wherein said method comprises contacting said sample with an antibody according to any of claims 1 to 5.
- 20 12. A method according to claims 11, for the *in vitro* diagnosis of tauopathy.
13. A pharmaceutical composition comprising the antibody or a fragment according to any of claims 1 to 5.
14. A pharmaceutical composition according to claims 13, for use in the treatment of tauopathy.
- 25 15. Use of pharmaceutical composition according to claims 14, for the treatment of Alzheimer's Disease, Down syndrome; Guam parkinsonism dementia complex; Dementia pugilistica; myotonic dystrophies; Niemann-Pick disease type C; Pick disease; argyrophilic grain disease; Fronto-temporal dementia; Cortico-basal degeneration; Pallido-ponto-nigral degeneration; Progressive supranuclear palsy; and Prion disorders such as Gerstmann-Sträussler-Scheinker disease with tangles.
- 30

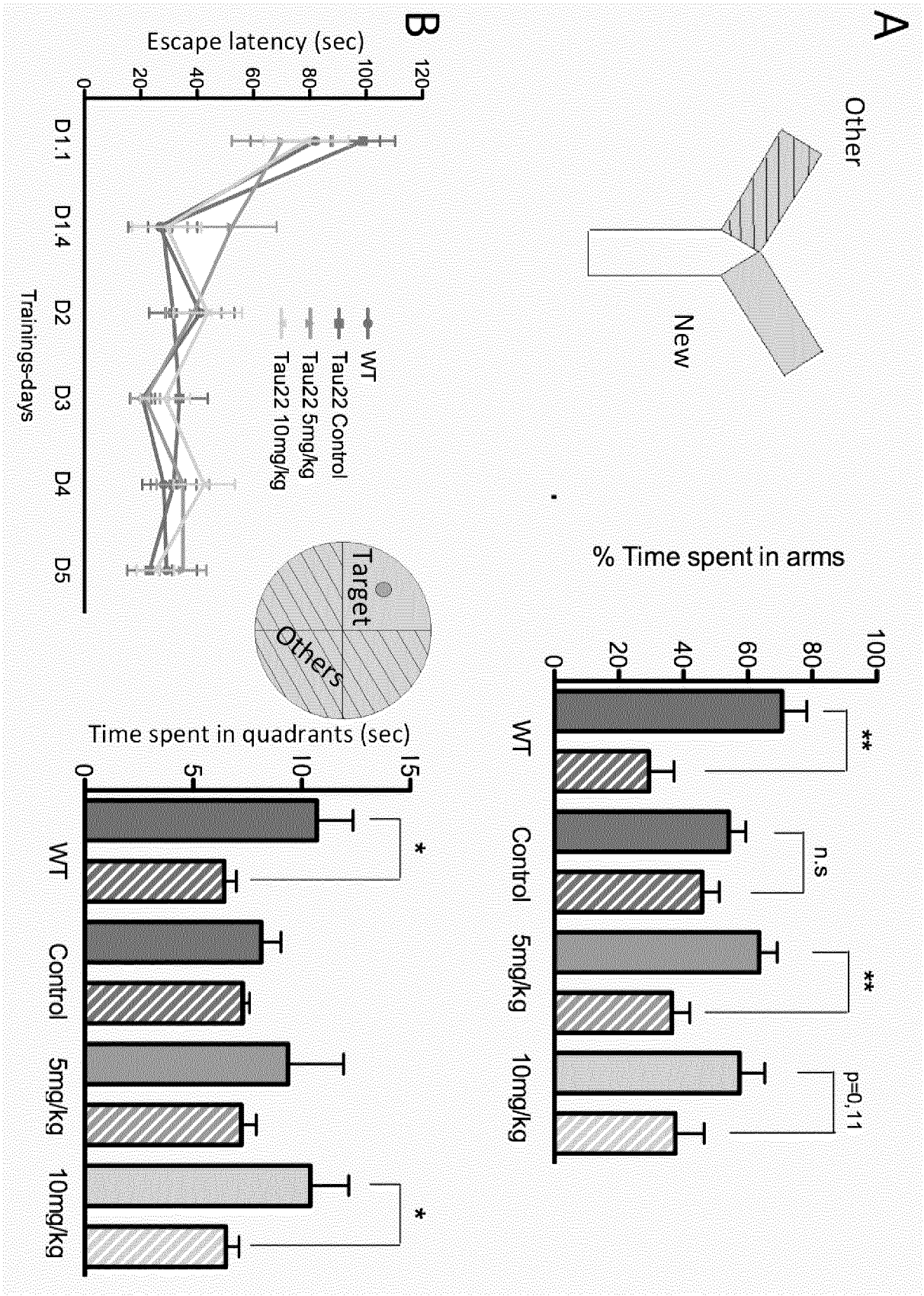
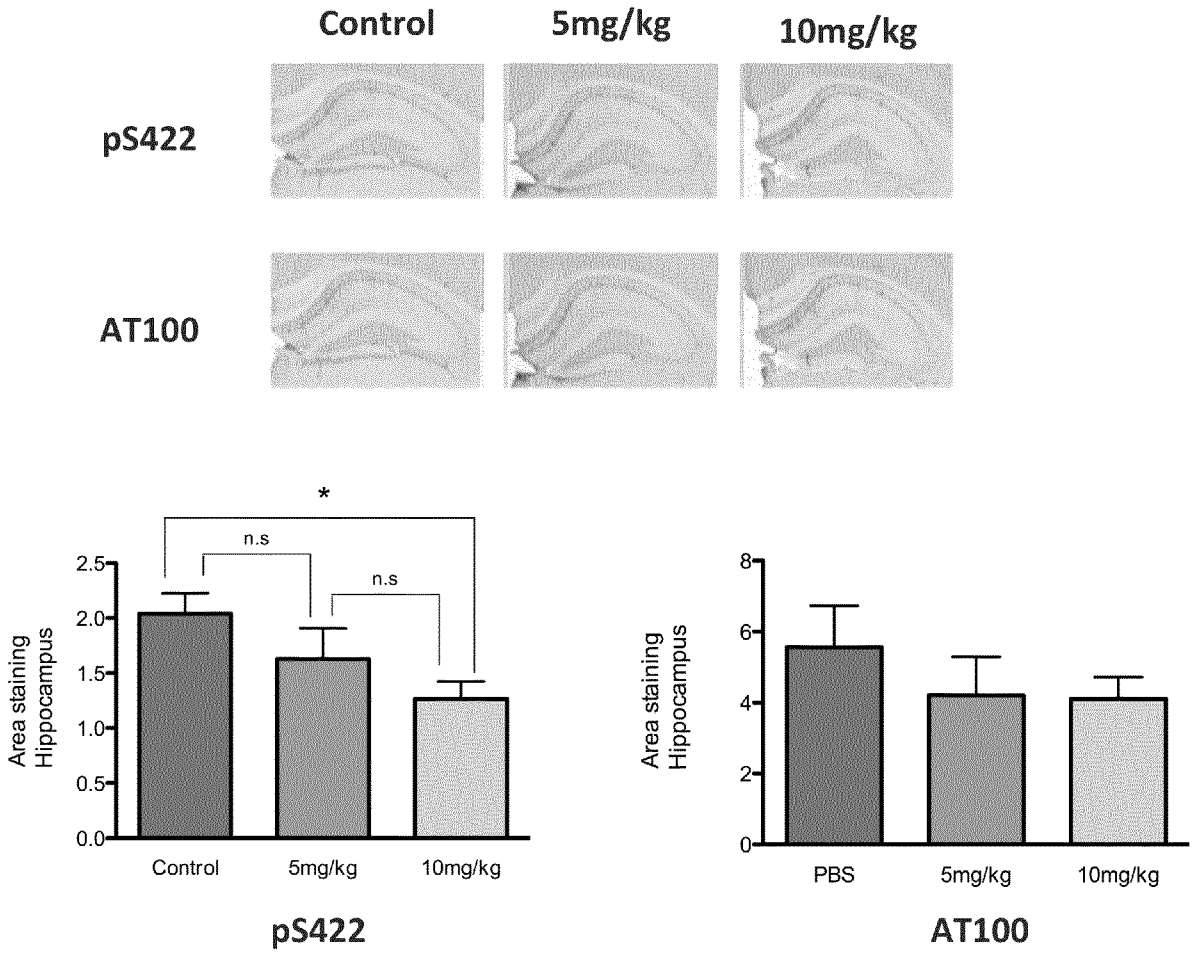


Figure 2

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A.



B.

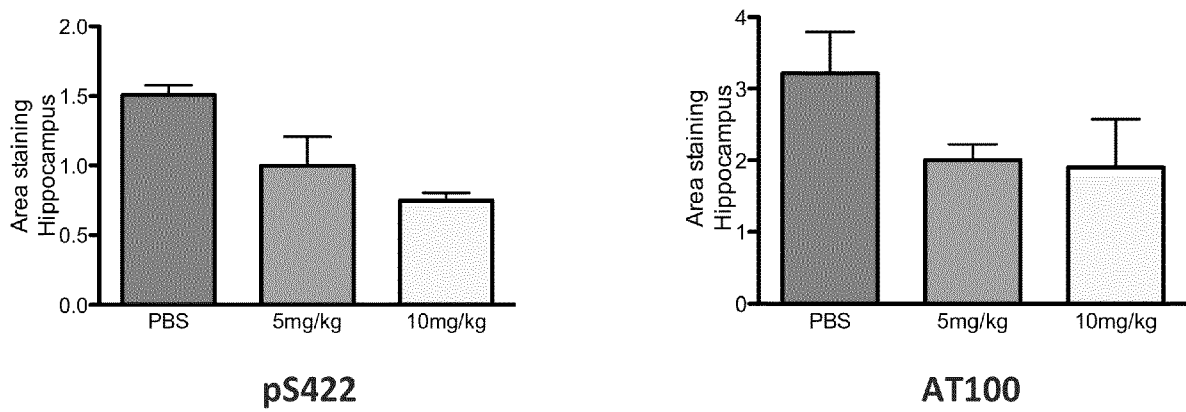


Figure 3

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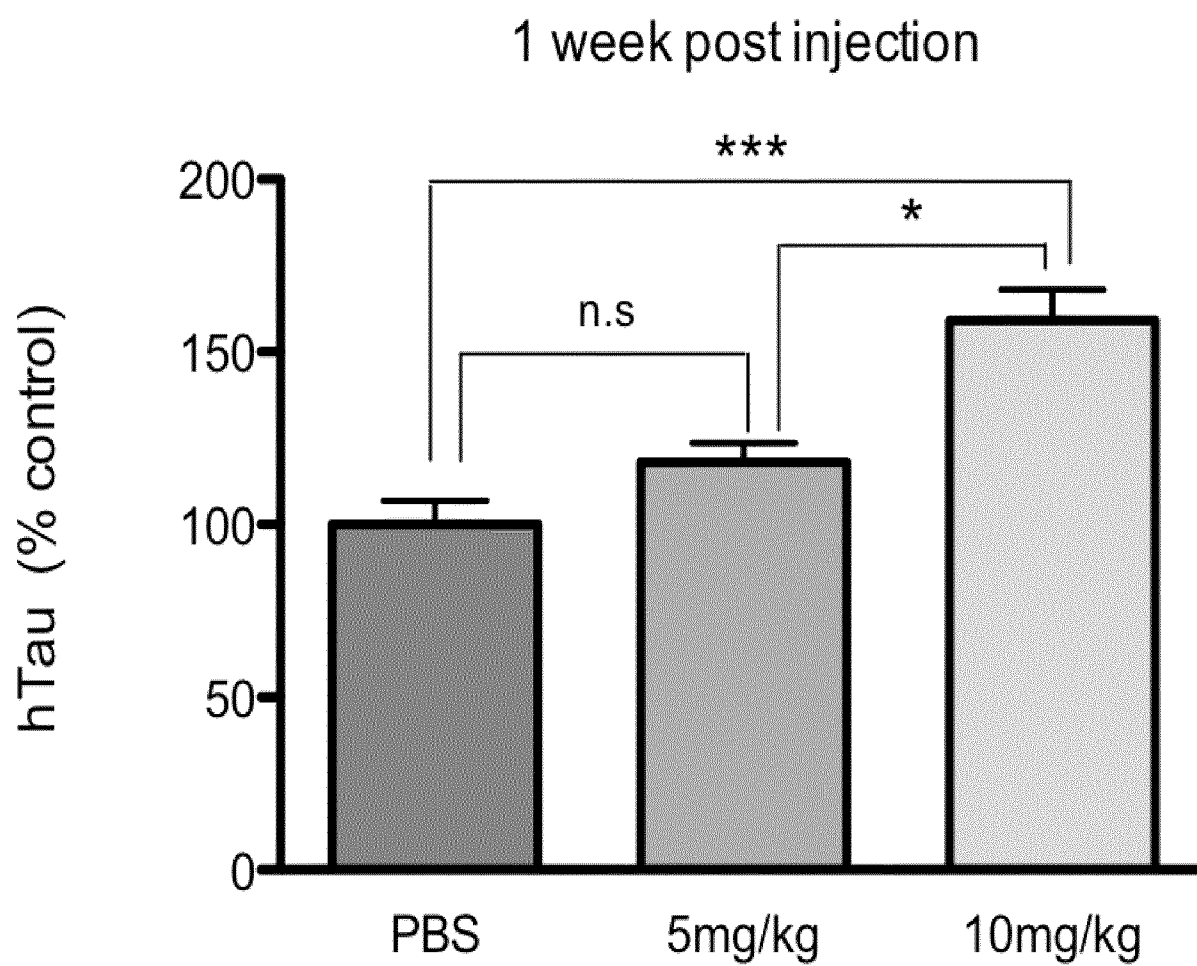
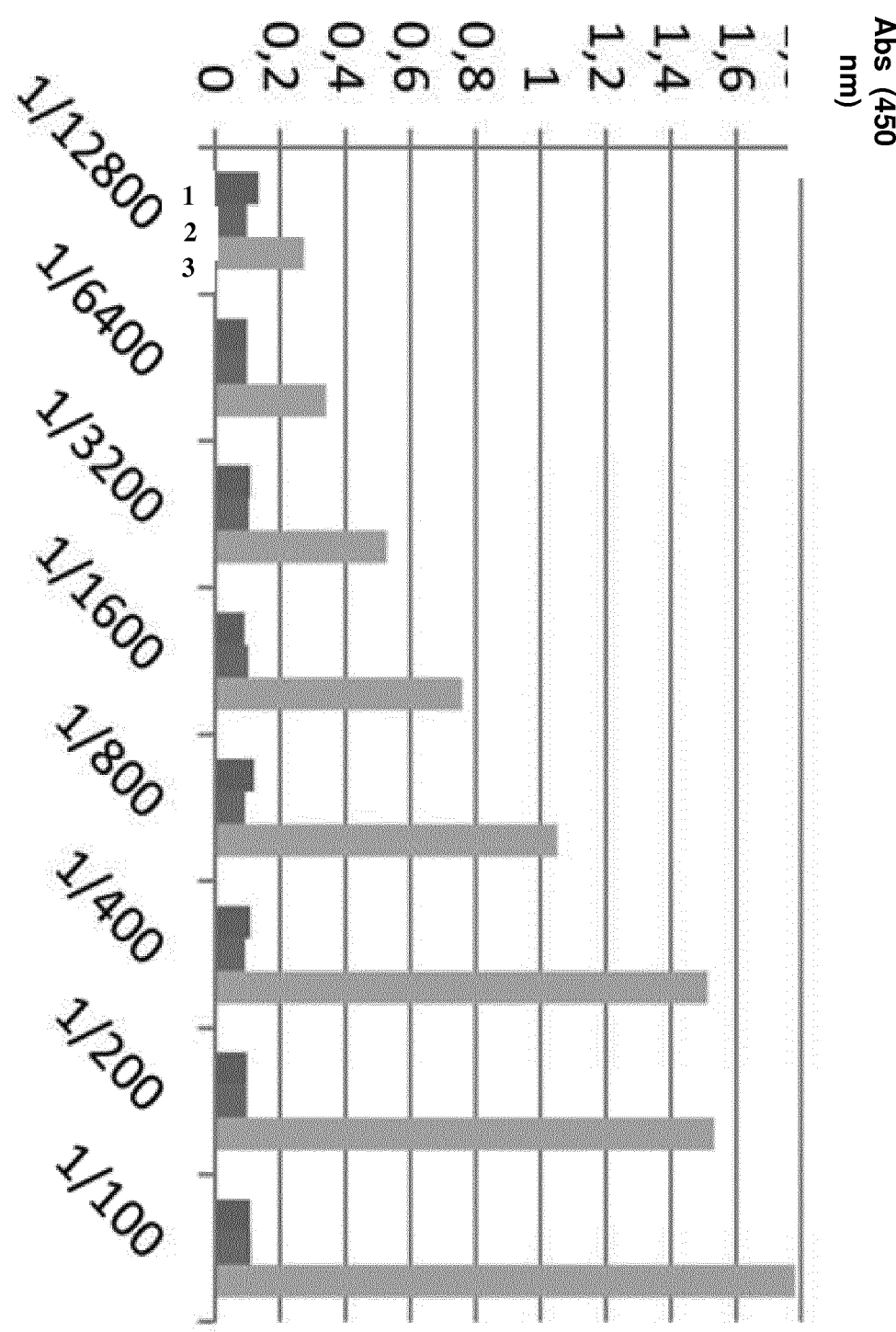


Figure 4



- 1 Irrelevant Peptide
- 2 S422 Tau peptide
- 3 Phospho S422 Tau peptide

Figure 5

INTERNATIONAL SEARCH REPORT

International application No
PCT/EP2013/077608

A. CLASSIFICATION OF SUBJECT MATTER INV. C07K16/18 C07K16/44 A61K39/395 A61P25/28 ADD.		
According to International Patent Classification (IPC) or to both national classification and IPC		
B. FIELDS SEARCHED Minimum documentation searched (classification system followed by classification symbols) C07K		
Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched		
Electronic data base consulted during the international search (name of data base and, where practicable, search terms used) EPO-Internal		
C. DOCUMENTS CONSIDERED TO BE RELEVANT		
Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 2010/142423 A2 (HOFFMANN LA ROCHE [CH]; BOHRMANN BERND [CH]; GOEPFERT ULRICH [DE]; GRU) 16 December 2010 (2010-12-16) cited in the application	1-8, 10-12
Y	the whole document table 3	13-15
X	----- US 2002/086009 A1 (ISHIGURO KOICHI [JP] ET AL) 4 July 2002 (2002-07-04)	1-8, 10-12
Y	example 1 ----- -/-	13-15
☒ Further documents are listed in the continuation of Box C. ☒ See patent family annex.		
* Special categories of cited documents : "A" document defining the general state of the art which is not considered to be of particular relevance "E" earlier application or patent but published on or after the international filing date "L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified) "O" document referring to an oral disclosure, use, exhibition or other means "P" document published prior to the international filing date but later than the priority date claimed "T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention "X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone "Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art "&" document member of the same patent family		
Date of the actual completion of the international search 20 February 2014		Date of mailing of the international search report 03/03/2014
Name and mailing address of the ISA/ European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Fax: (+31-70) 340-3016		Authorized officer Bumb, Peter

INTERNATIONAL SEARCH REPORT

International application No
PCT/EP2013/077608

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT		
Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	TROQUIER L ET AL: "Targeting phospho-Ser422 by active Tau immunotherapy in the THY-Tau22 mouse model: A suitable therapeutic approach", CURRENT ALZHEIMER RESEARCH, SAIF ZONE, SHARJAH [U.A.] : BENTHAM, AE, vol. 9, no. 4, 1 May 2012 (2012-05-01), pages 397-405, XP008160263, ISSN: 1567-2050, DOI: 10.2174/156720512800492503 page 397, right-hand column page 401 -----	13-15
Y,P	YANAMANDRA KIRAN ET AL: "Anti-tau antibodies that block tau aggregate seeding in vitro markedly decrease pathology and improve cognition in vivo.", NEURON 16 OCT 2013, vol. 80, no. 2, 16 October 2013 (2013-10-16), pages 402-414, XP002720174, ISSN: 1097-4199 the whole document -----	13-15

INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No

PCT/EP2013/077608

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO 2010142423 A2	16-12-2010	AR 077016 A1	27-07-2011
		AU 2010257799 A1	01-12-2011
		CA 2762594 A1	16-12-2010
		CN 102459333 A	16-05-2012
		CO 6450613 A2	31-05-2012
		CR 20110606 A	12-01-2012
		EC SP11011514 A	31-01-2012
		EP 2440578 A2	18-04-2012
		JP 2012529275 A	22-11-2012
		KR 20120024952 A	14-03-2012
		MA 33339 B1	01-06-2012
		NZ 596187 A	29-11-2013
		PE 05432012 A1	23-05-2012
		RU 2011152816 A	20-07-2013
		SG 176754 A1	30-01-2012
		TW 201103560 A	01-02-2011
		US 2011059093 A1	10-03-2011
		US 2013310541 A1	21-11-2013
		WO 2010142423 A2	16-12-2010

US 2002086009 A1	04-07-2002	US 2002086009 A1	04-07-2002
		WO 9734145 A1	18-09-1997
